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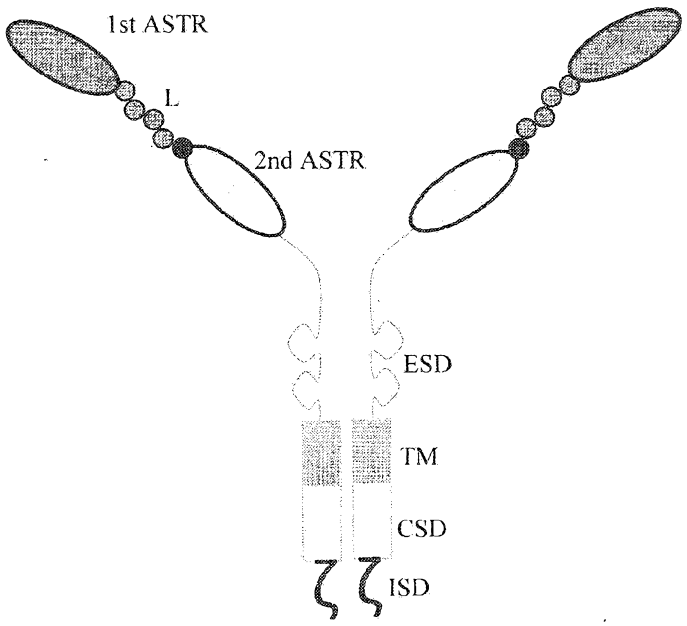
(54) Title: BISPECIFIC CHIMERIC ANTIGEN RECEPTORS AND THERAPEUTIC USES THEREOF

Figure 1



(57) Abstract: The invention is directed to a bispecific chimeric antigen receptor, comprising: (a) at least two antigen-specific targeting regions; (b) an extracellular spacer domain; (c) a transmembrane domain; (d) at least one co-stimulatory domain; and (e) an intracellular signaling domain, wherein each antigen-specific targeting region comprises an antigen-specific single chain Fv (scFv) fragment, and binds a different antigen, and wherein the bispecific chimeric antigen receptor is co-expressed with a therapeutic control. The invention also provides methods and uses of the bispecific chimeric antigen receptors.

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BISPECIFIC CHIMERIC ANTIGEN RECEPTORS AND THERAPEUTIC USES THEREOF

FIELD OF INVENTION

The invention relates to chimeric antigen receptors and to genetically engineered cells using the same.

BACKGROUND OF THE INVENTION

Current immunotherapies are designed to target single antigens on cancer cells. However, for example, cancer cells are unstable and some cells may no longer possess the target antigen. These cells, referred to as antigen loss escape variants, escape destruction by the therapy and may continue to grow and spread unchecked. Therefore there is a need in the art for therapies which prevent or minimize therapeutic failures in cancer and other diseases.

SUMMARY OF THE INVENTION

In an embodiment, the invention provides a bispecific chimeric antigen receptor, comprising (a) at least two antigen-specific targeting regions, (b) an extracellular spacer domain, (c) a transmembrane domain, (d) at least one co-stimulatory domain and (e) an intracellular signaling domain, wherein each antigen-specific targeting region comprises an antigen-specific single chain Fv (scFv) fragment, and binds a different antigen, and wherein the bispecific chimeric antigen receptor is co-expressed with a therapeutic control.

In an embodiment, the invention further provides a combination of a bispecific chimeric antigen receptor and a therapeutic control, wherein the bispecific chimeric antigen receptor comprises (a) at least two antigen-specific targeting regions, (b) an extracellular spacer domain, (c) a transmembrane domain, (d) at least one co-stimulatory domain and (e) an intracellular signaling domain, wherein each antigen-specific targeting region comprises an antigen-specific single chain Fv (scFv) fragment, and binds a different antigen.

In an embodiment, the invention further provides a bispecific chimeric antigen receptor, comprising (a) at least two antigen-specific targeting regions, (b) an extracellular spacer domain, (c) a transmembrane domain, (d) at least one co-stimulatory domain and (e) an intracellular signaling domain, wherein each antigen-specific targeting region comprises an antigen-specific single chain Fv (scFv) fragment, and binds a different antigen, and wherein the bispecific chimeric antigen receptor is co-expressed with truncated epidermal growth factor receptor (EGFRt).

In an embodiment, the invention further provides a bispecific chimeric antigen receptor, comprising (a) at least two antigen-specific targeting regions, (b) a CD8αhinge extracellular spacer domain, (c) a CD8α transmembrane domain, (d) a 4-1BB co-stimulatory domain and (vi) a CD3 zeta intracellular signaling domain, wherein each antigen-specific targeting region comprises an antigen-specific single chain Fv (scFv) fragment, and binds a different antigen, wherein the bispecific chimeric antigen receptor is co-expressed with EGFRt and wherein the bispecific chimeric antigen receptor and EGFRt are linked via a T2A linker.

In an embodiment, also provided are pharmaceutical compositions comprising the above-described bispecific chimeric antigen receptors, a combination of the bispecific chimeric antigen receptors and therapeutic controls, polypeptides encoding the bispecific chimeric antigen receptors, vectors, viruses and genetically engineered cells comprising the bispecific chimeric antigen receptors, vectors, viruses and genetically engineered cells comprising a combination of the bispecific chimeric antigen receptors and therapeutic controls, or combinations thereof, and a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF FIGURES

Exemplary embodiments are illustrated in the referenced figures. It is intended that the embodiments and figures disclosed herein are to be considered illustrative rather than restrictive.

Figure 1 depicts a schematic representation of a chimeric antigen receptor of the invention, in accordance with an embodiment of the present invention. ASTR is an

antigen-specific targeting region, L is a linker, ESD is an extracellular spacer domain, TM is a transmembrane domain, CSD is a co-stimulatory domain, and ISD is an intracellular signaling domain,

Figure 2 depicts (a) the components of an anti-CD19xCD20 CAR, and (b) a complete cDNA packaged into an epHIV-7 lentivirus vector transfer plasmid, in accordance with an embodiment of the present invention.

Figure 3 depicts, in accordance with an embodiment of the present invention, the nucleic acid sequence of a bispecific CAR CD19scFv-Gly4Ser1linker-CD20scFv-IgG4Hinge-CD28tm-41BB-CD3zeta-T2A-EGFRt_epHIV7.

Figure 4 depicts, in accordance with an embodiment of the present invention, the nucleic acid and amino acid sequences of a bispecific CAR CD19scFv-Gly4Ser1linker-CD20scFv-IgG4Hinge-CD28tm-41BB-CD3zeta-T2A-EGFRt_epHIV7.

Figure 5 depicts, in accordance with an embodiment of the present invention, a CD19scFv-Gly4Ser1linker-CD20scFv-IgG4hinge-CD28tm-CD28gg-CD3Zeta transgene construct.

Figure 6 depicts, in accordance with an embodiment of the present invention, development of a CγCR platform to support exogenous γc independent growth. (a) Schematic diagrams of wild type versus chimeric cytokine receptors. The IL-7Rα constitutive cytokine receptor (CγCR7) consists of the human IL-7 cytokine tethered to the full length human IL-7Rα chain via a $(G_4S)_2$ linker. The IL-2Rβ constitutive cytokine receptor (CγCR2) is identical to CγCR7 except that the IL-7Rα intracellular signaling domain is replaced with the human IL-2/IL-15Rβ cytoplasmic domain. (b) Diagram of the expression construct CγCR-T2A-CD19t.

Figure 7 depicts, in accordance with an embodiment of the present invention, the nucleic acid and amino acid sequences of an embodiment of the invention, namely a backbone CAR comprising the hinge region of IgG4, the transmembrane domain of CD28, the costimulatory domain of 4-1BB and the cytoplasmic domain of CD3zeta.

Figure 8 depicts, in accordance with an embodiment of the present invention, the nucleic acid sequence of an embodiment of the invention, namely GMCSFRss-CD19scFv-Gly4Serlinker-CD20scFv-huIgGHinge/CH2/CH3-CD28tm/CD28cyto-41BB-CD3zeta. GMCSFRss is the signal sequence from GMCSFR.

Figure 9 depicts, in accordance with an embodiment of the present invention, the nucleic acid and amino acid sequences of an embodiment of the invention, namely GMCSFRss-CD19scFv-Gly4Serlinker-CD20scFv-huIgGHinge/CH2/CH3-CD28tm/CD28cyto-41BB-CD3zeta. GMCSFRss is the signal sequence from GMCSFR.

Figure 10 depicts, in accordance with an embodiment of the present invention, the nucleic acid sequence of an embodiment of the invention, namely the GMCSFRss-CD19scFv-Gly4Serlinker-CD20scFv-CD8αHinge-CD8αtm-41BB-CD3zeta-T2A-EGFRt. GMCSFRss is the signal sequence from GMCSFR.

Figure 11 depicts, in accordance with an embodiment of the present invention, the nucleic acid and amino acid sequences of an embodiment of the invention, namely GMCSFRss-CD19scFv-Gly4Serlinker-CD20scFv-CD8αHinge-CD8αtm-41BB-CD3zeta-T2A-EGFRt. GMCSFRss is the signal sequence from GMCSFR.

Figure 12 depicts, in accordance with an embodiment of the present invention, the nucleic acid sequence of an embodiment of an invention namely T2A-EGFRt.

Figure 13 depicts, in accordance with an embodiment of the present invention, the nucleic acid and amino acid sequences of an embodiment of the invention, namely T2A-EGFRt.

DETAILED DESCRIPTION OF THE INVENTION

All references cited herein are incorporated by reference in their entirety as though fully set forth. Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton *et al.*, *Dictionary of Microbiology and Molecular Biology* 3rd *ed.*, J. Wiley & Sons (New York, NY 2001); March, *Advanced Organic Chemistry Reactions, Mechanisms and Structure* 5th *ed.*, J. Wiley & Sons (New York, NY 2001); and Sambrook and Russel, *Molecular Cloning: A Laboratory Manual 3rd ed.*, Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY 2001), provide one skilled in the art with a general guide to many of the terms used in the present application.

One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. Indeed, the present invention is in no way limited to the methods and materials described. For purposes of the present invention, the following terms are defined below.

The invention described herein provides chimeric antigen receptors. Chimeric antigen receptors are engineered receptors which graft an immune specificity onto a genetically engineered cell. By housing specificities to multiple antigens in a single chimeric antigen receptor (CAR), various benefits may be achieved, including, among others, a significant reduction in effort as compared to making multiple T-cell products per patient.

Definitions

Components of the Chimeric Antigen Receptors

"Antigen-specific targeting region" (ASTR) as used herein refers to the region of the CAR which targets specific antigens. The CARs of the invention comprise at least two targeting regions which target at least two different antigens. In an embodiment, CARs comprise three or more targeting regions which target at least three or more different antigens. The targeting regions on the CAR are extracellular. In some embodiments, the antigen-specific targeting regions comprise an antibody or a functional equivalent thereof or a fragment thereof or a derivative thereof and each of the targeting regions target a

different antigen. The targeting regions may comprise full length heavy chain, Fab fragments, single chain Fv (scFv) fragments, divalent single chain antibodies or diabodies, each of which are specific to the target antigen. There are, however, numerous alternatives, such as linked cytokines (which leads to recognition of cells bearing the cytokine receptor), affibodies, ligand binding domains from naturally occurring receptors, soluble protein/peptide ligand for a receptor (for example on a tumor cell), peptides, and vaccines to prompt an immune response, which may each be used in various embodiments of the invention. In fact, almost any molecule that binds a given antigen with high affinity can be used as an antigen-specific targeting region, as will be appreciated by those of skill in the art.

"Chimeric antigen receptor" or "CAR" or "CARs" as used herein refers to engineered receptors, which graft an antigen specificity onto cells (for example T cells such as naïve T cells, central memory T cells, effector memory T cells or combination thereof). CARs are also known as artificial T-cell receptors, chimeric T-cell receptors or chimeric immunoreceptors. The CARs of the invention comprise at least two antigen-specific targeting regions, an extracellular domain, a transmembrane domain, one or more co-stimulatory domains, and an intracellular signaling domain. The two or more antigen-specific targeting regions target at least two different antigens and may be arranged in tandem and separated by linker sequences. In an embodiment, the extracellular spacer domain is optional. In another embodiment, the CAR is a bispecific CAR. A bispecific CAR is specific to two different antigens.

"Co-stimulatory domain" (CSD) as used herein refers to the portion of the CAR which enhances the proliferation, survival and/or development of memory cells. The CARs of the invention may comprise one or more co-stimulatory domains. Each co-stimulatory domain comprises the costimulatory domain of any one or more of, for example, members of the TNFR superfamily, CD28, CD137 (4-1BB), CD134 (OX40), Dap10, CD27, CD2, CD5, ICAM-1, LFA-1(CD11a/CD18), Lck, TNFR-I, TNFR-II, Fas, CD30, CD40 or combinations thereof. Other co-stimulatory domains (e.g., from other proteins) will be apparent to those of skill in the art and may be used in connection with alternate embodiments of the invention.

"Extracellular spacer domain" (ESD) as used herein refers to the hydrophilic region which is between the antigen-specific targeting region and the transmembrane domain. In some embodiments, the CARs of the invention comprise an extracellular spacer domain. In other embodiments, the CARs of the invention do not comprise an extracellular spacer domain. The extracellular spacer domains include but are not limited to Fc fragments of antibodies or fragments or derivatives thereof, hinge regions of antibodies or fragments or derivatives thereof, CH2 regions of antibodies, CH3 regions of antibodies, artificial spacer sequences or combinations thereof. Examples of extracellular spacer domains include but are not limited to CD8α hinge, and artificial spacers made of polypeptides which may be as small as, for example, Gly3 or CH1 and CH3 domains of IgGs (such as human IgG4). In some embodiments, the extracellular spacer domain is any one or more of (i) a hinge, CH2 and CH3 regions of IgG4, (ii) a hinge region of IgG4, (iii) a hinge and CH2 of IgG4, (iv) a hinge region of CD8α, (v) a hinge, CH2 and CH3 regions of IgG1, (vi) a hinge region of IgG1 or (vi) a hinge and CH2 region of IgG1. Other extracellular spacer domains will be apparent to those of skill in the art and may be used in connection with alternate embodiments of the invention.

"Intracellular signaling domain" (ISD) or "cytoplasmic domain" as used herein refer to the portion of the CAR which transduces the effector function signal and directs the cell to perform its specialized function. Examples of domains that transduce the effector function signal include but are not limited to the ζ chain of the T-cell receptor complex or any of its homologs (*e.g.*, η chain, FcεR1γ and β chains, MB1 (Igα) chain, B29 (Igβ) chain, etc.), human CD3 zeta chain, CD3 polypeptides (Δ, δ and ε), syk family tyrosine kinases (Syk, ZAP 70, etc.), src family tyrosine kinases (Lck, Fyn, Lyn, etc.) and other molecules involved in T-cell transduction, such as CD2, CD5 and CD28. Other intracellular signaling domains will be apparent to those of skill in the art and may be used in connection with alternate embodiments of the invention.

"Linker" (L) or "linker domain" or "linker region" as used herein refer to an oligo- or polypeptide region from about 1 to 100 amino acids in length, which links together any of the domains/regions of the CAR of the invention. Linkers may be composed of flexible residues like glycine and serine so that the adjacent protein domains are free to move relative to one another. Longer linkers may be used when it is desirable to ensure that

two adjacent domains do not sterically interfere with one another. Linkers may be cleavable or non-cleavable. Examples of cleavable linkers include 2A linkers (for example T2A), 2A-like linkers or functional equivalents thereof and combinations thereof. In some embodiments, the linkers include the picornaviral 2A-like linker, CHYSEL sequences of porcine teschovirus (P2A), *Thosea asigna* virus (T2A) or combinations, variants and functional equivalents thereof. In other embodiments, the linker sequences may comprise Asp-Val/Ile-Glu-X-Asn-Pro-$Gly^{(2A)}$–$Pro^{(2B)}$ motif, which results in cleavage between the 2A glycine and the 2B proline. Other linkers will be apparent to those of skill in the art and may be used in connection with alternate embodiments of the invention.

"Transmembrane domain" (TMD) as used herein refers to the region of the CAR which crosses the plasma membrane. The transmembrane domain of the CAR of the invention is the transmembrane region of a transmembrane protein (for example Type I transmembrane proteins), an artificial hydrophobic sequence or a combination thereof. Other transmembrane domains will be apparent to those of skill in the art and may be used in connection with alternate embodiments of the invention.

Others

"Antigen loss escape variants" as used herein refer to cells which exhibit reduced or loss of expression of the target antigen, which antigens are targeted by the CARs of the invention.

"B-cell associated diseases" as used herein include B-cell immunodeficiencies, autoimmune diseases and/or excessive/uncontrolled cell proliferation associated with B-cells (including lymphomas and/or leukemias). Examples of such diseases, wherein bispecific CARs of the invention may be used for therapeutic approaches include but are not limited to systemic lupus erythematosus (SLE), diabetes, rheumatoid arthritis (RA), reactive arthritis, multiple sclerosis (MS), pemphigus vulgaris, celiac disease, Crohn's disease, inflammatory bowel disease, ulcerative colitis, autoimmune thyroid disease, X-linked agammaglobulinaemis, pre-B acute lymphoblastic leukemia, systemic lupus erythematosus, common variable immunodeficiency, chronic lymphocytic leukemia, diseases associated with selective IgA deficiency and/or IgG subclass deficiency, B

lineage lymphomas (Hodgkin's lymphoma and/or non-Hodgkin's lymphoma), immunodeficiency with thymoma, transient hypogammaglobulinaemia and/or hyper IgM syndrome, as well as virally-mediated B-cell diseases such as EBV mediated lymphoproliferative disease, and chronic infections in which B-cells participate in the pathophysiology.

"Beneficial results" may include, but are in no way limited to, lessening or alleviating the severity of the disease condition, preventing the disease condition from worsening, curing the disease condition, preventing the disease condition from developing, lowering the chances of a patient developing the disease condition and prolonging a patient's life or life expectancy.

"Cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to B-cell lymphomas (Hodgkin's lymphomas and/or non-Hodgkins lymphomas), brain tumor, breast cancer, colon cancer, lung cancer, hepatocellular cancer, gastric cancer, pancreatic cancer, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, thyroid cancer, renal cancer, carcinoma, melanoma, head and neck cancer, brain cancer, and prostate cancer, including but not limited to androgen-dependent prostate cancer and androgen-independent prostate cancer.

"Co-express" as used herein refers to simultaneous expression of two or more genes. Genes may be nucleic acids encoding, for example, a single protein or a chimeric protein as a single polypeptide chain. For example, the CARs of the invention may be co-expressed with a therapeutic control (for example truncated epidermal growth factor (EGFRt)), wherein the CAR is encoded by a first polynucleotide chain and the therapeutic control is encoded by a second polynucleotide chain. In an embodiment, the first and second polynucleotide chains are linked by a nucleic acid sequence that encodes a cleavable linker. The polynucleotides encoding the CAR and the therapeutic control system may be linked by IRES sequences. Alternately, the CAR and the therapeutic control are encoded by two different polynucleotides that are not linked via a linker but are instead encoded by, for example, two different vectors. Further, the CARs of the invention may be co-expressed with a therapeutic control and CCR, a therapeutic control and DHFR (for example mutant DHFR) or a therapeutic control and CCR and DHFR (for

example mutant DHFR). The CAR, therapeutic control and CCR may be co-expressed and encoded by first, second and third polynucleotide sequences, respectively, wherein the first, second and third polynucleotide sequences are linked via IRES sequences or sequences encoding cleavable linkers. Alternately, these sequences are not linked via linkers but instead are encoded via, for example, separate vectors. The CAR, therapeutic control and DHFR (for example mutant DHFR) may be co-expressed and encoded by first, second and fourth polynucleotide sequences, respectively, wherein the first, second and fourth polynucleotide sequences are linked via IRES sequences or via sequences encoding cleavable linkers. Alternately, these sequences are not linked via linkers but instead encoded via, for example, separate vectors. The CAR, therapeutic control, CCR and DHFR (for example mutant DHFR) may be co-expressed and encoded by first, second, third and fourth polynucleotide sequences, respectively, wherein the first, second, third and fourth polynucleotide sequences are linked via IRES sequences or sequences encoding cleavable linkers. Alternately, these sequences are not linked via linkers but instead are encoded via, for example, separate vectors. If the aforementioned sequences are encoded by separate vectors, these vectors may be simultaneously or sequentially transfected.

"Conditions", "disease conditions," "diseases" and "disease state" as used herein include physiological states in which diseased cells may be targeted with the CARs of the invention, expressing, for example, antibodies against specific antigens on the diseased cells. Examples of antigens which may be targeted include but are not limited to antigens expressed on B-cells (such as CD19 and CD20), antigens expressed on carcinomas, sarcomas, lymphomas, leukemia, germ cell tumors, blastomas, antigens expressed on various immune cells, and antigens expressed on cells associated with various hematologic diseases, autoimmune diseases, and/or inflammatory diseases.

"Disease targeted by genetically modified cells" as used herein encompasses the targeting of any cell involved in any manner in any disease by the genetically modified cells of the invention, irrespective of whether the genetically modified cells target diseased cells or healthy cells to effectuate a therapeutically beneficial result. The genetically modified cells include but are not limited to genetically modified T-cells, NK cells, hematopoietic stem cells, pluripotent embryonic stem cells or embryonic stem cells. The genetically modified cells express the CARs of the invention, which CARs may target any of the

antigens expressed on the surface of target cells. Examples of antigens which may be targeted include but are not limited to antigens expressed on B-cells; antigens expressed on carcinomas, sarcomas, lymphomas, leukemia, germ cell tumors, and blastomas; antigens expressed on various immune cells; and antigens expressed on cells associated with various hematologic diseases, autoimmune diseases, and/or inflammatory diseases. Other antigens that may be targeted will be apparent to those of skill in the art and may be targeted by the CARs of the invention in connection with alternate embodiments thereof.

"Effector function" refers to the specialized function of a differentiated cell. Effector function of a T-cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines.

"Genetically modified cells", "redirected cells", "genetically engineered cells" or "modified cells" as used herein refer to cells that express the CAR of the invention.

"Immune cell" as used herein refers to the cells of the mammalian immune system including but not limited to antigen presenting cells, B-cells, basophils, cytotoxic T-cells, dendritic cells, eosinophils, granulocytes, helper T-cells, leukocytes, lymphocytes, macrophages, mast cells, memory cells, monocytes, natural killer cells, neutrophils, phagocytes, plasma cells and T-cells.

"Immune response" as used herein refers to immunities including but not limited to innate immunity, humoral immunity, cellular immunity, immunity, inflammatory response, acquired (adaptive) immunity, autoimmunity and/or overactive immunity.

"Mammal" as used herein refers to any member of the class *Mammalia*, including, without limitation, humans and nonhuman primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats and guinea pigs, and the like. The term does not denote a particular age or sex. Thus, adult and newborn subjects, as well as fetuses, whether male or female, are intended to be included within the scope of this term.

"Polynucleotide" as used herein includes but is not limited to DNA, RNA, cDNA (complementary DNA), mRNA (messenger RNA), rRNA (ribosomal RNA), shRNA (small hairpin RNA), snRNA (small nuclear RNA), snoRNA (short nucleolar RNA), miRNA (microRNA), genomic DNA, synthetic DNA, synthetic RNA, and/or tRNA.

"Naked DNA" as used herein refers to DNA encoding a CAR cloned in a suitable expression vector in proper orientation for expression. Viral vectors which may be used include but are not limited SIN lentiviral vectors, retroviral vectors, foamy virus vectors, adeno-associated virus (AAV) vectors, hybrid vectors and/or plasmid transposons (for example sleeping beauty transposon system) or integrase based vector systems. Other vectors that may be used in connection with alternate embodiments of the invention will be apparent to those of skill in the art.

"Single chain variable fragment", "single-chain antibody variable fragments" or "scFv" antibodies as used herein refer to forms of antibodies comprising the variable regions of only the heavy and light chains, connected by a linker peptide.

"Target cell" as used herein refers to cells which are involved in a disease and can be targeted by the genetically modified cells of the invention (including but not limited to genetically modified T-cells, NK cells, hematopoietic stem cells, pluripotent stem cells, and embryonic stem cells). Other target cells will be apparent to those of skill in the art and may be used in connection with alternate embodiments of the invention.

The terms "T-cell" and "T-lymphocyte" are interchangeable and used synonymously herein. Examples include but are not limited to naïve T cells, central memory T cells, effector memory T cells or combinations thereof.

"Therapeutic agents" as used herein refers to agents that are used to, for example, treat, inhibit, prevent, mitigate the effects of, reduce the severity of, reduce the likelihood of developing, slow the progression of and/or cure, a disease. Diseases targeted by the therapeutic agents include but are not limited to carcinomas, sarcomas, lymphomas, leukemia, germ cell tumors, blastomas, antigens expressed on various immune cells, and antigens expressed on cells associated with various hematologic diseases, autoimmune diseases, and/or inflammatory diseases.

"Therapeutic controls" as used herein refers to agents that regulate cell proliferation, facilitate cell selection (for example selecting cells which express the chimeric antigen receptors of the invention), facilitate cell tracking or a combination thereof. In one embodiment, regulating cell proliferation comprises up-regulating cell proliferation to promote cell propagation. In another embodiment, regulating cell proliferation comprises down-regulating cell proliferation so as to reduce or inhibit cell propagation. In some embodiments, the agents that serve as therapeutic controls may promote enrichment of cells which express the bispecific chimeric antigen receptors which may result in a therapeutic advantage.

"Transduction" as used herein refers to the introduction of a foreign nucleic acid into a cell using a viral vector.

"Transfection" as used herein refers to the introduction of a foreign nucleic acid into a cell using recombinant DNA technology. The term "transformation" means the introduction of a "foreign" (*i.e.* extrinsic or extracellular) gene, DNA or RNA sequence to a host cell, so that the host cell will express the introduced gene or sequence to produce a desired substance, such as a protein or enzyme coded by the introduced gene or sequence. The introduced gene or sequence may also be called a "cloned" or "foreign" gene or sequence, may include regulatory or control sequences, such as start, stop, promoter, signal, secretion, or other sequences used by a cell's genetic machinery. The gene or sequence may include nonfunctional sequences or sequences with no known function. A host cell that receives and expresses introduced DNA or RNA has been "transformed" and is a "transformant" or a "clone." The DNA or RNA introduced to a host cell can come from any source, including cells of the same genus or species as the host cell, or cells of a different genus or species

"Treatment" and "treating," as used herein refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic condition, prevent the pathologic condition, pursue or obtain beneficial results, or lower the chances of the individual developing the condition even if the treatment is ultimately unsuccessful. Those in need of treatment include those

already with the condition as well as those prone to have the condition or those in whom the condition is to be prevented.

"Tumor," as used herein refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues.

"Vector", "cloning vector" and "expression vector" as used herein refer to the vehicle by which a polynucleotide sequence (*e.g.* a foreign gene) can be introduced into a host cell, so as to transform the host and promote expression (*e.g.* transcription and translation) of the introduced sequence. Vectors include plasmids, phages, viruses, etc.

Description of the Invention

Chimeric Antigen Receptors

While not wishing to be limited by any one premise, it is believed that the chimeric antigen receptors (for example bispecific CARs) of the instant invention may overcome conventional therapeutic failures due to, for example, outgrowth of antigen loss escape variants that can arise in the course of various therapies when a single antigen is targeted. Accordingly, the invention is directed to, among other things, nucleic acid sequences and amino acid sequences encoding CARs, vectors comprising CARs, viruses comprising CARs, genetically modified cells comprising the CARs (redirected cells) and methods of making and using them. In some embodiments, the CARs are bispecific CARs. In other embodiments, the CARs target and bind three or more different antigens.

In general embodiments, the present invention relates to CARs (for example bispecific CARs), nucleic acid sequences encoding the CARs (for example bispecific CARs), the vectors comprising the nucleic acids encoding the CARs (for example bispecific CARs), viruses comprising the nucleic acid sequences encoding the CARs (for example bispecific CARs), host cells (such as genetically modified cells) expressing the CARs (for example bispecific CARs), combinations of CARs (for example bispecific CARs) and therapeutic controls and methods of making and using the CARs (for example bispecific CARs) as therapeutic agents.

The CARs of the invention target at least two different antigens. The CARs (such as bispecific CARs) are co-expressed with a therapeutic control; for instance, truncated epidermal growth factor receptor (EGFRt), chimeric cytokine receptors (CCR) and/or dihydroxyfolate receptor (DHFR) (e.g., mutant DHFR). The polynucleotides encoding the CAR and the therapeutic control(s) may be linked via IRES sequences or via polynucleotide sequences encoding cleavable linkers. The CARs of the invention are constructed so that they may be expressed in cells, which in turn proliferate in response to the presence of at least one molecule that interacts with at least one antigen-specific targeting region, for instance, an antigen.

In some embodiments, therapeutic controls for use with the CARs of the invention comprise any one or more of truncated epidermal growth factor receptor (EGFRt), thymidine kinase, cytosine deaminase, nitroreductase, xanthine-guanine phosphoribosyl transferase, human caspase 8, human caspase 9, purine nucleoside phosphorylase, linamarase/linamarin/glucose oxidase, deoxyribonucleoside kinase, horseradish peroxidase (HRP)/indole-3-acetic (IAA), Gamma-glutamylcysteine synthetase, CD20/alphaCD20, CD34/thymidine kinase chimera, dox-depedent caspase-2, mutant thymidine kinase (HSV-TKSR39) or AP1903/Fas system. In an embodiment, the CARs of the invention are linked to EGFRt via a cleavable linker or IRES sequences. In another embodiment, a bispecific CAR is linked to EGFRt via a cleavable linker or IRES sequences.

The CARs described herein may be synthesized as single polypeptide chains and may comprise at least two antigen-specific targeting regions, an extracellular spacer domain, a transmembrane domain, one or more co-stimulatory domains and an intracellular signaling domain. In this embodiment, the antigen-specific targeting regions are at the N-terminus, arranged in tandem and are separated by a linker peptide. The antigen-specific targeting region is linked to an extracellular spacer domain which is linked to the transmembrane domain. The transmembrane domain is linked to the co-stimulatory domain. The co-stimulatory domain is linked to the intracellular signaling domain which is at the C-terminus. If more than one co-stimulatory domain is used, the multiple co-stimulatory domains may be arranged in tandem with the transmembrane domain at its N-terminus and the intracellular signaling domain at its C-terminus. Polynucleotides encoding these polypeptides may further comprise an N-terminal signal sequence which

directs the CAR to the cell surface as a type I transmembrane protein. The antigen-specific targeting region may be extracellular-facing and the intracellular signaling domain may be cytoplasmic.

Figure 1 shows a schematic of a chimeric antigen receptor of the invention.

In an embodiment, an extracellular spacer domain in the CAR is optional. In such a CAR, the antigen-specific targeting regions are at the N-terminus, arranged in tandem, and separated by a linker peptide. The antigen-specific targeting region may be linked to the transmembrane domain. The transmembrane domain may be linked to the co-stimulatory domain. The co-stimulatory domain may be linked to the intracellular signaling domain, which is at the C-terminus. If more than one co-stimulatory domain is used, the multiple co-stimulatory domains may be arranged in tandem with the transmembrane domain at its N-terminus and the intracellular signaling domain at its C-terminus. Polynucleotides encoding these polypeptides may further comprise an N-terminal signal sequence which directs the CAR to the cell surface as a type I transmembrane protein. The antigen-specific targeting region may be extracellular-facing and the intracellular signaling domain may be cytoplasmic.

Antigen-Specific Targeting Regions of Chimeric Antigen Receptors

The CARs of the invention may target several (such as two or more, three or more) different antigens. In an embodiment, the CAR is a bispecific CAR and targets two different antigens. As described above, the antigen-specific targeting regions of the CAR may be arranged in tandem and may be separated by linker peptides. The antigens targeted by the CAR may be antigens on single diseased cell (such as a cancerous B-cell) or antigens that are expressed on separate cells that each contribute to the disease. The antigens targeted by the CAR are antigens which are either directly or indirectly involved in the disease.

In a bispecific CAR, at least two different antigen-specific antibodies or fragments thereof or derivatives thereof may be cloned into the antigen-specific targeting region. The antibodies may be specific for any, but at least two, distinct antigens of choice. The

antibody specific to the antigen may be the Fab fragment of the antibody or the single chain variable fragment (scFv) of the antibody.

For example, Figure 2 shows an embodiment of the invention depicting a CAR specific to CD19 and CD20. Using methods well known to one skilled in the art, scFvs specific to multiple, but at least two different antigens, may be cloned upstream (i.e., to N-terminus) of the IgG_4-CD28-zeta domains so long as the target-antigens are expressed on cells that are targetable by the genetically modified cells described below. Such techniques are explained fully in the literature. (Sambrook et al, "Molecular Cloning: A Laboratory Manual" (1989), Current Protocols in Molecular Biology. Volumes I-III [Ausubel, R. M., ed. (1994)], Cell Biology: A Laboratory Handbook. Volumes I-III [J. E. Celis, ed. (1994))], Current Protocols in Immunology. Volumes I-III [Coligan, J. E., ed. (1994)], Oligonucleotide Synthesis. (M. J. Gait ed. 1984), Nucleic Acid Hybridization [B. D. Hames & S. J. Higgins eds. (1985)], Transcription And Translation [B. D. Hames & S. J. Higgins, eds. (1984)], Animal Cell Culture [R. I. Freshney, ed. (1986)], Immobilized Cells And Enzymes [IRL Press, (1986)], Practical Guide To Molecular Cloning B. Perbal (1984), Current Prptocols in Immunology (J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach and W. Strober, eds., 1991), Annual Review of Immunology as well as monographs in journals such as Advances in Immunology).

In one embodiment, each antigen-specific targeting region comprises the full-length IgG heavy chain (specific for the target antigen) having the V_H, CH1, hinge, and the CH2 and CH3 (Fc) Ig domains, if the V_H domain alone is sufficient to confer antigen-specificity ("single-domain antibodies"). The full length IgG heavy chain may be linked to the co-stimulatory domain and the intracellular signaling domain via the appropriate transmesmbrane domain. If both, the V_H and the V_L domains, are necessary to generate a fully active antigen-specific targeting region, the V_H-containing CAR and the full-length lambda light chain (IgL) are both introduced into the cells to generate an active antigen-specific targeting region. In an embodiment, an extracelluar spacer domain may be linked between the antigen-specific binding domain and the transmembrane domain. The cells include but are not limited to T-lymphocytes (T-cells), natural killer cells, hematopoietic stem cells and/or pluripotent embryonic/induced stem cells capable of giving rise to therapeutically relevant progeny.

In another embodiment, each antigen-specific targeting region of the CAR comprises at least two single chain antibody variable fragments (scFv), each specific for a different target antigen. scFvs, in which the C-terminus of one variable domain (V_H or V_L) is tethered to the N-terminus of the other (V_L or V_H, respectively) via a polypeptide linker, have been developed without significantly disrupting antigen binding or specificity of the binding. (Chaudhary et al., A recombinant single-chain immunotoxin composed of anti-Tac variable regions and a truncated diphtheria toxin. 1990 *Proc. Natl. Acad. Sci.*, 87:9491; Bedzyk et al. Immunological and structural characterization of a high affinity anti-fluorescein single-chain antibody. 1990 *J. Biol. Chem.*, 265:18615). The linker connects the N-terminus of the V_H with the C-terminus of V_L or the C-terminus of V_H with the N-terminus of V_L. These scFvs lack the constant regions (Fc) present in the heavy and light chains of the native antibody. The scFvs, specific for at least two different antigens, are arranged in tandem and linked to the co-stimulatory domain and the intracellular signaling domain via a transmembrane domain. In an embodiment, an extracelluar spacer domain may be linked between the antigen-specific binding region and the transmembrane domain.

In another aspect, each scFv fragment may be fused to all or a portion of the constant domains of the heavy chain. The resulting antigen-specific targeting region, specific for at least two different antigens, is joined to the co-stimulatory domain and the intracellular signaling domain via a transmembrane domain. In an embodiment, an extracelluar spacer domain may be linked between the antigen-specific binding domain and the transmembrane domain.

In a further embodiment, each antigen-specific targeting region of the CAR comprises a divalent (or bivalent) single-chain variable fragment (di-scFvs, bi-scFvs). In CARs comprising di-scFVs, two scFvs specific for each antigen are linked together by producing a single peptide chain with two V_H and two V_L regions, yielding tandem scFvs. (Xiong, Cheng-Yi; Natarajan, A; Shi, XB; Denardo, GL; Denardo, SJ (2006). "Development of tumor targeting anti-MUC-1 multimer: effects of di-scFv unpaired cysteine location on PEGylation and tumor binding". *Protein Engineering Design and Selection* 19 (8): 359–367; Kufer, Peter; Lutterbüse, Ralf; Baeuerle, Patrick A. (2004). "A revival of bispecific antibodies". *Trends in Biotechnology* 22 (5): 238–244). CARs comprising at least two antigen-specific targeting regions would express two scFvs

specific for each of the two antigens. The resulting antigen-specific targeting region, specific for at least two different antigens, is joined to the co-stimulatory domain and the intracellular signaling domain via a transmembrane domain. In an embodiment, an extracelluar spacer domain may be linked between the antigen-specific binding domain and the transmembrane domain.

In an additional embodiment, each antigen-specific targeting region of the CAR comprises a diabody. In a diabody, the scFvs are created with linker peptides that are too short for the two variable regions to fold together, driving the scFvs to dimerize. Still shorter linkers (one or two amino acids) lead to the formation of trimers, the so-called triabodies or tribodies. Tetrabodies may also be used.

To create the CARs of the present invention, two or more individual antigen-specific targeting regions are connected to each other, either covalently or noncovalently, on a single protein molecule. An oligo- or polypeptide linker, an Fc hinge or membrane hinge region may be used to connect these domains to each other. The CARs of the present invention may comprise two or more of the different antigen-specific targeting regions connected together in different combinations. For example, two or more antigen-specific targeting regions containing immunoglobulin sequences (*e.g.* scFvs and/or single-domain antibodies) may be linked to each other.

Targets of Antigen-specific targeting regions of chimeric antigen receptors

In some embodiments, the antigen-specific targeting region of the CAR (for example bispecific CAR) targets antigens specific for cancer, inflammatory disease, neuronal-disorders, diabetes, cardiovascular disease, infectious diseases or a combination thereof. Examples of antigens which may be targeted by the CARs (for example bispecific CARs) of the invention include but are not limited to antigens expressed on B-cells, antigens expressed on carcinomas, sarcomas, lymphomas, leukemia, germ cell tumors, blastomas, antigens expressed on various immune cells, and antigens expressed on cells associated with various hematologic diseases, autoimmune diseases, and/or inflammatory diseases. The CARs of the invention, which are specific for at least two different target antigens, may be capable of redirecting the effector function of the expressing-cells to either of both of the target antigens. This feature of the construct may overcome the issue of

antigen loss escape variants when targeting, for example, genetically unstable B-cell lineage malignancies using single antigen-specificity.

Antigens specific for cancer which may be targeted by the CARs (for example bispecific CARs) of the invention include but are not limited to any one or more of 4-1BB, 5T4, adenocarcinoma antigen, alpha-fetoprotein, BAFF, B-lymphoma cell, C242 antigen, CA-125, carbonic anhydrase 9 (CA-IX), C-MET, CCR4, CD152, CD19, CD20, CD200, CD22, CD221, CD23 (IgE receptor), CD28, CD30 (TNFRSF8), CD33, CD4, CD40, CD44 v6, CD51, CD52, CD56, CD74, CD80, CEA, CNTO888, CTLA-4, DR5, EGFR, EpCAM, CD3, FAP, fibronectin extra domain-B, folate receptor 1, GD2, GD3 ganglioside, glycoprotein 75, GPNMB, HER2/neu, HGF, human scatter factor receptor kinase, IGF-1 receptor, IGF-I, IgG1, L1-CAM, IL-13, IL-6, insulin-like growth factor I receptor, integrin $\alpha 5\beta 1$, integrin $\alpha v\beta 3$, MORAb-009, MS4A1, MUC1, mucin CanAg, N-glycolylneuraminic acid, NPC-1C, PDGF-R α, PDL192, phosphatidylserine, prostatic carcinoma cells, RANKL, RON, ROR1, SCH 900105, SDC1, SLAMF7, TAG-72, tenascin C, TGF beta 2, TGF-β, TRAIL-R1, TRAIL-R2, tumor antigen CTAA16.88, VEGF-A, VEGFR-1, VEGFR2 or vimentin. Other antigens specific for cancer will be apparent to those of skill in the art and may be used in connection with alternate embodiments of the invention. Examples of CARs which target the above antigens include but are not limited to bispecific CARs, bispecific CARs co-expressed with EGFRt, bispecific CARs co-expressed with EGFRt and CCR, bispecific CARs co-expressed with EGFRt and DHFR (for example mutant DHFR) or bispecific CARs co-expressed with EGFRt and CDR and DHFR (for example mutant DHFR).

In some embodiments, the bispecific chimeric antigen receptors target and bind at least two different antigens. Examples of pairings of at least two antigens bound by the bispecific CARs of the invention include but are not limited to CD19 and CD20, CD19 and CD22, CD20 and L1-CAM, L1-CAM and GD2, EGFR and L1-CAM, EGFR and C-MET, EGFR and HER2, C-MET and HER2 and EGFR and ROR1. Other pairings of antigens specific for cancer will be apparent to those of skill in the art and may be used in connection with alternate embodiments of the invention. In yet other embodiments, the bispecific chimeric antigen receptor targets CD19 and CD20. Examples of CARs which target the above antigens include but are not limited to bispecific CARs, bispecific CARs co-expressed with EGFRt, bispecific CARs co-expressed with EGFRt and CCR,

bispecific CARs co-expressed with EGFRt and DHFR (for example mutant DHFR) or bispecific CARs co-expressed with EGFRt and CDR and DHFR (for example mutant DHFR).

Antigens specific for inflammatory diseases which may be targeted by the CARs of the invention include but are not limited to any one or more of AOC3 (VAP-1), CAM-3001, CCL11 (eotaxin-1), CD125, CD147 (basigin), CD154 (CD40L), CD2, CD20, CD23 (IgE receptor), CD25 (α chain of IL-2 receptor), CD3, CD4, CD5, IFN-α, IFN-γ, IgE, IgE Fc region, IL-1, IL-12, IL-23, IL-13, IL-17, IL-17A, IL-22, IL-4, IL-5, IL-5, IL-6, IL-6 receptor, integrin α4, integrin α4β7, Lama glama, LFA-1 (CD11a), MEDI-528, myostatin, OX-40, rhuMAb β7, scleroscin, SOST, TGF beta 1, TNF-α or VEGF-A. Other antigens specific for inflammatory diseases will be apparent to those of skill in the art and may be used in connection with alternate embodiments of the invention. Examples of CARs which target the above antigens include but are not limited to bispecific CARs, bispecific CARs co-expressed with EGFRt, bispecific CARs co-expressed with EGFRt and CCR, bispecific CARs co-expressed with EGFRt and DHFR (for example mutant DHFR) or bispecific CARs co-expressed with EGFRt and CDR and DHFR (for example mutant DHFR).

Antigens specific for neuronal disorders which may be targeted by the CARs of the invention include but are not limited to any one or more of beta amyloid or MABT5102A. Other antigens specific for neuronal disorders will be apparent to those of skill in the art and may be used in connection with alternate embodiments of the invention. Examples of CARs which target the above antigens include but are not limited to bispecific CARs, bispecific CARs co-expressed with EGFRt, bispecific CARs co-expressed with EGFRt and CCR, bispecific CARs co-expressed with EGFRt and DHFR (for example mutant DHFR) or bispecific CARs co-expressed with EGFRt and CDR and DHFR (for example mutant DHFR).

Antigens specific for diabetes which may be targeted by the CARs of the invention include but are not limited to any one or more of L-1β or CD3. Other antigens specific for diabetes or other metabolic disorders will be apparent to those of skill in the art and may be used in connection with alternate embodiments of the invention. Examples of

CARs which target the above antigens include but are not limited to bispecific CARs, bispecific CARs co-expressed with EGFRt, bispecific CARs co-expressed with EGFRt and CCR, bispecific CARs co-expressed with EGFRt and DHFR (for example mutant DHFR) or bispecific CARs co-expressed with EGFRt and CDR and DHFR (for example mutant DHFR).

Antigens specific for cardiovascular diseases which may be targeted by the CARs of the invention include but are not limited to any one or more of C5, cardiac myosin, CD41 (integrin alpha-IIb), fibrin II, beta chain, ITGB2 (CD18) and sphingosine-1-phosphate. Other antigens specific for cardiovascular diseases will be apparent to those of skill in the art and may be used in connection with alternate embodiments of the invention. Examples of CARs which target the above antigens include but are not limited to bispecific CARs, bispecific CARs co-expressed with EGFRt, bispecific CARs co-expressed with EGFRt and CCR, bispecific CARs co-expressed with EGFRt and DHFR (for example mutant DHFR) or bispecific CARs co-expressed with EGFRt and CDR and DHFR (for example mutant DHFR).

Antigens specific for infectious diseases which may be targeted by the CARs of the invention include but are not limited to any one or more of anthrax toxin, CCR5, CD4, clumping factor A, cytomegalovirus, cytomegalovirus glycoprotein B, endotoxin, Escherichia coli, hepatitis B surface antigen, hepatitis B virus, HIV-1, Hsp90, Influenza A hemagglutinin, lipoteichoic acid, Pseudomonas aeruginosa, rabies virus glycoprotein, respiratory syncytial virus and TNF-α. Other antigens specific for infectious diseases will be apparent to those of skill in the art and may be used in connection with alternate embodiments of the invention. Examples of CARs which target the above antigens include but are not limited to bispecific CARs, bispecific CARs co-expressed with EGFRt, bispecific CARs co-expressed with EGFRt and CCR, bispecific CARs co-expressed with EGFRt and DHFR (for example mutant DHFR) or bispecific CARs co-expressed with EGFRt and CDR and DHFR (for example mutant DHFR).

Further examples of target antigens include but are not limited to surface proteins found on cancer cells in a specific or amplified fashion (*e.g.* the IL-14 receptor, CD19, CD20 and CD40 for B-cell lymphoma, the Lewis Y and CEA antigens for a variety of carcinomas, the Tag72 antigen for breast and colorectal cancer, EGF-R for lung cancer,

folate binding protein and the HER-2 protein which is often amplified in human breast and ovarian carcinomas), or viral proteins (*e.g.* gp120 and gp41 envelope proteins of HIV, envelope proteins from the Hepatitis B and C viruses, the glycoprotein B and other envelope glycoproteins of human cytomegalovirus, the envelope proteins from oncoviruses such as Kaposi's sarcoma-associated Herpes virus). Other potential targets of the CARs of the invention include CD4, where the ligand is the HIV gp120 envelope glycoprotein, and other viral receptors, for example ICAM, which is the receptor for the human rhinovirus, and the related receptor molecule for poliovirus.

Additional targets of the CARs of the invention include antigens involved in B-cell associated diseases. Yet further targets of the CARs of the invention will be apparent to those of skill in the art and may be used in connection with alternate embodiments of the invention.

Co-stimulatory domains of chimeric antigen receptors

The CARs of the invention may also comprise a co-stimulatory domain. This domain may enhance cell proliferation, cell survival and development of memory cells. The CARs of the invention may comprise one or more co-stimulatory domains. Each co-stimulatory domain comprises the co-stimulatory domain of any one or more of, for example, members of the TNFR super family, CD28, CD137 (4-1BB), CD134 (OX40), Dap10, CD27, CD2, CD5, ICAM-1, LFA-1, Lck, TNFR-1, TNFR-II, Fas, CD30, CD40 or combinations thereof. Co-stimulatory domains from other proteins may also be used with the CARs of the invention. Additional co-stimulatory domains will be apparent to those of skill in the art and may be used in connection with alternate embodiments of the invention. If a CAR comprises more than one co-stimulatory domain, these domains may be arranged in tandem, optionally separated by a linker.

Extracellular spacer domain of chimeric antigen receptor

The CARs of the invention may further comprise an extracellular spacer domain. In some embodiments, this domain facilitates proper protein folding. The extracellular spacer domain comprises a hydrophilic region which is attached to the antigen-specific targeting region and the transmembrane domain. Extracellular spacer domains may

include, but are not limited to, Fc fragments of antibodies or fragments or derivatives thereof, hinge regions of antibodies or fragments or derivatives thereof, CH2 regions of antibodies, CH3 regions antibodies, artificial spacer sequences or combinations thereof. Examples of extracellular spacer domains include but are not limited to CD8α hinge, artificial spacers made of polypeptides such as Gly3, or CH1, CH3 domains of IgG's (such as human IgG4). Specifically, the extracellular spacer domain may be (i) a hinge, CH2 and CH3 regions of IgG4, (ii) a hinge region of IgG4, (iii) a hinge and CH2 of IgG4, (iv) a hinge region of CD8α, (v) a hinge, CH2 and CH3 regions of IgG1, (vi) a hinge region of IgG1 or (vi) a hinge and CH2 of IgG1 or a combination thereof. Additional extracellular spacer domains will be apparent to those of skill in the art and may be used in connection with alternate embodiments of the invention.

Transmembrane domain of chimeric antigen receptors

The CARs of the invention may also comprise a transmembrane domain. The transmembrane domain may comprise the transmembrane sequence from any protein which has a transmembrane domain, including any of the type I, type II or type III transmembrane proteins. The transmembrane domain of the CAR of the invention may also comprise an artificial hydrophobic sequence. The transmembrane domains of the CARs of the invention may be selected so as not to dimerize. Additional transmembrane domains will be apparent to those of skill in the art and may be used in connection with alternate embodiments of the invention.

Intracellular signaling domain of chimeric antigen receptors

The CARs of the invention may also comprise an intracellular signaling domain. This domain may be cytoplasmic and may transduce the effector function signal and direct the cell to perform its specialized function. Examples of intracellular signaling domains include, but are not limited to, ζ chain of the T-cell receptor or any of its homologs (*e.g.*, η chain, FcεR1γ and β chains, MB1 (Igα) chain, B29 (Igβ) chain, etc.), CD3 polypeptides (Δ, δ and ε), syk family tyrosine kinases (Syk, ZAP 70, etc.), src family tyrosine kinases (Lck, Fyn, Lyn, etc.) and other molecules involved in T-cell transduction, such as CD2, CD5 and CD28. Specifically, the intracellular signaling domain may be human CD3 zeta

chain, FcγRIII, FcεRI, cytoplasmic tails of Fc receptors, immunoreceptor tyrosine-based activation motif (ITAM) bearing cytoplasmic receptors or combinations thereof. Additional intracellular signaling domains will be apparent to those of skill in the art and may be used in connection with alternate embodiments of the invention.

Linkers in chimeric antigen receptors

In some embodiments, two or more components of the CARs of the invention are separated by one or more linkers. For example, in CARs comprising at least two antigen-specific targeting regions, the first targeting region on the CAR may be separated from the second targeting region on the CAR via a linker. Additionally, the CAR may be linked to therapeutic controls via a linker. Linkers are oligo- or polypeptides region from about 1 to 100 amino acids in length, that link together any of the domains/regions of the CAR of the invention. In some embodiments, the linkers may be for example, 5-12 amino acids in length, 5-15 amino acids in length or 5 to 20 amino acids in length. Linkers may be composed of flexible residues like glycine and serine so that the adjacent protein domains are free to move relative to one another. Longer linkers, for example those longer than 100 amino acids, may be used in connection with alternate embodiments of the invention, and may be selected to, for example, ensure that two adjacent domains do not sterically interfere with one another. Examples of linkers which may be used in the instant invention include but are not limited to 2A linkers (for example T2A), 2A-like linkers or functional equivalents thereof.

Therapeutic controls

Therapeutic controls regulate cell proliferation, facilitate cell selection (for example selecting cells which express the chimeric antigen receptors of the invention) or a combination thereof. In one embodiment, regulating cell proliferation comprises up-regulating cell proliferation to promote cell propagation. In another embodiment, regulating cell proliferation comprises down-regulating cell proliferation so as to reduce or inhibit cell propagation. In some embodiments, the agents that serve as therapeutic controls may promote enrichment of cells which express the bispecific chimeric antigen receptors which may result in a therapeutic advantage. In some embodiments, agents

which serve as therapeutic controls may biochemically interact with additional compositions so as to regulate the functioning of the therapeutic controls. For example, EGFRt (a therapeutic control) may biochemically interact with cetuximab so as to regulate the function of EGFRt in selection, tracking, cell ablation or a combination thereof.

Examples of therapeutic controls include but are not limited to any one or more of truncated epidermal growth factor receptor (EGFRt), thymidine kinase, cytosine deaminase, nitroreductase, xanthine-guanine phosphoribosyl transferase, human caspase 8, human caspase 9, purine nucleoside phosphorylase, linamarase/linamarin/glucose oxidase, deoxyribonucleoside kinase, horseradish peroxidase (HRP)/indole-3-acetic (IAA), Gamma-glutamylcysteine synthetase, CD20/alphaCD20, CD34/thymidine kinase chimera, dox-depedent caspase-2, mutant thymidine kinase (HSV-TKSR39), AP1903/Fas system, a chimeric cytokine receptor (CCR), a selection marker, and combinations thereof. In some embodiments, the therapeutic controls are co-expressed with the bispecific chimeric antigen receptor.

Examples of agents which regulate the functioning of the therapeutic controls include but are not limited to any one or more of Herceptin, methotrexate, cetuximab, thymidine analogs (for example ganciclovir), (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU), 5-flurocytosine (5-FC), 5-(azaridin-1-yl)-2, 4-dinitrobenzamide (CB1954), 6-thioguanine, a synthetic dimerizing drug (for example AP1903), fludarabine phosphate, linamarin (lin), nucleoside analogs (for exmaple BVDU, difluorodeoxycytidine (dFdC), 1-β-D-arabinofuranosylthymine (ara-T)), indole-3-acetic (IAA), l-buthionine-S,R-sulfoximine (BSO), rituximab (RTX), doxycycline, tyrosine kinase inhibitors or combinations thereof. These agents may be administered before, during or after the use of the therapeutic controls.

As described above, the CARs of the invention may be synthesized as single polypeptide chains. If the CAR is a bispecific CAR, the polynucleotide sequence encoding the CAR may be, for example, in the following configuration in the N-terminal to C-terminal direction: N-terminal signal sequence - antigen-specific targeting region 1 – linker – antigen-specific targeting region 2 – extracellular spacer domain – transmembrane

domain – co-stimulatory domain – intracellular signaling domain. In an embodiment, such a CAR may comprise two or more co-stimulatory domains.

Alternatively, the polynucleotide sequence encoding the CAR may be in the following configuration in the N-terminal to C-terminal direction: N-terminal signal sequence - antigen-specific targeting region 1 – linker – antigen-specific targeting region 2 – transmembrane domain – co-stimulatory domain – intracellular signaling domain. In an embodiment, such a CAR may comprise two or more co-stimulatory domains.

If a CAR comprises more than two antigen-specific targeting regions, the polynucleotide sequence encoding the CAR may be in the following configuration in the N-terminal to C-terminal direction: N-terminal signal sequence - antigen-specific targeting region 1 – linker – antigen-specific targeting region 2 - linker – $(\text{antigen-specific targeting region})_n$ – transmembrane domain – co-stimulatory domain – intracellular signaling domain. Such a CAR may further comprise an extracellular spacer domain. Each antigen-specific targeting region may be separated by a linker. In an embodiment, such a CAR may comprise two or more co-stimulatory domains.

The invention provides a nucleic acid sequence of the backbone of an exemplary CAR of the invention comprising an extracellular spacer domain, a transmembrane domain, a co-stimulatory domain and an intracellular signaling domain. Specifically, an exemplary backbone for a may CAR comprise, in the N-terminus to C-terminus orientation, IgG4hinge-CD28tm-41BB-CD3zeta, wherein the extracellular spacer domain is the IgG4 hinge region, the transmembrane domain is the transmembrane region from CD28, the co-stimulatory domain is from 4-1BB and the intracellular signaling domain is from the CD3 zeta chain (Figure 7). At least two or more antigen-specific targeting regions may be inserted N-terminal to the IgG4 hinge.

The invention provides nucleic acid sequences of an exemplary embodiment of the invention where the CAR is specific to CD19 and CD20. In one embodiment, the sequence encoding a bispecific anti-CD19xCD20 CAR is set forth in Figure 3, 8 or 10. In another embodiment, the sequence encoding a bispecific anti-CD19xCD20 CAR is set forth in Figure 4, 9 or 11. In this exemplary embodiment, the bispecific CAR comprises

scFvs specific for CD19 and CD20 with each scFv separated by a linker, joined to an extracellular spacer domain, which is joined to the co-stimulatory and intracellular signaling domains via a transmembrane domain. Although the exemplary CAR depicts a set of scFv sequences, any scFv specific for CD19 and CD20 may be used. In a particular embodiment, the bispecific CAR specific for CD19 and CD20 is CD19scFv-Gly4Serlinker-CD20scFv-IgG4-Hinge-CD28tm-41BB(cyto)-zeta(cyto) and is encoded by the sequences set forth in Figures 3 and 4. This bispecific CAR comprises single chain Fv fragments specific for CD19 and CD20 linked by a Gly4Ser linker, an IgG4 hinge extracellular spacer domain, a CD28 transmembrane domain, a 41BB costimulatory domain and the cytoplasmic domain from CD3 zeta chain.

In another embodiment, the bispecific CAR specific for CD19 and CD20 comprises CDl9scFv-Gly4serlinker-CD20scFv-hulgG4-hingeCH2CH3-CD28tm/cyto-41BB-zeta (Figures 9-10). This bispecific CAR comprises single chain Fv fragments specific for CD19 and CD20 linked by a Gly4Ser linker, a human IgG4 hinge, CH2 and CH3 extracellular spacer domain, a CD28 transmembrane domain, a 4-1BB costimulatory domain and the cytoplasmic domain from CD3 zeta chain.

In a further embodiment, the bispecific CAR specific for CD19 and CD20 is CD19-Gly4serlinker-CD20scFv-CD8αhinge-CD8αTM-41BBcostim-zetacyto (Figures 11-12). This bispecific CAR comprises single chain Fv fragments specific for CD19 and CD20 linked by a Gly4Ser linker, a CD8alpha hinge extracellular spacer domain, a CD8alpha transmembrane domain, a 41BB costimulatory domain and the cytoplasmic domain from CD3 zeta chain.

Truncated epidermal growth factor receptor (EGFRt)

Human epidermal growth factor receptor (huEGFR)(EGFR; ErbB-1, HER1 in humans) is a receptor tyrosine kinase of the ErbB family of growth factor receptors that is not expressed by cells of the hematopoietic and lymphopoietic systems. Ligand (EGF, TGF-α) binding occurs within N-terminal extracellular domains I and II of EGFR resulting from transition of receptor tyrosine kinase inactive monomers to active homodimers.

Extracellular domain III of EGFR contains the binding sites of antibodies (for example cetuximab (Erbitux), an IgG1 chimeric antibody). It is believed that human EGFR may be rendered incapable of binding ligands (EGF, TGF-α) by removal of domains I and II, and devoid of signaling activity by deletion of its cytoplasmic tail, while retaining an intact antibody binding site (for example cetuximab binding site), for example in extracellular domain III, IV or a combination thereof (Wang et al., A transgene-encoded cell surface polypeptide for selection, in vivo tracking, and ablation of engineered cells *Blood* 118(5)1255-1263).

A truncated EGFRt polypeptide described herein has at least three uses for genetic engineering of cell-based therapies: ex vivo cell purification, in vivo cell tracking, and cell ablation. In an embodiment, EGFRt, for use as a therapeutic control with the CARs of the invention, binds any one or more of EGFR-specific siRNA, a small molecule that targets EGFR, an anti-EGFR-antibody or a combination thereof. In another embodiment, EGFRt comprises the sequence set forth in Figures 12 or 13 or sequences that are about 70%, about 75%, about 80%, about 85%, about 90% or about 95% homologous to the sequences set forth in Figures 12 or 13.

In an embodiment of the invention, huEGFRt may be co-expressed with the CARs of the invention so as to purify cells expressing the CARs (for example ex vivo cell purification), track cells (for example in vitro or in vivo cell tracking) expressing the CARs or regulate cells (for example in vivo or in vitro or ex vivo) expressing the CARs by triggering cell ablation as required. In one embodiment, the CARs are bispecific CARs.

Chimeric cytokine receptor (CCR)

Based on the limitations of using exogenous γc cytokines in adoptive immunotherapy, the invention provides T cells with an intrinsic γc cytokine signaling mechanism. The utility of forced constitutive chimeric cytokine receptors IL-2/IL-15Rβ (CγCR2) and IL-7Rα (CγCR7) receptor signals were compared. As described below, the chimeric cytokine receptors have the ability to improve the survival, persistence, and *in vivo* engraftment of cytotoxic T cells (CTLs).

Accordingly, in an embodiment of the invention, the CARs of the invention may be co-expressed with CCR. For example, a bispecific CAR may be co-expressed with EGFRt and CCR. Alternately, a bispecific CAR may be co-expressed with CCR. Examples of chimeric cytokine receptor include but are not limited to IL-7 cytokine-linker- IL7Rα, IL-7 cytokine-linker-extracellular domain of IL-7Rα-transmembrane domain of IL-7Rα-cytoplasmic domain of IL-2Rβ, IL-7 cytokine-linker-IL2Rβ.

A CCR comprising IL–7 cytokine-linker- IL7Rα comprises an N-terminal signal sequence joined to the N-terminus of the IL-7 cytokine which is linked via a linker to extracellular, transmembrane and cytoplasmic domains of IL-7Rα (the alpha chain of the IL-7 receptor).

A CCR comprising IL-7 cytokine-linker-extracellular domain of IL-7Rα-transmembrane domain of IL-7Rα-cytoplasmic domain of IL-2Rβ comprises an N-terminal signal sequence joined to the N-terminus of the IL-7 cytokine which is linked via a linker to the extracellular domain and transmembrane domain of IL-7Rα and to the cytoplasmic domain of IL-2Rβ (the beta chain of the IL-2 receptor).

A CCR comprising IL-7 cytokine-linker-IL2Rβ comprises N-terminal signal sequence joined to the N-terminus of the IL-7 cytokine which is linked via a linker to extracellular, transmembrane and cytoplasmic domains of IL-2Rβ.

Dihydroxyfolate Receptor (DHFR)

Genetic modification of T cells to co-express a therapeutic transgene and a drug resistant transgene that confers resistance to lymphotoxic drugs provides the opportunity to select for therapeutic cells both *in vivo* and *ex vivo*. A mutated human enzyme transgene, dihydrofolate reductase double mutant ($DHFR^{FS}$; L22F, F31S), which confers resistance of engineered T cells to methotrexate (MTX), allowing selection of cells co-expressing a CD19-specific chimeric antigen receptor (CD19CAR) that specifically targets B-lineage tumor cells.

In an embodiment, the CARs of the invention (for example bispecific CARs) may be co-expressed with DHFR (for example mutant DHFR). In a further embodiment, the bispecific CAR may be co-expressed with EGFRt, CCR and DHFR (including mutant DHFR). Alternately, the bispecific CAR may be co-expressed with EGFRt and DHFR (including mutant DHFR).

Other selection markers that may be used with the CARs of the invention include but are not limited to methylated-DNA-protein-cysteine methyltransferase (MDMT), inosine monophosphate dehydrogenase II (IMDHP2) or a combination thereof. MDMT makes cells resistant to chemotherapy and therefore may be used if synergy between chemotherapy and T cell therapy is desired.

Vectors encoding the CARs of the invention are also provided herein. Vectors encoding CARs also encode EGFRt. In some embodiments, vectors encoding CARs and EGFRt also encode CCR or DHFR (for example mutant DHFR). In other embodiments, vectors encoding CARs and EGFRt also encode CCD and DHFR (for example mutant DHFR). In some specific embodiments, the vectors may encode a bispecific CAR and EGFRt, a bispecific CAR and EGFRt and CCR, a bispecific CAR and EGFRt and DHFR (for example mutant DHFR) or a bispecific CAR and EGFRt and CCR and DHFR (for example mutant DHFR). Vectors which may be used to express the CARs of the invention include but are not limited to lentivirus vectors, gamma retrovirus vectors, foamy virus vectors, AAV vectors, adeno virus vectors, engineered hybrid viruses, naked DNA (including but not limited to transposon mediated vectors, such as Sleeping Beauty, Piggybak, and Integrases such as Phi31.

In an exemplary embodiment of the invention, the bisepcific CAR specific to CD19 and CD20 disclosed herein is expressed via a lentiviral vector as illustrated in Figure 5.

Genetically engineered cells of the invention

The invention also provides genetically engineered cells which comprise and stably express the CAR of the invention. The CAR expressed by the genetically engineered cell may comprise at least two antigen-specific targeting regions, an extracellular domain, a transmembrane domain, one or more co-stimulatory domains and an intracellular

signaling domain. The polynucleotide sequence encoding the CAR may also comprise an N-terminal signal sequence. In an embodiment, the CAR is a bispecific CAR. Each of the at least two antigen-specific targeting regions, extracellular spacer domain, transmembrane domain, one or more co-stimulatory domains and an intracellular signaling domain are described above. The antigen-specific targeting domains may be capable of specifically binding, in an MHC unrestricted manner, an antigen which is not normally bound by a T-cell receptor in that manner.

In an embodiment, the genetically engineered cells that express the CARs (for example bispecific CARs) of the invention co-express EGFRt. In a further embodiment, the genetically engineered cells that express the CARs (for example bispecific CARs) co-express EGFRt and CCR. In an additional embodiment, the genetically engineered cells that express the CARs (for example bispecific CARs) co-express EGFRt and DHFR (for example mutant DHFR). In another embodiment, the genetically engineered cells that express the CARs (for example bispecific CARs) co-express EGFRt, CCR and DHFR (for example mutant DHFR).

The genetically engineered cells express a CAR having at least two antigen-specific targeting regions which are specific for at least two different target antigens. In one embodiment, the antigen-specific targeting regions comprise target-specific antibodies or functional equivalents or fragments or derivatives thereof. The antigen-specific antibody may be the Fab fragment of the antibody or the single chain variable fragment (scFv) of the antibody.

Genetically engineered cells which may comprise and express the CARs of the invention include, but are not limited to, T-lymphocytes (T-cells), naïve T cells (T_N), memory T cells (for example, central memory T cells (T_{CM}), effector memory cells (T_{EM})), natural killer cells, hematopoietic stem cells and/or pluripotent embryonic/induced stem cells capable of giving rise to therapeutically relevant progeny. In an embodiment, the genetically engineered cells are autologous cells. By way of example, individual T-cells of the invention may be CD4+/CD8-, CD4-/CD8+, CD4-/CD8- or CD4+/CD8+. The T-cells may be a mixed population of CD4+/CD8- and CD4-/CD8+ cells or a population of a single clone. CD4+ T-cells of the invention may produce IL-2, IFNγ, TNFα and other

T-cell effector cytokines when co-cultured *in vitro* with cells expressing the target antigens (for example CD20+ and/or CD19+ tumor cells). $CD8^+$ T-cells of the invention may lyse antigen-specific target cells when co-cultured *in vitro* with the target cells. In some embodiments, T cells may be any one or more of $CD45RA^+$ $CD62L^+$ naïve cells, $CD45RO^+$ $CD62L^+$ central memory cells, $CD62L^-$ effector memory cells or a combination thereof (Berger et al., Adoptive transfer of virus-specific and tumor-specific T cell immunity. *Curr Opin Immunol* 2009 21(2)224-232).

Genetically modified cells may be produced by stably transfecting cells with DNA encoding the CAR of the invention. DNA encoding the CAR of the invention (for example bispecific CAR) may also encode EGFRt, CCR and/or DHFR (for example mutant DHFR). In one embodiment, a first polynucleotide encodes the CAR (for example bispecific CAR) and is linked via IRES sequences or a polynucleotide that encodes a cleavable linker, to a second polynucleotide that encodes EGFRt. In another embodiment, the first polynucleotide encodes the CAR (for example bispecific CAR) and is linked via IRES sequences or a polynucleotide that encodes a cleavable linker, to a second polynucleotide that encodes EGFRt and the first or second polynucleotides are linked to a third polynucleotide that encodes CCR or DHFR (for example mutant DHFR), also via IRES sequences or a polynucleotide that encodes a cleavable linker. In a further embodiment, the first polynucleotide encodes the CAR (for example bispecific CAR) and is linked via IRES sequences or a polynucleotide that encodes a cleavable linker, to a second polynucleotide that encodes EGFRt and the first and second polynucleotides are linked to a third polynucleotide that encodes CCR and a fourth polynucleotide that encodes DHFR (for example mutant DHFR) via IRES sequences or a polynucleotide that encodes a cleavable linker. Viral vectors are commonly used to carry heterologous genes into cells (*e.g.*, T-cells). Examples of viral vectors which may be used to generate genetically modified cells include but are not limited to SIN lentiviral vectors, retroviral vectors, foamy virus vectors, adeno-associated virus (AAV) vectors and/or plasmid transposons (*e.g.*, sleeping beauty transposon system).

Various methods produce stable transfectants which express the CARs of the invention. In one embodiment, a method of stably transfecting and re-directing cells is by electroporation using naked DNA. By using naked DNA, the time required to produce redirected cells may be significantly reduced. Additional methods to genetically engineer

cells using naked DNA encoding the CAR of the invention include but are not limited to chemical transformation methods (*e.g.*, using calcium phosphate, dendrimers, liposomes and/or cationic polymers), non-chemical transformation methods (*e.g.*, electroporation, optical transformation, gene electrotransfer and/or hydrodynamic delivery) and/or particle-based methods (*e.g.*, impalefection, using a gene gun and/or magnetofection). The transfected cells demonstrating presence of a single integrated un-rearranged vector and expression of the CAR may be expanded *ex vivo*. In one embodiment, the cells selected for *ex vivo* expansion are $CD8^+$ and demonstrates the capacity to specifically recognize and lyse antigen-specific target cells.

Viral transduction methods may also be used to generate redirected cells which express the CAR of the invention. Cell types that may be used to generate genetically modified cells expressing the bispecific CAR of the invention include but are not limited to T-lymphocytes (T-cells), natural killer cells, hematopoietic stem cells and/or pluripotent embryonic/induced stem cells capable of giving rise to therapeutically relevant progeny.

Stimulation of the T-cells by an antigen under proper conditions results in proliferation (expansion) of the cells and/or production of IL-2. The cells comprising the CAR of the invention will expand in number in response to the binding of one or more antigens to the antigen-specific targeting regions of the CAR. The invention also provides a method of making and expanding cells expressing a CAR. The method comprises transfecting or transducing the cells with the vector expressing the CAR and stimulating the cells with cells expressing the target antigens, recombinant target antigens, or an antibody to the receptor to cause the cells to proliferate, so as to make and expand T-cells. In an embodiment, the cells may be any one or more of T-lymphocytes (T-cells), natural killer (NK) cells, hematopoietic stem cells (HSCs) or pluripotent embryonic/induced stem cells capable of giving rise to therapeutically relevant progeny.

In an exemplary embodiment, the genetically engineered cells of the invention express a bispecific CAR which is specific for CD19 and CD20 antigens. In a further embodiment, a genetically engineered T-cell expresses the bispecific CARs CDl9scFv-Gly4ser-linker-CD20scFv-hulgG4-hinge-CD28-41BB(cyto)-zeta(cyto) or CDl9scFv-Gly4ser-linker-CD20scFv-hulgG4-hingeCH2CH3-CD28tm/cyto-zeta or CD19-Gly4serlinker-CD20scFv-CD8alphahinge-CD8alphaTM-41BBcostim-zetacyto.

In an exemplary embodiment, the invention provides a method of making and expanding T-cells expressing a CD19-specific and CD20-specific CAR. The method comprises using a lentivirus to transduce CD3xCD28 bead-stimulated purified central memory T-cells (such as T-cells from peripheral blood) with the vector expressing the CD19 and CD20 bispecific CAR, growing the T-cells in the presence of rhuIL-2 and/or IL-15 and restimulating the T-cells with $CD19^+$ and $CD20^+$ cells, recombinant CD19 and CD20, or an antibody to the receptor to cause the T-cells to proliferate, so as to make and expand CD19-specific and CD20-specific T-cells.

Therapeutic methods of the invention

The CARs of the invention may be used to overcome therapeutic failures arising from antigen loss escape variants, to reduce resistance to existing therapies and/or to treat diseases associated with the antigens targeted by the CARs.

Accordingly, the invention also provides methods for treating a disease associated with the antigen targeted by the CAR of the invention in a subject in need thereof. The method comprises providing a composition comprising the CAR of the invention and administering an effective amount of the composition so as to treat the disease associated with the antigen in the subject.

The invention also provides methods for overcoming therapeutic failures arising from antigen loss escape variants in disease states (*e.g.*, B-cell diseases) in subjects in need thereof. The method comprises providing a composition comprising the CAR of the invention and administering an effective amount of the composition so as to treat the disease associated with the antigen in the subject.

In some embodiments, the composition comprises a polynucleotide encoding the CAR, a protein comprising the CAR or genetically modified cells comprising the CAR. In another embodiment, the genetically modified cells of the composition are T-lymphocytes (T-cells), naïve T cells (T_N), memory T cells (for example, central memory T cells (T_{CM}), effector memory cells (T_{EM})), natural killer (NK) cells, hematopoietic stem cells (HSCs) or pluripotent embryonic/induced stem cells capable of giving rise to therapeutically

relevant progeny, which express the CAR of the invention. The compositions of the invention may be administered alone or in conjunction with existing therapies. If other therapies are used in conjunction, the compositions of the invention may be administered concurrently or sequentially with the other the existing therapies.

Pharmaceutical compositions

In various embodiments, the present invention provides pharmaceutical compositions comprising a pharmaceutically acceptable excipient and a therapeutically effective amount of the CAR (for example, bispecific CAR) of the invention. The CAR of the invention in the composition may be any one or more of a polynucleotide encoding the CAR, a protein comprising the CAR or genetically modified cells comprising the CAR. The composition may further comprise polynucleotides encoding EGFRt, CCR and/or DHFR (for example mutant DHFR), proteins co-expressed with the CAR including EGFRt, CCR and/or DHFR or genetically modified cells that express the CAR and co-express EGFRt, CCR and/or DHFR. "Pharmaceutically acceptable excipient" means an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic, and desirable, and includes excipients that are acceptable for veterinary use as well as for human pharmaceutical use. Such excipients may be solid, liquid, semisolid, or, in the case of an aerosol composition, gaseous.

In various embodiments, the pharmaceutical compositions according to the invention may be formulated for delivery via any route of administration. "Route of administration" may refer to any administration pathway known in the art, including but not limited to aerosol, nasal, oral, intravenous, intramuscular, intraperitoneal, inhalation, transmucosal, transdermal, parenteral, implantable pump, continuous infusion, topical application, capsules and/or injections.

The pharmaceutical compositions according to the invention can also contain any pharmaceutically acceptable carrier. "Pharmaceutically acceptable carrier" as used herein refers to a pharmaceutically acceptable material, composition, or vehicle that is involved in carrying or transporting a compound of interest from one tissue, organ, or portion of the body to another tissue, organ, or portion of the body. For example, the carrier may be a liquid or solid filler, diluent, excipient, solvent, or encapsulating material, or a

combination thereof. Each component of the carrier must be "pharmaceutically acceptable" in that it must be compatible with the other ingredients of the formulation. It must also be suitable for use in contact with any tissues or organs with which it may come in contact, meaning that it must not carry a risk of toxicity, irritation, allergic response, immunogenicity, or any other complication that excessively outweighs its therapeutic benefits.

The pharmaceutical compositions according to the invention can also be encapsulated, tableted or prepared in an emulsion or syrup for oral administration. Pharmaceutically acceptable solid or liquid carriers may be added to enhance or stabilize the composition, or to facilitate preparation of the composition. Liquid carriers include syrup, peanut oil, olive oil, glycerin, saline, alcohols and water. Solid carriers include starch, lactose, calcium sulfate, dihydrate, terra alba, magnesium stearate or stearic acid, talc, pectin, acacia, agar or gelatin. The carrier may also include a sustained release material such as glyceryl monostearate or glyceryl distearate, alone or with a wax.

The pharmaceutical preparations are made following the conventional techniques of pharmacy involving milling, mixing, granulation, and compressing, when necessary, for tablet forms; or milling, mixing and filling for hard gelatin capsule forms. When a liquid carrier is used, the preparation will be in the form of syrup, elixir, emulsion or an aqueous or non-aqueous suspension. Such a liquid formulation may be administered directly p.o. or filled into a soft gelatin capsule.

The pharmaceutical compositions according to the invention may be delivered in a therapeutically effective amount. The precise therapeutically effective amount is that amount of the composition that will yield the most effective results in terms of efficacy of treatment in a given subject. This amount will vary depending upon a variety of factors, including but not limited to the characteristics of the therapeutic compound (including activity, pharmacokinetics, pharmacodynamics, and bioavailability), the physiological condition of the subject (including age, sex, disease type and stage, general physical condition, responsiveness to a given dosage, and type of medication), the nature of the pharmaceutically acceptable carrier or carriers in the formulation, and the route of administration. One skilled in the clinical and pharmacological arts will be able to determine a therapeutically effective amount through routine experimentation, for

instance, by monitoring a subject's response to administration of a compound and adjusting the dosage accordingly. For additional guidance, see *Remington: The Science and Practice of Pharmacy* (Gennaro ed. 20th edition, Williams & Wilkins PA, USA) (2000).

EXAMPLES

The following examples are provided to better illustrate the claimed invention and are not to be interpreted as limiting the scope of the invention. To the extent that specific materials are mentioned, it is merely for purposes of illustration and is not intended to limit the invention. One skilled in the art may develop equivalent means or reactants without the exercise of inventive capacity and without departing from the scope of the invention.

Example 1

Figure 1 is a schematic representation of the bispecific chimeric antigen receptor of the invention. In an exemplary embodiment of the invention, Figure 2 depicts the components of bispecific anti-CD19xanti-CD20 bispecific CAR. Figure 2 also depicts a schematic of the complete cDNA packaged into epHIV-7 lentivirus vector transfer plasmid. Figures 3 and 4 show the nucleic and amino acid sequences of an exemplary bispecific CAR, namely GMCSFss-CD19scFv-Gly4Ser1linker-CD20scFv-IgG4Hinge-CD28tm-41BBzeta-T2A-EGFRt_epHIV7.

Example 2

Figure 5 is a schematic showing the vector construct of an exemplary CAR of the invention, namely, the CD19scFv-CD20scFv-IgG4-CD28tm-CD28costim-CD3zeta transgene construct. The CD19scFv-CD20scFv-IgG4-CD28tmCD28costim-CD3zeta transgene was assembled using the one-step isothermal DNA assembly method previously described by Gibson et. al. (Enzymatic assembly of DNA molecules upto several hindred kilobases. *Nature Methods*. 2009;6:343-345). The V_L and V_H domains of the CD19 scFv construct was sequenced from a CD19CAR-CD28-Zeta transgene previously described. Schmitz N, Dreger P, Glass B, Sureda A. Allogeneic transplantation

in lymphoma: current status. *Haematologica.* 2007;92(11):1533-1548) through polymerase chain reaction (PCR). The V_H and V_L domains of the CD20 scFv were assembled by spliced-overlap polymerase chain reaction using a CD20R-CD28-Zeta transgene previously described (Michael Jensen et al., CD20 is a molecular target for scFvFc:zeta receptor redirected T-cells: implications for cellular immunotherapy of $CD20^+$ malignancy. *Biology of Blood and Marrow Transplant.* 1998;4:75-83). The V_H and the V_L domains of CD19 scFv and CD20 scFv were linked with an 18-residue linker peptide as previously described. The IgG4-CD28tm-CD28costim domain was sequenced using the CD19R-CD28-CD3zeta transgene by PCR. The CD3zeta-T2A-EGFRt_epHIV7 lentiviral destination vector was prepared by NheI and RsrII restriction digestion of the CD19R-CD28 portion from a CD19R-CD28-Zeta-T2A-EGFRt_ epHIV7 plasmid previously described (Seitaro Terakura et al., Generation of CD19-CAR modified CD8+ T-cells derived from virus-specific central memory T-cells. *Blood.* Oct. 26, 2011). The final CD19scFv-CD20scFv-IgG4-CD28tm-CD28costim-CD3zeta construct was assembled by the one-step isothermal Gibson DNA assembly method using the restriction digested Zeta-epHIV7 destination vector and the CD19scFv, CD20scFv, and IgG4-CD28tm-CD28costim- DNA fragments with primers for each containing a 30 bp overlap at the 5' terminus.

Table 1: Regulatory Elements Present in the bispecific CAR epHIV-7 Transfer Plasmid	
Regulatory Element	**Function**
U5	5' Unique sequence
Psi	Packaging signal
RRE	Rev-responsive element
flap	Contains polypurine track sequence and central termination sequence to facilitate nuclear import of pre-integration complex
EF1p Promoter	EF1-alpha Eukaryotic Promoter sequence driving expression of CD19xCD20 CAR
WPRE	Woodchuck hepatitis virus derived regulatory element to enhance viral RNA transportation
delU3	3' U3 with deletion to generate SIN vector
R	Repeat sequence within LTR
U5	3' U5 sequence in LTR
Amp^R	Ampicillin-resistance gene
CoEl ori	Replication origin of plasmid
SV40 ori	Replication origin of SV40
CMV promoter	CMV promoter to generate viral genome RNA
R	Repeat sequence within LTR

Example 3

HEK 293T-cells were transfected with anti-CD19xCD20CAR-T2A-EGFRt epHIV-7 transfer plasmid or with anti-CD20xCD19CAR-T2A-EGFRt epHIV-7 transfer plasmid. Transfected cells were stained with biotinylated anti-Fc antibodies and streptavidin PE (SA-PE) and then were subjected to flow cytometric analysis for detection of expression of the above two CARs. Both the anti-CD19xCD20 CAR and the anti-CD20xCD19 CAR were expressed on transfected HEK 293T cells.

The epHIV-7 transfer plasmid co-expressed EGFRt with the above two bispecific CARs. EGFRt co-expression was detected on the same transfected cells using a combination of biotinlylated anti-EGFR antibodies/SA-PE staining and flow cytometric analysis.

Example 4

Primary human peripheral blood derived T-cells were activated with OKT3 and then were lentivirally transduced with monospecific anti-CD19 CAR, monospecific anti-CD20 CAR or bispecific anti-CD19xCD20CAR-T2A-EGFRt epHIV7 lentivirus vector. epHIV7 lentivirus vector also encoded EGFRt together with monospecific anti-CD19 CAR, monospecific anti-CD20 CAR or bispecific anti-CD19xCD20. Thus, cells expressing the CARs co-expressed EGFRt. Transfected cells were stained with biotinlylated anti-EGFR antibodies and SA-PE and then were subjected to flow cytometric analysis for detection of EGFRt expression and co-expression of monospecific or bispecific CARs. Of the cells transfected with monospecific anti-CD19 CAR, 51% expressed EGFRt; of the cells transfected with monospecific anti-CD20 CAR, 38.5% expressed EGFRt; of the cells transfected with the bispecific anti-CD19xCD20 CAR, 63.8% expressed EGFRt.

T cell receptor (TCR) complex in transfected cells was also detected in the same transfected cells using FITC-conjugated anti-TCRα and anti-TCRβ antibodies staining and flow cytometric analysis.

Example 5

H9 cells were genetically modified to express CD19, or CD20, or both CD19 and CD20. Cells were stained with anti-CD19 and anti-CD20 antibodies and then were subject to flow cytometric analysis to detect the expression of CD19 and CD20. Cytometric analysis confirmed the desired expression profile of $CD19^{+}CD20^{-}$, $CD19^{-}CD20^{+}$, and $CD19^{+}CD20^{+}$ H9 cells, namely, genetically engineered H9 cells expressed CD19, or CD20, or both CD19 and CD20 thereby simulating cancer target cells, which contain antigen-negative antigen loss escape variants. As described later, these cell lines were subsequently used as target cells to stimulate CAR-expressing T-cell lines, which act as effector cells to kill target cells.

Also, endogenous levels of CD19 and CD20 expression in SUP-B15 and DHL-6 cell lines was analyzed using anti-CD19 APC and anti-CD20 PE staining and flow cytometric analysis. SUP-B15 cell line expressed high level of CD19 with low level of CD20 (thus $CD19^{+}CD20^{-}$), and DHL-16 cell line expressed high level of CD20 with low level of CD19 (thus $CD19^{-}CD20^{+}$).

Example 6

A 4-hour chromium release assay was used to measure the lysis of the target cells by the effector cells. Effector cells are primary human T-cells lentivirally transduced to express monospecific anti-CD19 CAR, monospecific anti-CD20 CAR or bispecific anti-CD19xCD20 CAR. The bispecific anti-CD19xCD20 CAR effector T-cells effectively lysed all $CD19^{+}CD20^{-}$, $CD19^{-}CD20^{+}$, and $CD19^{+}CD20^{+}$ target cells, which include $CD19^{+}CD20^{-}$ H9 cells, $CD19^{-}CD20^{+}$ H9 cells, $CD19^{+}CD20^{+}$ H9 cells and SUP-B15 cells. At effector to target ratios of 1:1, 3:1, 10:1, and 30:1, about 25%, 45%, 50% and 60%, respectively, target cells were lysed.

In contrast, monospecific CAR expressing T-cell lines fail to lyse antigen-negative antigen loss escape variants, which escaped from the monospecific CAR effector cells. The anti-CD19 CAR effector T-cells failed to lyse $CD19^{-}CD20^{+}$ targets and the anti-CD20 CAR effector T-cells failed to lyse $CD19^{+}CD20^{-}$ targets.

Example 7

Bispecific CAR-expressing CD4 enriched T-cells were activated for cytokine secretion (Interferon gamma (IFN-g, IFN-γ)) upon stimulation by $CD19^{+}CD20^{-}$, $CD19^{-}CD20^{+}$, and $CD19^{+}CD20^{+}$ target cells, which include $CD19^{+}CD20^{-}$ H9 cells, $CD19^{-}CD20^{+}$ H9 cells, $CD19^{+}CD20^{+}$ H9 cells and SUP-B15 cells. IFN-γ content was measured by cytokine bead array of culture supernatants of T-cells and target cells after 24-hours of co-culture. Activated bispecific CAR-expressing CD4 enriched T-cells secreted at least 2500 pg/ml INF-g upon stimulation by every type of target cells. In contrast, monospecific CAR expressing T-cell lines were not activated for cytokine INF-g secretion upon stimulation by antigen-negative antigen loss escape variants, which escaped from the monospecific

CAR effector cells. CD19 CAR T-cells failed to secrete IGN-γ upon co-culture with $CD19^{-}CD20^{+}$ target cells and CD20 CAR T-cells failed to secrete IGN-γ upon co-culture with $CD19^{+}CD20^{-}$ target cells.

In-vitro Stimulation Assay

- Stimulators (3x10^5):
  - TM-LCL — H9 parent
  - OKT3-TM-LCL — H9 CD19R
  - SUP-B15 — H9 CD20R
  - DHL-6 — H9 CD19/20R
- Responders (1x10^6 on $S_1R_2D_{17}$):
  - CD4 enriched mock — CD8 enriched mock
  - CD4 enriched CD19R — CD8 enriched CD19R
  - CD4 enriched CD20R — CD8 enriched CD20R
  - CD4 enriched CD19/20R — CD8 enriched CD19/20R
- Cells incubated for 24 hrs, and cell free supernatant will be harvested today for BioPlex assay

Example 8

The example below describes a CD19 specific chimeric antigen receptor linked to truncated epidermal growth factor receptor (EGFRt) via a T2A sequence. EGFRt may be linked to and co-expressed with other chimeric antigen receptors, for example, bispecific chimeric antigen receptors.

Applicants demonstrated the utility of such a truncated EGFR (huEGFRt) expressed by transduced T cells for immunomagnetic purification using biotinylated cetuximab, cell tracking by flow cytometry and immunohistochemistry, and in vivo cell ablation after systemic cetuximab administration. In this exemplary embodiment, domain I and II of EGFRt have been deleted while domains III and IV have been retained.

The CD19CAR-T2A-EGFRt-epHIV7 lentiviral construct contains: (1) the chimeric antigen receptor (CAR) sequence consisting of the V_H and V_L gene segments of the CD19-specific FMC63 monoclonal antibody (mAb), an IgG4 hinge-C_{H2}-C_{H3}, the transmembrane, and cytoplasmic signaling domains of the co-stimulatory molecule

CD28, and the cytoplasmic domain of the CD3ζ chain (Kowolik CK. et al., CD28 costimuation provided through a CD19-specific chimeric antigen receptor enhances in vivo persistence and antitumor efficacy of adoptively transferred T cells. *Cancer Res.* 2006, 66(22):10995-11004); (2) the self-cleaving *T2A* sequence (Szymczak AL. et al., Correction of multi-gene deficiency in vivo using a "self-cleaving" 2A peptide-based retroviral vector. *Nat Biotechnol* 2004; 22(5)589-594); and (3) the truncated *EGFR* sequence as indicated.

Immunomagnetic enrichment of huEGFRt$^+$ human T cells after lentiviral transduction

The biotinylated cetuximab was used for either immunomagnetic selection or FACS sorting of huEGFRt$^+$ cells. Applicants used biotinylated cetuximab in conjunction with commercially available antibiotin microbeads for the immunomagnetic selection of human T cells transduced with a self-inactivating lentivirus that directs the co-expression of CD19CAR and huEGFRt.

PBMCs or purified central memory (CD45RO$^+$CD62L$^+$ T_{CM}) or effector memory (CD45RO$^+$CD62L$^+$ T_{EM}) T-cell subsets were stimulated with anti-CD3/anti-CD28 beads and then transduced by lentiviral vector to generate a panel of primary human T-cell lines, of which 2.6%-40% expressed huEGFRt and CAR. The unselected cells were labeled with biotinylated cetuximab and anti-biotin microbeads; and then were separated to consistently obtain a selected cell population, of which 90% express huEGFRt and CAR.

Unselected T cells and selected fraction were stained with biotinylated-cetuximab and either PE-conjugated streptavidin or PE-conjugated anti-biotin Ab, and then were subject to flow cytometric analysis. Selection of CD19CAR$^+$EGFRt$^+$ cells was performed either 3 days after transduction of OKT3 blasts (enriched from 38% to 98%), or after 1 rapid expansion cycle of transduced effector memory CD62LCD45RO$^+$-derived cells (enriched from 20% to 96%), after 3 rapid expansion cycles of transduced CMVpp65-specific TCM-derived cells (enriched from 12% to 91%), or after 2 rapid expansion cycles of transduced CD8$^+$TCM-derived cells (enriched from 3% to 97%). Selection of CD19CAR$^+$EGFRt$^+$IMPDH2dm$^+$ cells was performed after 1 rapid expansion cycle of transduced TCM-derived cells (enriched from 25 to 92%).

CD19CAR-T2A-EGFRt-IMPDH2dm constructs contained in lentiviral vectors include codon optimized sequence portions of the CD19-specific, CD28 co-stimulatory CAR (CD19CAR), followed by the self-cleavable T2A, and selection markers huEGFRt and IMPDH2dm (a double mutant of the inosine monophosphate dehydrogenase 2 gene that allows for cell survival upon addition of mycophenolate 27), along with the Elongation Factor 1 promoter sequences (EF-1p), the GM-CSF receptor alpha chain signal sequences (GMCSFRss), and the 3 nucleotide stop codon.

Before immunomagnetic selection, a proliferative advantage of huEGFRt$^-$ cells over huEGFRt$^+$ cells was observed in cultures of unselected transduced T cells subjected to OKT3-mediated expansion. However, after immunomagnetic selection, the level of huEGFRt expression and the frequency of expressing cells remained stable over 3 consecutive 14-day cycles of OKT3-based expansion[14]. The fold expansion of EGFRt$^+$ cells after immunomagnetic selection was significantly enhanced over that of huEGFRt$^+$ cells in the unselected cultures.

These data demonstrate that huEGFRt can serve as a cell surface marker unique to transduced human T cells and enable subsequent cetuximab-based immunomagnetic purification of stable huEGFRt-expressing cell populations which also express CARs.

Tracking of adoptively transferred huEGFRt$^+$ T cells using flow cytometry and immunohistochemistry

To test the utility of huEGFRt for tracking the engraftment of adoptively transferred T cells, Applicants harvested blood and bone marrow specimens from NOD/*Scid* IL-2RγC ^{null} mice engrafted with CD19CAR$^+$EGFRt$^+$ human T cells.

First, unfixed peripheral blood and bone marrow mononuclear cell samples were subjected to flow cytometric analysis after being stained with biotinylated cetuximab and PE-conjugated streptavidin. Although the level of human CD45$^+$ T-cell engraftment (20%-25%) was similar in animals administered either EGFRt-negative or -positive T cells, double staining for human CD45 and EGFR allowed for the resolution of huEGFRt$^+$ (ie, transgeneexpressing) human T cells from their huEGFRt-negative counterparts.

Second, Applicants sought to determine whether standard paraffin embedded fixed tissue specimens were amenable to detection of huEGFRt$^+$ T-cell infiltrates using EGFR-specific diagnostic kits. Applicants performed immunohistochemical analysis of paraffin-embedded femurs from engrafted mice and detected huEGFRt$^+$ cells in the bone marrow. These data support the utility of huEGFRt to serve as a tracking marker for quantifying the frequency and tissue distribution of adoptively transferred T cells.

Cetuximab binding to huEGFRt sensitizes human T cells to ADCC

A valuable feature of a cell surface selection/tracking marker would be its capacity to serve as a target for in vivo cell ablation. Applicants evaluated the extent to which Cetuximab bound to huEGFRt on T cells activates ADCC of huEGFRt$^+$ T cells *in vitro*, and whether Cetuximab administration could attenuate the engraftment of adoptively transferred huEGFRt$^+$ T cells in NOD/*scid* mice.

^{51}Cr-labeled huEGFRt$^+$ T cells as the target cells and human GM-CSF activated fresh PBMCs as effectors were co-cultured. Then, the addition of Cetuximab specifically sensitized huEGFRt$^+$ T cells to ADCC cytolysis by effectors. Lysis of huEGFRt$^+$ T cells was measured by 4-hour chromium release assay and results showed that Cetuximab addition significantly increased lysis from less than 5% to about 50%, 45%, 40% and 15% respectively at effector to target (effector:target) ratios 50:1, 25:1, 5:1 and 1:1.

In contrast, the addition of the CD20-specific mAb Rituxan had no effect on triggering ADCC of huEGFRt$^+$ T cells in this assay.

Applicants next derived huEGFRt$^+$ CTLL-2 murine T cells that were additionally modified to secrete autocrine IL-2 and express the firefly luciferase biophotonic reporter, and adoptively transferred these ffLuc$^+$huEGFRt$^+$ CTLL-2 cells via intravenous injection to NOD/*scid* mice, which subsequently received Cetuximab or Rituxan. The *in vivo* engraftment of transferred CTLL-2, as measured by in vivo biophotonic imaging, was significantly inhibited (97%, $P < .05$) in mice that received Erbitux (1 mg intraperitoneally daily). The Cetuximab-mediated elimination of the ffLuc$^+$huEGFRt$^+$ CTLL-2 cells

occurred between 4 and 6 days. These data support the use of Cetuximab administration as a therapeutic control for patients receiving huEGFRt$^+$ T cells.

Example 9

This example describes T cells with an intrinsic γc cytokine signaling mechanism, and shows that chimeric cytokine receptors (CCR) IL-2/IL-15Rβ (CγCR2) and IL-7Rα (CγCR7) have the ability to improve the survival, persistence, and *in vivo* engraftment of cytotoxic T cells (CTLs). Truncated CD19 antigen (CD19t) was linked to CγCR via a T2A linker to show the expression of CγCR on the cell surface. The chimeric cytokine receptors described herein may be linked to the chimeric antigen receptors of the invention, such as bispecific CARs described herein.

To develop a cell-intrinsic, ligand-independent γc cytokine platform, Applicants engineered chimeric γc cytokine receptors (CγCR) comprised of the IL-7 cytokine tethered by ten amino acids to the extracellular domain of IL-7Rα. To engineer a CγCR that confers an IL-7R signal, IL-7 cytokine was tethered to the full length IL-7Rα chain (CγCR7). A CγCR that provides an IL-2/IL-15Rβ signal was engineered by tethering the IL-7 cytokine to the extracellular and transmembrane domain of IL-7Rα fused to the cytoplasmic domain of IL-2/IL-15Rβ (CγCR2). These single chain chimeric receptors are expected to require endogenous γc chain for signaling.

Constructs were then generated where the CγCR transgenes were followed by the self-cleavable T2A sequence, and a cytoplasmically truncated CD19 antigen (CD19t). CγCR and CD19t are expressed as a single transcript and cleaved post-translationally at the C-terminus of the T2A self-cleaving peptide to yield two separate type 1 membrane proteins CγCR(T2A) and CD19t. Based on expression of two proteins from a single transcript, the ratio of CγCR(T2A) to CD19t expression is 1:1, therefore, cell surface CD19t is an indication of CγCR cell surface expression. Lentiviral transduction and expression of these constructs could then be measured by surface CD19t expression, such as that seen in both Jurkat and NK-92 cell lines.

A third CγCR was also engineered, having IL-7 cytokine tethered to a truncated IL-7Rα (CγCR7t), which is missing amino acids 1-126 from the extracellular domain of the IL-

7Rα . A molecular model of CγCR7t dimerization with the endogenous γc chain is necessary for signal transduction. The lack of amino acids 1-126 of the extracellular domain of IL-7Rα renders the CγCR7t nonfunctional.

Truncated CγCR7 expression does not functionally signal or support cytokine independent cell growth. Flow cytometric detected cell-surface CD19t on lenti-transduced Jurkat (95% $CD19t^{+}C\gamma CR7t^{+}$) and Teff cell lines (97% $CD19t^{+}C\gamma CR7t^{+}$). Western blot analysis of STAT5 phosphorylation within CγCR7t expressing Jurkat cell line did not detect obvious increase of phosphorylated STAT5 as compared to non-transduced control Jurkat cell line. Positive controls OKT3 stimulated PBMC cultured in 50U/ml IL-2 and 10ng/ml IL-15 and K562 showed activation of increased phosphorylated STAT5. Accordingly, expansion and viability of CTLs transduced with CγCR7t cultured for 20 days were still dependent on cytokines.

To determine if functional CγCRs such as CγCR2 and CγCR7 could support the growth of $CD8^{+}$ human primary T cells in the absence of exogenous cytokine, we measured the expansion of CTLs expressing each CγCR. Human primary T cells expressing CγCR7t were unable to expand in the absence of exogenous cytokine . Both CγCR2 and CγCR7 were able to support the survival and proliferation of the $CD8^{+}$ T cells through maintenance of viability, in a manner similar to that of parental cells cultured in 5U/ml and 0.5 U/ml IL-2, respectively. The increased total cell expansion measured for $C\gamma CR2^{+}$ versus $C\gamma CR7^{+}$ CTL correlates with increased expression (i.e., MFI of 26 for CγCR7 versus 52 for CγCR2) of Ki67, a nuclear antigen protein present in G1, S, G2, and M phase of the cell cycle. Higher Bcl-2, an key antiapoptotic protein induced in response to IL-2 and IL-7 signaling, expression was observed for $C\gamma CR7^{+}$ versus $C\gamma CR2^{+}$ CTL, supporting the ability of CγCR7 to maintain the survival of the human primary T cells. Together this data suggests that, although both CγCRs support cytokine-independent T cell viability and expansion, CγCR2 provides a proliferative advantage while CγCR7 maintains survival for effector $CD8^{+}$ CTLs.

CγCR expressing CD8$^+$ T cells exhibit cytokine independent engraftment in vivo

Studies by our lab and others indicate that human CTL engraftment in NOD/*Scid* IL-2RγC ^{null} mice is dependent on exogenous administration of human IL-15 or IL-2. To test the potential of CγCR expression in CTLs to overcome this dependence, parental effector T cells, CγCR7$^+$ CTLs, and CγCR2$^+$ CTLs were injected into the tail vein of immunodeficient NOD/*Scid* IL-2RγC ^{null} mice in the absence of exogenous cytokine administration. Total engraftment was compared by harvesting at least four mice per group at day 8, 17, 24, and 48 and analyzing T cell levels in the blood and bone marrow.

In the blood, CγCR2$^+$ CTLs had impressive significant (P<0.007) exogenous cytokine independent engraftment compared to CγCR7$^+$ CTLs and the parental cells. In the bone marrow, both CγCR7$^+$ CTLs (P<0.03) and CγCR2$^+$ CTLs (P<0.0005) had significant exogenous cytokine independent engraftment compared to the parental cells. CγCR2$^+$ CTLs had higher engraftment compared to CγCR7$^+$ CTLs. This indicates that both CγCR7$^+$ CTLs and CγCR2$^+$ CTLs are capable of supporting exogenous cytokine independent engraftment but the total percentage of cells was different. The blood supported higher percent engraftment of CγCR2$^+$ CTLs compared to bone marrow. The bone marrow supported the engraftment of CγCR7$^+$ CTLs over a longer period of time. Importantly, the engraftment was not infinite as the cells were no longer present in the blood and bone marrow at day 48 in either group.

Cell intrinsic γc cytokine signals can replace the need for exogenous cytokine administration for the support of adoptively transferred CTLs. Providing cell intrinsic cytokine receptors can overcome the major limitation of adoptive immunotherapy; the long-term persistence of adoptively transferred CTL. This may eliminate the need for administration of exogenous cytokine, which may reduce toxicities and bystander effects on endogenous cell types.

Example 10

This example shows that CD19 chimeric antigen receptor linked to EGFRt and DHFR can be regulated by methotrexate. Using the methods described herein, the

dihydroxyfolate receptor described herein may be linked to the bispecific chimeric antigen receptors of the invention.

Applicants developed a human selectable transgene using a variant of human dihydrofolate reductase (hDHFR) that would enable selection of T cells with the less toxic, pharmaceutically available drug methotrexate (MTX). MTX exerts its anti-proliferative effect through competitive inhibition of DHFR, a key enzyme essential for *de novo* synthesis of thymidylate nucleotides.

In the instant example, Applicants evaluated the potential of $DHFR^{FS}$ (hDHFR L22F/F31S variant) mediated *in vitro* selection of primary human T cells that co-express a CD19-specific chimeric antigen receptor (CD19CAR for targeting of CD19-expressing tumors). In this strategy, we hypothesized that exposure of a transduced mixed population of T cells to the lymphotoxic drug MTX should lead to elimination of untransduced T cells and selective expansion of $DHFR^{FS}$/CD19CAR T cells co-expressing T cells, increasing the anti-tumor efficacy of the T cell population as a whole. Here Applicants show that $DHFR^{FS}$-mediated selection of gene modified T cells enforced the CD19CAR therapeutic transgene expression, and allowed for the derivation of CAR^{+} stable integrants in the presence of clinically attainable concentrations of MTX (e.g., 0.1 μM MTX).

To translate the $hDHFR^{FS}$ selection approach for potential therapeutic utility, Applicants designed a lentiviral vector co-expressing $hDHFR^{FS}$ in conjunction with a CD19-specific chimeric antigen receptor (CD19CAR) and a truncated human EGFR polypeptide as a tracking marker (huEGFRt) each separated by a ribosomal skip T2A sequence.

CTLL2 T cells were first transduced with this CD19CAR-huEGFRt-$hDHFR^{FS}$ lentiviral vector and evaluated for their resistance to MTX. Ten days after lenti-transduction, 7-8 % of the cells were positive for CD19CAR and huEGFRt expression.

In the absence of MTX, the non-transduced and transduced CTLL2 cells expanded at an equivalent rate (21- and 27-fold respectively). After incubation with MTX (0-0.1 μM) for 8 days, a 7-fold expansion with 80% survival was observed with transduced cells, while

exposure of non-transduced CTLL2 cells to ≥ 0.05 μM MTX resulted in strong inhibition of non-transduced CTLL2 cell expansion and viability.

Evaluation of huEGFRt expression levels of transduced CTLL2 cells after 8 days in culture with varying concentrations of MTX further revealed significant MTX-mediated enrichment of transgene-expressing huEGFRt$^+$ cells (49%, 93%, 98.5%, 99% at 0.01, 0.025, 0.05 and 0.1 μM MTX respectively).

To further characterize the maximum dose of MTX that could be tolerated by selected CTLL2 cells, transduced CTLL2 cells that had been cultured in 0.1 μM MTX for 8 days were re-plated at a wider range of MTX concentrations (up to 0.75 μM). These transduced and pre-MTX selected cells were able to expand 90-100 fold at MTX concentrations up to 0.25 μM, which is equivalent to non-transduced control CTLL2 expansion in the absence of MTX.

Applicants transduced primary human T cells with the same CD19CAR-huEGFRt-hDHFR^{FS} lentiviral vector. Purified CD62L$^+$CD45RO$^+$ T cells were used as a starting population based on their potential for persistence after adoptive transfer. Ten days after transduction, these T cells were cultured in varying concentrations of MTX and assessed for cell number and viability over time. After 10 days, transduced and non-transduced T cells expanded equally (80-fold) in the absence of MTX. Furthermore, even at 0.1 μM MTX, transduced T cells maintained a viability of 63%, while non-transduced primary human T cells exhibited strong inhibition of both viability and fold-expansion starting at concentrations as low as 0.025 μM MTX.

Flow cytometric evaluation of transduced T cells after 10 days in culture with varying concentrations of MTX revealed significant MTX-mediated enrichment of transgene-expressing cells (e.g., 0.025μM MTX enriched about 54% CD19CAR$^+$ and 79% EGFRt$^+$; 0.05μM MTX enriched about 76% CD19CAR$^+$ and 89% EGFRt$^+$)

Comparison of CD19CAR and EGFRt expression at day 6 vs. day 10 of culture revealed the steady progression of this MTX/DHFR^{FS}-mediated selection over time (Day 0: 18% CD19CAR$^+$, 28% EGFRt$^+$; Day 6: 48% CD19CAR$^+$, 71% EGFRt$^+$; Day 10: 70% CD19CAR$^+$, 88% EGFRt$^+$).

All references cited herein are incorporated by reference in their entirety as though fully set forth. Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton *et al.*, *Dictionary of Microbiology and Molecular Biology* 3^{rd} *ed.*, J. Wiley & Sons (New York, NY 2001); March, *Advanced Organic Chemistry Reactions, Mechanisms and Structure* 5^{th} *ed.*, J. Wiley & Sons (New York, NY 2001); and Sambrook and Russel, *Molecular Cloning: A Laboratory Manual 3rd ed.*, Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY 2001), provide one skilled in the art with a general guide to many of the terms used in the present application.

One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. Indeed, the present invention is in no way limited to the methods and materials described. For purposes of the present invention, the following terms are defined below.

While these descriptions directly describe the above embodiments, it is understood that those skilled in the art may conceive modifications and/or variations to the specific embodiments shown and described herein. Any such modifications or variations that fall within the purview of this description are intended to be included therein as well. Unless specifically noted, it is the intention of the inventors that the words and phrases in the specification and claims be given the ordinary and accustomed meanings to those of ordinary skill in the applicable art(s).

The foregoing description of various embodiments of the invention known to the applicant at this time of filing the application has been presented and is intended for the purposes of illustration and description. The present description is not intended to be exhaustive nor limit the invention to the precise form disclosed and many modifications and variations are possible in the light of the above teachings. The embodiments described serve to explain the principles of the invention and its practical application and to enable others skilled in the art to utilize the invention in various embodiments and with various modifications as are suited to the particular use contemplated. Therefore, it is intended that the invention not be limited to the particular embodiments disclosed for carrying out the invention.

While particular embodiments of the present invention have been shown and described, it will be obvious to those skilled in the art that, based upon the teachings herein, changes and modifications may be made without departing from this invention and its broader aspects. It will be understood by those within the art that, in general, terms used herein are generally intended as "open" terms (*e.g.*, the term "including" should be interpreted as "including but not limited to," the term "having" should be interpreted as "having at least," the term "includes" should be interpreted as "includes but is not limited to," etc.).

What is claimed is:

1. A bispecific chimeric antigen receptor, comprising:
   a. at least two antigen-specific targeting regions;
   b. an extracellular spacer domain;
   c. a transmembrane domain;
   d. at least one co-stimulatory domain; and
   e. an intracellular signaling domain,
   wherein each antigen-specific targeting region comprises an antigen-specific single chain Fv (scFv) fragment, and binds a different antigen, and
   wherein the bispecific chimeric antigen receptor is co-expressed with a therapeutic control.

2. The bispecific chimeric antigen receptor of claim 1, wherein the therapeutic control comprises any one or more of truncated epidermal growth factor receptor (EGFRt), thymidine kinase, cytosine deaminase, nitroreductase, xanthine-guanine phosphoribosyl transferase, human caspase 8, human caspase 9, purine nucleoside phosphorylase, linamarase/linamarin/glucose oxidase, deoxyribonucleoside kinase, horseradish peroxidase (HRP)/indole-3-acetic (IAA), Gamma-glutamylcysteine synthetase, CD20/alphaCD20, CD34/thymidine kinase chimera, dox-depedent caspase-2, mutant thymidine kinase (HSV-TKSR39), AP1903/Fas system, a chimeric cytokine receptor (CCR), a selection marker, and combinations thereof.

3. The bispecific chimeric antigen receptor of claim 2, wherein the EGFRt binds any one or more of an EGFR-specific siRNA , a small molecule, an anti-EGFR antibody or a fragment thereof, or a combination thereof.

4. The bispecific chimeric antigen receptor of claim 2, wherein the selection marker comprises any one or more of dihydroxyfolate receptor (DHFR), mutant DHFR, methylated-DNA-protein-cysteine methyltransferase, inosine monophosphate dehydrogenase II (IMDHP2) and combinations thereof.

5. The bispecific chimeric antigen receptor of claim 2, wherein the CCR comprises any one or more of (i) IL-7 cytokine-linker- IL7Rα, (ii) IL-7 cytokine-linker-extracellular domain of IL-7Rα-transmembrane domain of IL-7Rα-cytoplasmic domain of IL-2Rβ, (iii) IL-7 cytokine-linker-IL2Rβ, and (iv) combinations thereof.

6. The bispecific chimeric antigen receptor of claim 1, wherein the bispecific chimeric antigen receptor and the therapeutic control are linked via a cleavable linker.

7. The bispecific chimeric antigen receptor of claim 6, wherein the cleavable linker is a self-cleaving cleavable linker.

8. The bispecific chimeric antigen receptor of claim 7, wherein the cleavable linker is any one or more of a 2A linker, 2A-like linker or a functional equivalent thereof.

9. The bispecific chimeric antigen receptor of claim 1, wherein the extracellular spacer domain comprises any one or more of an Fc fragment of an antibody or a functional equivalent, fragment or derivative thereof, a hinge region of an antibody or a functional equivalent, fragment or derivative thereof, a CH2 region of an antibody, a CH3 region of an antibody, an artificial spacer sequence and combinations thereof.

10. The bispecific chimeric antigen receptor of claim 9, wherein the extracellular spacer domain comprises any one or more of (i) a hinge, CH2 and CH3 region of IgG4, (ii) a hinge region of IgG4, (iii) a hinge and CH2 region of IgG4, (iv) a hinge region of CD8α, (v) a hinge, CH2 and CH3 region of IgG1, (vi) a hinge region of IgG1, (vi) a hinge and CH2 region of IgG1, or (vii) combinations thereof.

11. The bispecific chimeric antigen receptor of claim 1, wherein the transmembrane domain comprises any one or more of a transmembrane region of a Type I transmembrane protein, an artificial hydrophobic sequence, and combinations thereof.

12. The bispecific chimeric antigen receptor of claim 11, wherein the transmembrane domain comprises any one or more of a transmembrane domain of a zeta chain of a T cell receptor complex, CD28, CD8α, and combinations thereof.

13. The bispecific chimeric antigen receptor of claim 1, wherein the co-stimulatory domain comprises a signaling domain from any one or more of CD28, CD137 (4-1BB), CD134 (OX40), Dap10, CD27, CD2, CD5, ICAM-1, LFA-1, Lck, TNFR-I, TNFR-II, Fas, CD30, CD40 and combinations thereof.

14. The bispecific chimeric antigen receptor of claim 1, wherein the intracellular signaling domain comprises a signaling domain of one or more of a human CD3 zeta chain, FcγRIII, FcεRI, a cytoplasmic tail of a Fc receptor, an immunoreceptor tyrosine-based activation motif (ITAM) bearing cytoplasmic receptors, and combinations thereof.

15. The bispecific chimeric antigen receptor of claim 1, wherein each of the at least two antigen-specific targeting domains target an antigen independently selected from the group consisting of antigens specific for cancer, an inflammatory disease, a neuronal disorder, diabetes, a cardiovascular disease, an infectious disease, an autoimmune disease, and combinations thereof.

16. The bispecific chimeric antigen receptor of claim 15, wherein the antigen specific for cancer comprises any one or more of 4-1BB, 5T4, adenocarcinoma antigen, alpha-fetoprotein, BAFF, B-lymphoma cell, C242 antigen, CA-125, carbonic anhydrase 9 (CA-IX), C-MET, CCR4, CD152, CD19, CD20, CD200, CD22, CD221, CD23 (IgE receptor), CD28, CD30 (TNFRSF8), CD33, CD4, CD40, CD44 v6, CD51, CD52, CD56, CD74, CD80, CEA, CNTO888, CTLA-4, DR5, EGFR, EpCAM, CD3, FAP, fibronectin extra domain-B, folate receptor 1, GD2, GD3 ganglioside, glycoprotein 75, GPNMB, HER2/neu, HGF, human scatter factor receptor kinase, IGF-1 receptor, IGF-I, IgG1, L1-CAM, IL-13, IL-6, insulin-like growth factor I receptor, integrin α5β1, integrin αvβ3, MORAb-009, MS4A1, MUC1, mucin CanAg, N-glycolylneuraminic acid, NPC-1C, PDGF-R α, PDL192, phosphatidylserine, prostatic carcinoma cells, RANKL, RON, ROR1, SCH 900105, SDC1, SLAMF7, TAG-72, tenascin C, TGF beta 2, TGF-β, TRAIL-R1, TRAIL-R2, tumor antigen CTAA16.88, VEGF-A, VEGFR-1, VEGFR2, vimentin, and combinations thereof.

17. The bispecific chimeric antigen receptor of claim 1, wherein the at least two antigen-specific targeting regions bind (i) CD19 and CD20, (ii) CD20 and L1-CAM, (iii) L1-

CAM and GD2, (iv) EGFR and L1-CAM, (v) CD19 and CD22, (vi) EGFR and C-MET, (vii) EGFR and HER2, (viii) C-MET and HER2, or (ix) EGFR and ROR1.

18. The bispecific chimeric antigen receptor of claim 1, wherein the at least two antigen-specific targeting regions bind CD19 and CD20.

19. The bispecific chimeric antigen receptor of claim 15, wherein the antigen specific for an inflammatory disease comprises any one or more of AOC3 (VAP-1), CAM-3001, CCL11 (eotaxin-1), CD125, CD147 (basigin), CD154 (CD40L), CD2, CD20, CD23 (IgE receptor), CD25 (α chain of IL-2 receptor), CD3, CD4, CD5, IFN-α, IFN-γ, IgE, IgE Fc region, IL-1, IL-12, IL-23, IL-13, IL-17, IL-17A, IL-22, IL-4, IL-5, IL-5, IL-6, IL-6 receptor, integrin α4, integrin α4β7, Lama glama, LFA-1 (CD11a), MEDI-528, myostatin, OX-40, rhuMAb β7, scleroscin, SOST, TGF beta 1, TNF-α, VEGF-A, and combinations thereof.

20. The bispecific chimeric antigen receptor of claim 15, wherein the antigen specific for a neuronal disorder comprises any one or more of beta amyloid, MABT5102A, and combinations thereof.

21. The bispecific chimeric antigen receptor of claim 15, wherein the antigen specific for diabetes comprises any one or more of L-1β, CD3, and combinations thereof.

22. The bispecific chimeric antigen receptor of claim 15, wherein the antigen-specific for a cardiovascular disease comprises any one or more of C5, cardiac myosin, CD41 (integrin alpha-IIb), fibrin II, beta chain, ITGB2 (CD18), sphingosine-1-phosphate, and combinations thereof.

23. The bispecific chimeric antigen receptor of claim 15, wherein the antigen specific for an infectious disease comprises any one or more of anthrax toxin, CCR5, CD4, clumping factor A, cytomegalovirus, cytomegalovirus glycoprotein B, endotoxin, Escherichia coli, hepatitis B surface antigen, hepatitis B virus, HIV-1, Hsp90, Influenza A hemagglutinin, lipoteichoic acid, Pseudomonas aeruginosa, rabies virus glycoprotein, respiratory syncytial virus, TNF-α, and combinations thereof.

24. In combination, the bispecific chimeric antigen receptor of claim 1 and the therapeutic control.

25. The combination of claim 24, wherein the therapeutic control comprises any one or more of truncated epidermal growth factor receptor (EGFRt), thymidine kinase, cytosine deaminase, nitroreductase, xanthine-guanine phosphoribosyl transferase, human caspase 8, human caspase 9, purine nucleoside phosphorylase, linamarase/linamarin/glucose oxidase, deoxyribonucleoside kinase, horseradish peroxidase (HRP)/indole-3-acetic (IAA), Gamma-glutamylcysteine synthetase, CD20/alphaCD20, CD34/thymidine kinase chimera, dox-depedent caspase-2, mutant thymidine kinase (HSV-TKSR39), AP1903/Fas system, a chimeric cytokine receptor (CCR), a selection marker, and combinations thereof.

26. The combination of claim 25, wherein the EGFRt binds any one or more of an EGFR-specific siRNA, a small molecule, an anti-EGFR antibody or a fragment thereof, or a combination thereof.

27. The combination of claim 25, wherein the selection marker comprises any one or more of dihydroxyfolate receptor (DHFR), mutant DHFR, methylated-DNA-protein-cysteine methyltransferase, inosine monophosphate dehydrogenase II (IMDHP2) and combinations thereof.

28. The combination of claim 25, wherein the CCR comprises any one or more of (i) IL-7 cytokine-linker- IL7Rα, (ii) IL-7 cytokine-linker-extracellular domain of IL-7Rα-transmembrane domain of IL-7Rα-cytoplasmic domain of IL-2Rβ, (iii) IL-7 cytokine-linker-IL2Rβ, and (iv) combinations thereof.

29. The combination of claim 24, wherein the bispecific chimeric antigen receptor and the therapeutic control are linked via a cleavable linker.

30. The combination of claim 29, wherein the cleavable linker is a self-cleaving cleavable linker.

31. The combination of claim 29, wherein the cleavable linker is any one or more of a 2A linker, 2A-like linker or a functional equivalent thereof.

32. A polynucleotide encoding the bispecific chimeric antigen receptor of claim 1 or the combination of claim 24.

33. A polypeptide encoded by the polynucleotide of claim 32.

34. A vector comprising the polynucleotide of claim 32.

35. A virus comprising the polynucleotide of claim 32.

36. The virus of claim 35, wherein the virus is an RNA virus.

37. The virus of claim 35, wherein the virus is a retrovirus, an adenovirus, an adeno-associated virus, a lentivirus, a pox virus or a herpes virus.

38. A genetically engineered cell, comprising the polynucleotide of claim 32, the chimeric antigen receptor of claim 1, or the combination of claim 24.

39. The genetically engineered cell of claim 38, wherein the cell is a T-lymphocyte (T-cell)

40. The genetically engineered cell of claim 39, wherein the cell is a naïve T cells, a central memory T cells, an effector memory T cell or a combination thereof.

41. The genetically engineered cell of claim 38, wherein the cell is a natural killer (NK) cell, a hematopoietic stem cell (HSC), an embryonic stem cell, or a pluripotent stem cell.

42. A pharmaceutical composition, comprising:
    a. any one or more of the bispecific chimeric antigen receptor of claim 1, the combination of claim 24, the polypeptide of claim 32, the vector of claim 34, the virus of claim 35, the genetically engineered cell of claim 38, and combinations thereof; and
    b. a pharmaceutically acceptable carrier.

43. In combination, the pharmaceutical composition of claim 42 and a composition adapted to biochemically interact with the therapeutic control to inhibit proliferation of a cell expressing the therapeutic control.

44. The combination of claim 43, wherein the composition adapted to biochemically interact with the therapeutic control is any one or more of Herceptin, methotrexate, cetuximab, thymidine analogs (for example ganciclovir), (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU), 5-flurocytosine (5-FC), 5-(azaridin-1-yl)-2, 4-dinitrobenzamide (CB1954), 6-thioguanine, a synthetic dimerizing drug (for example AP1903), fludarabine phosphate, linamarin (lin), nucleoside analogs (for exmaple BVDU, difluorodeoxycytidine (dFdC), 1-β-D-arabinofuranosylthymine (ara-T)), indole-3-acetic (IAA), l-buthionine-S,R-sulfoximine (BSO), rituximab (RTX), doxycycline, tyrosine kinase inhibitors or combinations thereof.

45. A method of producing a quantity of T-cells expressing a chimeric antigen receptor, comprising:

(i) transfecting one or more T-cells with the vector of claim 34; and

(ii) stimulating the one or more T-cells with cells expressing antigens targeted by the at least two antigen-specific targeting regions, recombinant antigens targeted by the at least two antigen-specific targeting regions, or an antibody to the chimeric antigen receptor, whereby the T-cells proliferate so as to produce the quantity of T-cells.

46. A method for treating a disease in a subject in need thereof, comprising:

(i) providing the composition of claim 42; and

(ii) administering a therapeutically effective amount of the composition to the subject so as to treat the disease,

wherein the at least two antigen-specific targeting regions each target an antigen, and at least one such antigen is associated with the disease.

47. A bispecific chimeric antigen receptor, comprising:

a. at least two antigen-specific targeting regions;

b. an extracellular spacer domain;

c. a transmembrane domain;

d. at least one co-stimulatory domain; and

e. an intracellular signaling domain,

wherein each antigen-specific targeting region comprises an antigen-specific single chain Fv (scFv) fragment, and binds a different antigen, and

wherein the bispecific chimeric antigen receptor is co-expressed with truncated epidermal growth factor receptor (EGFRt).

48. The bispecific chimeric antigen receptor of claim 47, wherein the bispecific chimeric antigen receptor is co-expressed with a therapeutic control, comprising any one or more of thymidine kinase, cytosine deaminase, nitroreductase, xanthine-guanine phosphoribosyl transferase, human caspase 8, human caspase 9, purine nucleoside phosphorylase, linamarase/linamarin/glucose oxidase, deoxyribonucleoside kinase, horseradish peroxidase (HRP)/indole-3-acetic (IAA), Gamma-glutamylcysteine synthetase, CD20/alphaCD20, CD34/thymidine kinase chimera, dox-depedent caspase-2, mutant thymidine kinase (HSV-TKSR39), AP1903/Fas system, a chimeric cytokine receptor (CCR), a selection marker, and combinations thereof.

49. The bispecific chimeric antigen receptor of claim 47, wherein the EGFRt binds any one or more of an EGFR-specific siRNA, a small molecule, an anti-EGFR antibody or a fragment thereof, or a combination thereof.

50. The bispecific chimeric antigen receptor of claim 48, wherein the selection marker comprises any one or more of dihydroxyfolate receptor (DHFR), mutant DHFR, methylated-DNA-protein-cysteine methyltransferase, inosine monophosphate dehydrogenase II (IMDHP2) and combinations thereof.

51. The bispecific chimeric antigen receptor of claim 48, wherein the CCR comprises any one or more of (i) IL-7 cytokine-linker- IL7Rα, (ii) IL-7 cytokine-linker-extracellular domain of IL-7Rα-transmembrane domain of IL-7Rα-cytoplasmic domain of IL-2Rβ, (iii) IL-7 cytokine-linker-IL2Rβ, and (iv) combinations thereof.

52. The bispecific chimeric antigen receptor of claim 47, wherein the bispecific chimeric antigen receptor and the therapeutic control are linked via a cleavable linker.

53. The bispecific chimeric antigen receptor of claim 52, wherein the cleavable linker is a self-cleaving cleavable linker.

54. The bispecific chimeric antigen receptor of claim 52, wherein the cleavable linker is any one or more of a 2A linker, 2A-like linker or a functional equivalent thereof.

55. The bispecific chimeric antigen receptor of claim 47, wherein the extracellular spacer domain comprises any one or more of an Fc fragment of an antibody or a functional equivalent, fragment or derivative thereof, a hinge region of an antibody or a functional equivalent, fragment or derivative thereof, a CH2 region of an antibody, a CH3 region of an antibody, an artificial spacer sequence and combinations thereof.

56. The bispecific chimeric antigen receptor of claim 55, wherein the extracellular spacer domain comprises any one or more of (i) a hinge, CH2 and CH3 region of IgG4, (ii) a hinge region of IgG4, (iii) a hinge and CH2 region of IgG4, (iv) a hinge region of CD8α, (v) a hinge, CH2 and CH3 region of IgG1, (vi) a hinge region of IgG1, (vi) a hinge and CH2 region of IgG1, and (vii) combinations thereof.

57. The bispecific chimeric antigen receptor of claim 47, wherein the transmembrane domain comprises any one or more of a transmembrane region of a Type I transmembrane protein, an artificial hydrophobic sequence, and combinations thereof.

58. The bispecific chimeric antigen receptor of claim 57, wherein the transmembrane domain comprises any one or more of a transmembrane domain of a zeta chain of a T cell receptor complex, CD28, CD8α, and combinations thereof.

59. The bispecific chimeric antigen receptor of claim 47, wherein the co-stimulatory domain comprises a signaling domain from any one or more of CD28, CD137 (4-1BB), CD134 (OX40), Dap10, CD27, CD2, CD5, ICAM-1, LFA-1, Lck, TNFR-I, TNFR-II, Fas, CD30, CD40 and combinations thereof.

60. The bispecific chimeric antigen receptor of claim 47, wherein the intracellular signaling domain comprises a signaling domain of one or more of a human CD3 zeta chain,

FcγRIII, FcεRI, a cytoplasmic tail of a Fc receptor, an immunoreceptor tyrosine-based activation motif (ITAM) bearing cytoplasmic receptors, and combinations thereof.

61. The bispecific chimeric antigen receptor of claim 47, wherein each of the at least two antigen-specific targeting domains target an antigen independently selected from the group consisting of antigens specific for cancer, an inflammatory disease, a neuronal disorder, diabetes, a cardiovascular disease, an infectious disease, an autoimmune disease, and combinations thereof.

62. The bispecific chimeric antigen receptor of claim 61, wherein the antigen specific for cancer comprises any one or more of 4-1BB, 5T4, adenocarcinoma antigen, alpha-fetoprotein, BAFF, B-lymphoma cell, C242 antigen, CA-125, carbonic anhydrase 9 (CA-IX), C-MET, CCR4, CD152, CD19, CD20, CD200, CD22, CD221, CD23 (IgE receptor), CD28, CD30 (TNFRSF8), CD33, CD4, CD40, CD44 v6, CD51, CD52, CD56, CD74, CD80, CEA, CNTO888, CTLA-4, DR5, EGFR, EpCAM, CD3, FAP, fibronectin extra domain-B, folate receptor 1, GD2, GD3 ganglioside, glycoprotein 75, GPNMB, HER2/neu, HGF, human scatter factor receptor kinase, IGF-1 receptor, IGF-I, IgG1, L1-CAM, IL-13, IL-6, insulin-like growth factor I receptor, integrin α5β1, integrin αvβ3, MORAb-009, MS4A1, MUC1, mucin CanAg, N-glycolylneuraminic acid, NPC-1C, PDGF-R α, PDL192, phosphatidylserine, prostatic carcinoma cells, RANKL, RON, ROR1, SCH 900105, SDC1, SLAMF7, TAG-72, tenascin C, TGF beta 2, TGF-β, TRAIL-R1, TRAIL-R2, tumor antigen CTAA16.88, VEGF-A, VEGFR-1, VEGFR2, vimentin, and combinations thereof.

63. The bispecific chimeric antigen receptor of claim 47, wherein the at least two antigen-specific targeting regions bind (i) CD19 and CD20, (ii) CD20 and L1-CAM, (iii) L1-CAM and GD2, (iv) EGFR and L1-CAM, (v) CD19 and CD22, (vi) EGFR and C-MET, (vii) EGFR and HER2, (viii) C-MET and HER2, or (ix) EGFR and ROR1.

64. The bispecific chimeric antigen receptor of claim 47, wherein the at least two antigen-specific targeting regions bind CD19 and CD20.

65. The bispecific chimeric antigen receptor of claim 61, wherein the antigen specific for an inflammatory disease comprises any one or more of AOC3 (VAP-1), CAM-3001, CCL11 (eotaxin-1), CD125, CD147 (basigin), CD154 (CD40L), CD2, CD20, CD23 (IgE receptor), CD25 (α chain of IL-2 receptor), CD3, CD4, CD5, IFN-α, IFN-γ, IgE, IgE Fc region, IL-1, IL-12, IL-23, IL-13, IL-17, IL-17A, IL-22, IL-4, IL-5, IL-5, IL-6, IL-6 receptor, integrin α4, integrin α4β7, Lama glama, LFA-1 (CD11a), MEDI-528, myostatin, OX-40, rhuMAb β7, scleroscin, SOST, TGF beta 1, TNF-α, VEGF-A, and combinations thereof.

66. The bispecific chimeric antigen receptor of claim 61, wherein the antigen specific for a neuronal disorder comprises any one or more of beta amyloid, MABT5102A, and combinations thereof.

67. The bispecific chimeric antigen receptor of claim 61, wherein the antigen specific for diabetes comprises any one or more of L-1β, CD3, and combinations thereof.

68. The bispecific chimeric antigen receptor of claim 61, wherein the antigen-specific for a cardiovascular disease comprises any one or more of C5, cardiac myosin, CD41 (integrin alpha-IIb), fibrin II, beta chain, ITGB2 (CD18), sphingosine-1-phosphate, and combinations thereof.

69. The bispecific chimeric antigen receptor of claim 61, wherein the antigen specific for an infectious disease comprises any one or more of anthrax toxin, CCR5, CD4, clumping factor A, cytomegalovirus, cytomegalovirus glycoprotein B, endotoxin, Escherichia coli, hepatitis B surface antigen, hepatitis B virus, HIV-1, Hsp90, Influenza A hemagglutinin, lipoteichoic acid, Pseudomonas aeruginosa, rabies virus glycoprotein, respiratory syncytial virus, TNF-α, and combinations thereof.

70. In combination, the bispecific chimeric antigen receptor of claim 47 and the EGFRt.

71. The combination of claim 70, wherein the EGFRt binds any one or more of an EGFR-specific siRNA, a small molecule, an anti-EGFR antibody or a fragment thereof, or a combination thereof.

72. The combination of claim 70, wherein the bispecific chimeric antigen receptor and the EGFRt are linked via a cleavable linker.

73. The combination of claim 72, wherein the cleavable linker is a self-cleaving cleavable linker.

74. The combination of claim 72, wherein the cleavable linker is any one or more of a 2A linker, 2A-like linker or a functional equivalent thereof.

75. A polynucleotide encoding the bispecific chimeric antigen receptor of claim 47 or the combination of claim 70.

76. A polypeptide encoded by the polynucleotide of claim 75.

77. A vector comprising the polynucleotide of claim 75.

78. A virus comprising the polynucleotide of claim 75.

79. The virus of claim 78, wherein the virus is an RNA virus.

80. The virus of claim 78, wherein the virus is a retrovirus, an adenovirus, an adeno-associated virus, a lentivirus, a pox virus or a herpes virus.

81. A genetically engineered cell, comprising the polynucleotide of claim 75, the chimeric antigen receptor of claim 47, or the combination of claim 70.

82. The genetically engineered cell of claim 81, wherein the cell is a T-lymphocyte (T-cell)

83. The genetically engineered cell of claim 82, wherein the cell is a naïve T cells, a central memory T cells, an effector memory T cell or a combination thereof.

84. The genetically engineered cell of claim 81, wherein the cell is a natural killer (NK) cell, a hematopoietic stem cell (HSC), an embryonic stem cell, or a pluripotent stem cell.

85. A pharmaceutical composition, comprising:

a. any one or more of the bispecific chimeric antigen receptor of claim 47, the combination of claim 70, the polypeptide of claim 76, the vector of claim 77, the

virus of claim 78, the genetically engineered cell of claim 81, and combinations thereof; and

b. a pharmaceutically acceptable carrier.

86. In combination, the pharmaceutical composition of claim 85 and a composition adapted to biochemically interact with the therapeutic control to inhibit proliferation of a cell expressing the EGFRt.

87. The combination of claim 86, wherein the composition adapted to biochemically interact with the therapeutic control is any one or more of Herceptin, methotrexate, cetuximab, thymidine analogs (for example ganciclovir), (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU), 5-flurocytosine (5-FC), 5-(azaridin-1-yl)-2, 4-dinitrobenzamide (CB1954), 6-thioguanine, a synthetic dimerizing drug (for example AP1903), fludarabine phosphate, linamarin (lin), nucleoside analogs (for exmaple BVDU, difluorodeoxycytidine (dFdC), 1-β-D-arabinofuranosylthymine (ara-T)), indole-3-acetic (IAA), l-buthionine-S,R-sulfoximine (BSO), rituximab (RTX), doxycycline, tyrosine kinase inhibitors or combinations thereof.

88. A method of producing a quantity of T-cells expressing a chimeric antigen receptor, comprising:

(i) transfecting one or more T-cells with the vector of claim 77; and

(ii) stimulating the one or more T-cells with cells expressing antigens targeted by the at least two antigen-specific targeting regions, recombinant antigens targeted by the at least two antigen-specific targeting regions, or an antibody to the chimeric antigen receptor, whereby the T-cells proliferate so as to produce the quantity of T-cells.

89. A method for treating a disease in a subject in need thereof, comprising:

(i) providing the composition of claim 85; and

(ii) administering a therapeutically effective amount of the composition to the subject so as to treat the disease,

wherein the at least two antigen-specific targeting regions each target an antigen, and at least one such antigen is associated with the disease.

90. A bispecific chimeric antigen receptor comprising the sequence set forth in Figures 4, 9 or 11.

91. A bispecific chimeric antigen receptor, comprising:

a. at least two antigen-specific targeting regions;

b. a CD8αhinge extracellular spacer domain;

c. a CD8α transmembrane domain;

d. a 4-1BB co-stimulatory domain; and

e. a CD3 zeta intracellular signaling domain,

wherein each antigen-specific targeting region comprises an antigen-specific single chain Fv (scFv) fragment, and binds a different antigen,

wherein the bispecific chimeric antigen receptor is co-expressed with truncated epidermal growth factor receptor (EGFRt), and

wherein the bispecific chimeric antigen receptor and EGFRt are linked via a T2A linker.

92. The bispecific chimeric antigen receptor of claim 91, wherein the bispecific chimeric antigen receptor is co-expressed with a therapeutic control, comprising any one or more of thymidine kinase, cytosine deaminase, nitroreductase, xanthine-guanine phosphoribosyl transferase, human caspase 8, human caspase 9, purine nucleoside phosphorylase, linamarase/linamarin/glucose oxidase, deoxyribonucleoside kinase, horseradish peroxidase (HRP)/indole-3-acetic (IAA), Gamma-glutamylcysteine synthetase, CD20/alphaCD20, CD34/thymidine kinase chimera, dox-depedent caspase-2, mutant thymidine kinase (HSV-TKSR39), AP1903/Fas system, a chimeric cytokine receptor (CCR), a selection marker, and combinations thereof.

93. The bispecific chimeric antigen receptor of claim 91, wherein the EGFRt binds any one or more of an EGFR-specific siRNA, a small molecule, an anti-EGFR antibody or a fragment thereof, or a combination thereof.

94. The bispecific chimeric antigen receptor of claim 92, wherein the selection marker comprises any one or more of dihydroxyfolate receptor (DHFR), mutant DHFR,

methylated-DNA-protein-cysteine methyltransferase, inosine monophosphate dehydrogenase II (IMDHP2) and combinations thereof.

95. The bispecific chimeric antigen receptor of claim 92, wherein the CCR comprises any one or more of (i) IL-7 cytokine-linker- IL7Rα, (ii) IL-7 cytokine-linker-extracellular domain of IL-7Rα-transmembrane domain of IL-7Rα-cytoplasmic domain of IL-2Rβ, (iii) IL-7 cytokine-linker-IL2Rβ, and (iv) combinations thereof.

96. The bispecific chimeric antigen receptor of claim 91, wherein each of the at least two antigen-specific targeting domains target an antigen independently selected from the group consisting of antigens specific for cancer, an inflammatory disease, a neuronal disorder, diabetes, a cardiovascular disease, an infectious disease, an autoimmune disease, and combinations thereof.

97. The bispecific chimeric antigen receptor of claim 96, wherein the antigen specific for cancer comprises any one or more of 4-1BB, 5T4, adenocarcinoma antigen, alpha-fetoprotein, BAFF, B-lymphoma cell, C242 antigen, CA-125, carbonic anhydrase 9 (CA-IX), C-MET, CCR4, CD152, CD19, CD20, CD200, CD22, CD221, CD23 (IgE receptor), CD28, CD30 (TNFRSF8), CD33, CD4, CD40, CD44 v6, CD51, CD52, CD56, CD74, CD80, CEA, CNTO888, CTLA-4, DR5, EGFR, EpCAM, CD3, FAP, fibronectin extra domain-B, folate receptor 1, GD2, GD3 ganglioside, glycoprotein 75, GPNMB, HER2/neu, HGF, human scatter factor receptor kinase, IGF-1 receptor, IGF-I, IgG1, L1-CAM, IL-13, IL-6, insulin-like growth factor I receptor, integrin α5β1, integrin αvβ3, MORAb-009, MS4A1, MUC1, mucin CanAg, N-glycolylneuraminic acid, NPC-1C, PDGF-R α, PDL192, phosphatidylserine, prostatic carcinoma cells, RANKL, RON, ROR1, SCH 900105, SDC1, SLAMF7, TAG-72, tenascin C, TGF beta 2, TGF-β, TRAIL-R1, TRAIL-R2, tumor antigen CTAA16.88, VEGF-A, VEGFR-1, VEGFR2, vimentin, and combinations thereof.

98. The bispecific chimeric antigen receptor of claim 96, wherein the at least two antigen-specific targeting regions bind (i) CD19 and CD20, (ii) CD20 and L1-CAM, (iii) L1-CAM and GD2, (iv) EGFR and L1-CAM, (v) CD19 and CD22, (vi) EGFR and C-MET, (vii) EGFR and HER2, (viii) C-MET and HER2, or (ix) EGFR and ROR1.

99. The bispecific chimeric antigen receptor of claim 96, wherein the at least two antigen-specific targeting regions bind CD19 and CD20.

100. The bispecific chimeric antigen receptor of claim 96, wherein the antigen specific for an inflammatory disease comprises any one or more of AOC3 (VAP-1), CAM-3001, CCL11 (eotaxin-1), CD125, CD147 (basigin), CD154 (CD40L), CD2, CD20, CD23 (IgE receptor), CD25 (α chain of IL-2 receptor), CD3, CD4, CD5, IFN-α, IFN-γ, IgE, IgE Fc region, IL-1, IL-12, IL-23, IL-13, IL-17, IL-17A, IL-22, IL-4, IL-5, IL-5, IL-6, IL-6 receptor, integrin α4, integrin α4β7, Lama glama, LFA-1 (CD11a), MEDI-528, myostatin, OX-40, rhuMAb β7, scleroscin, SOST, TGF beta 1, TNF-α, VEGF-A, and combinations thereof.

101. The bispecific chimeric antigen receptor of claim 96, wherein the antigen specific for a neuronal disorder comprises any one or more of beta amyloid, MABT5102A, and combinations thereof.

102. The bispecific chimeric antigen receptor of claim 96, wherein the antigen specific for diabetes comprises any one or more of L-1β, CD3, and combinations thereof.

103. The bispecific chimeric antigen receptor of claim 96, wherein the antigen-specific for a cardiovascular disease comprises any one or more of C5, cardiac myosin, CD41 (integrin alpha-IIb), fibrin II, beta chain, ITGB2 (CD18), sphingosine-1-phosphate, and combinations thereof.

104. The bispecific chimeric antigen receptor of claim 96, wherein the antigen specific for an infectious disease comprises any one or more of anthrax toxin, CCR5, CD4, clumping factor A, cytomegalovirus, cytomegalovirus glycoprotein B, endotoxin, Escherichia coli, hepatitis B surface antigen, hepatitis B virus, HIV-1, Hsp90, Influenza A hemagglutinin, lipoteichoic acid, Pseudomonas aeruginosa, rabies virus glycoprotein, respiratory syncytial virus, TNF-α, and combinations thereof.

Figure 1

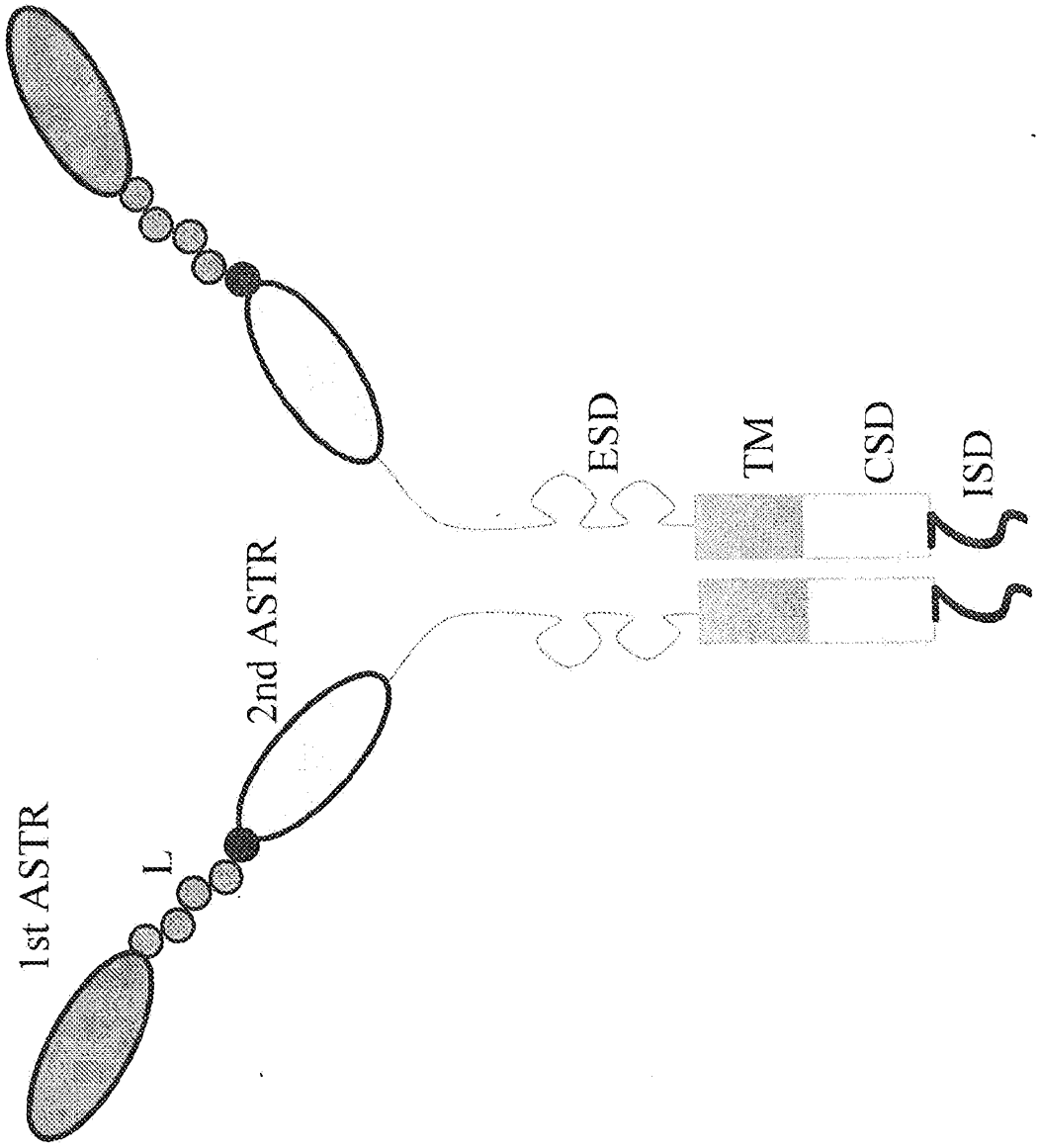

Figure 2

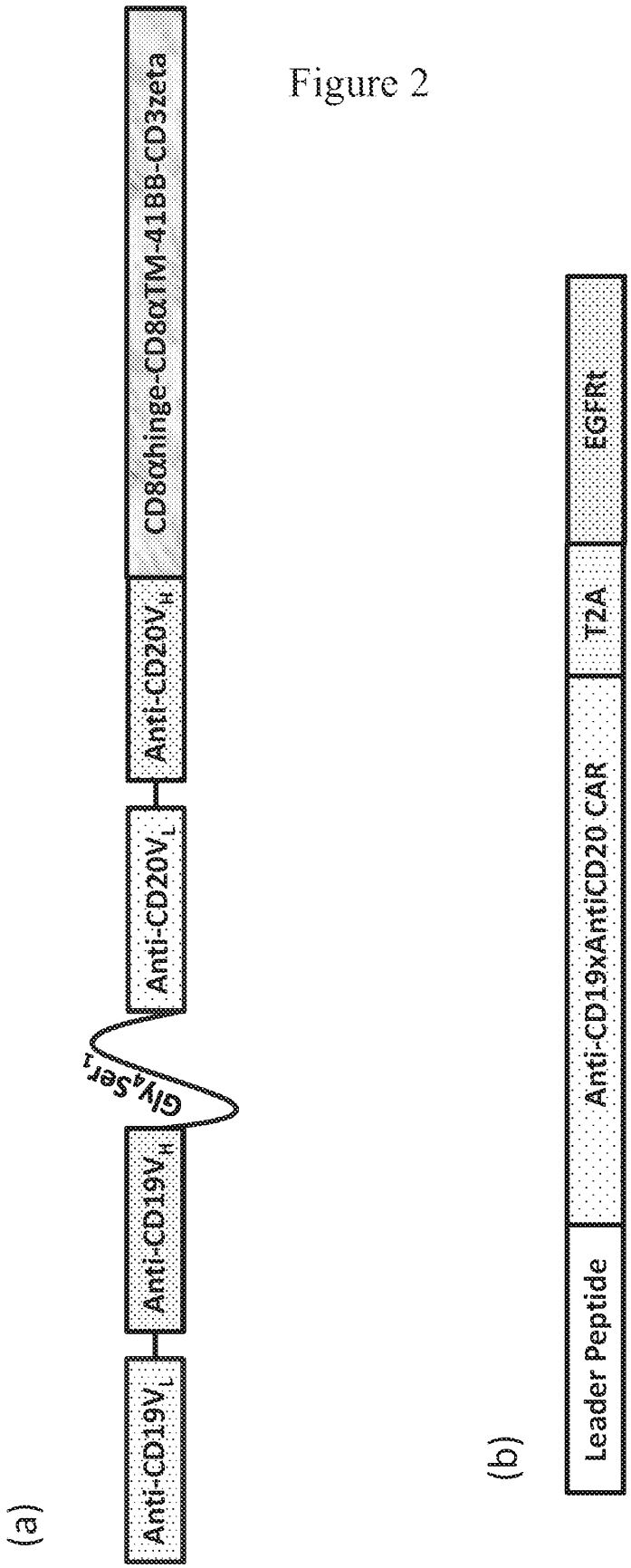
(a)
Anti-CD19V$_L$
Anti-CD19V$_H$
Gly$_4$Ser$_1$
Anti-CD20V$_L$
Anti-CD20V$_H$
CD8αhinge-CD8αTM-41BB-CD3zeta
(b)
Leader Peptide
Anti-CD19xAntiCD20 CAR
T2A
EGFRt

Figure 3

GMCSFRs.s CD19scFv-Gly4Ser1linker-CD20scFv-IgG4Hinge-CD28tm-41BB-CD3zeta-T2A-EGFRt_epHIV7

atgctgctgctggtgaccagcctgctgctgtgcgagctgccccacccccgcctttctgctgatccccatgacccagaccacctccagcctgagcgccagc
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Figure 4

GMCSFRs.s CD19scFv-Gly4Ser1linker-CD20scFv-IgG4Hinge-CD28tm-41BB-CD3zeta-T2A-EGFRt_epHIV7

```
DNA: ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAGCTGCCCCACCCCGCC
 AA: M  L  L  L  V  T  S  L  L  L  C  E  L  P  H  P  A
DNA: TTTCTGCTGATCCCCATGACCCAGACCACCTCCAGCCTGAGCGCCAGCCTG
 AA: F  L  L  I  P  M  T  Q  T  T  S  S  L  S  A  S  L
DNA: GGCGACCGGGTGACCATCAGCTGCCGGGCCAGCCAGGACATCAGCAAGTAC
 AA: G  D  R  V  T  I  S  C  R  A  S  Q  D  I  S  K  Y
DNA: CTGAACTGGTATCAGCAGAAGCCCGACGGCACCGTCAAGCTGCTGATCTAC
 AA: L  N  W  Y  Q  Q  K  P  D  G  T  V  K  L  L  I  Y
DNA: CACACCAGCCGGCTGCACAGCGGCGTGCCCAGCCGGTTTAGCGGCAGCGGC
 AA: H  T  S  R  L  H  S  G  V  P  S  R  F  S  G  S  G
DNA: TCCGGCACCGACTACAGCCTGACCATCTCCAACCTGGAACAGGAAGATATC
 AA: S  G  T  D  Y  S  L  T  I  S  N  L  E  Q  E  D  I
DNA: GCCACCTACTTTTGCCAGCAGGGCAACACACTGCCCTACACCTTTGGCGGC
 AA: A  T  Y  F  C  Q  Q  G  N  T  L  P  Y  T  F  G  G
DNA: GGAACAAAGCTGGAAATCACCGGCAGCACCTCCGGCAGCGGCAAGCCTGGC
 AA: G  T  K  L  E  I  T  G  S  T  S  G  S  G  K  P  G
DNA: AGCGGCGAGGGCAGCACCAAGGGCGAGGTGAAGCTGCAGGAAAGCGGCCCT
 AA: S  G  E  G  S  T  K  G  E  V  K  L  Q  E  S  G  P
DNA: GGCCTGGTGGCCCCCAGCCAGAGCCTGAGCGTGACCTGCACCGTGAGCGGC
 AA: G  L  V  A  P  S  Q  S  L  S  V  T  C  T  V  S  G
DNA: GTGAGCCTGCCCGACTACGGCGTGAGCTGGATCCGGCAGCCCCCCAGGAAG
 AA: V  S  L  P  D  Y  G  V  S  W  I  R  Q  P  P  R  K
DNA: GGCCTGGAATGGCTGGGCGTGATCTGGGGCAGCGAGACCACCTACTACAAC
 AA: G  L  E  W  L  G  V  I  W  G  S  E  T  T  Y  Y  N
DNA: AGCGCCCTGAAGAGCCGGCTGACCATCATCAAGGACAACAGCAAGAGCCAG
 AA: S  A  L  K  S  R  L  T  I  I  K  D  N  S  K  S  Q
DNA: GTGTTCCTGAAGATGAACAGCCTGCAGACCGACGACACCGCCATCTACTAC
 AA: V  F  L  K  M  N  S  L  Q  T  D  D  T  A  I  Y  Y
DNA: TGCGCCAAGCACTACTACTACGGCGGCAGCTACGCCATGGACTACTGGGGC
 AA: C  A  K  H  Y  Y  Y  G  G  S  Y  A  M  D  Y  W  G
DNA: CAGGGCACCAGCGTGACCGTGAGCAGCGGAGGTGGTGGATCCGAGGTGCAG
 AA: Q  G  T  S  V  T  V  S  S  G  G  G  G  S  E  V  Q
DNA: CTGCAGCAGTCTGGGGCTGAGCTGGTGAAGCCTGGGGCCTCAGTGAAGATG
 AA: L  Q  Q  S  G  A  E  L  V  K  P  G  A  S  V  K  M
DNA: TCCTGCAAGGCTTCTGGCTACACATTTACCAGTTACAATATGCACTGGGTA
 AA: S  C  K  A  S  G  Y  T  F  T  S  Y  N  M  H  W  V
DNA: AAGCAGACACCTGGACAGGGCCTGGAATGGATTGGAGCTATTTATCCAGGA
 AA: K  Q  T  P  G  Q  G  L  E  W  I  G  A  I  Y  P  G
DNA: AATGGTGATACTTCCTACAATCAGAAGTTCAAAGGCAAGGCCACATTGACT
 AA: N  G  D  T  S  Y  N  Q  K  F  K  G  K  A  T  L  T
DNA: GCAGACAAATCCTCCAGCACAGCCTACATGCAGCTCAGCAGCCTGACATCT
 AA: A  D  K  S  S  S  T  A  Y  M  Q  L  S  S  L  T  S
DNA: GAGGACTCTGCGGACTATTACTGTGCAAGATCTAATTATTACGGTAGTAGC
 AA: E  D  S  A  D  Y  Y  C  A  R  S  N  Y  Y  G  S  S
DNA: TACTGGTTCTTCGATGTCTGGGGCGCAGGGACCACGGTCACCGTCTCCTCA
 AA: Y  W  F  F  D  V  W  G  A  G  T  T  V  T  V  S  S
DNA: GGCAGTACTAGCGGTGGTGGCTCCGGGGGCGGTTCCGGTGGGGGCGGCAGC
 AA: G  S  T  S  G  G  G  S  G  G  G  S  G  G  G  G  S
DNA: AGCGACATTGTGCTGACCCAATCTCCAGCTATCCTGTCTGCATCTCCAGGG
 AA: S  D  I  V  L  T  Q  S  P  A  I  L  S  A  S  P  G
DNA: GAGAAGGTCACAATGACTTGCAGGGCCAGCTCAAGTGTAAATTACATGGAC
 AA: E  K  V  T  M  T  C  R  A  S  S  S  V  N  Y  M  D
```

```
DNA: TGGTACCAGAAGAAGCCAGGATCCTCCCCCAAACCCTGGATTTATGCCACA
 AA: W  Y  Q  K  K  P  G  S  S  P  K  P  W  I  Y  A  T
DNA: TCCAACCTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGG
 AA: S  N  L  A  S  G  V  P  A  R  F  S  G  S  G  S  G
DNA: ACCTCTTACTCTCTCACAATCAGCAGAGTGGAGGCTGAAGATGCTGCCACT
 AA: T  S  Y  S  L  T  I  S  R  V  E  A  E  D  A  A  T
DNA: TATTACTGCCAGCAGTGGAGTTTTAATCCACCCACGTTCGGAGGGGGGACC
 AA: Y  Y  C  Q  Q  W  S  F  N  P  P  T  F  G  G  G  T
DNA: AAGCTGGAAATAAAAGAGAGCAAGTACGGACCGCCCTGCCCCCCTTGCCCT
 AA: K  L  E  I  K  E  S  K  Y  G  P  P  C  P  P  C  P
DNA: ATGTTCTGGGTGCTGGTGGTGGTCGGAGGCGTGCTGGCCTGCTACAGCCTG
 AA: M  F  W  V  L  V  V  V  G  G  V  L  A  C  Y  S  L
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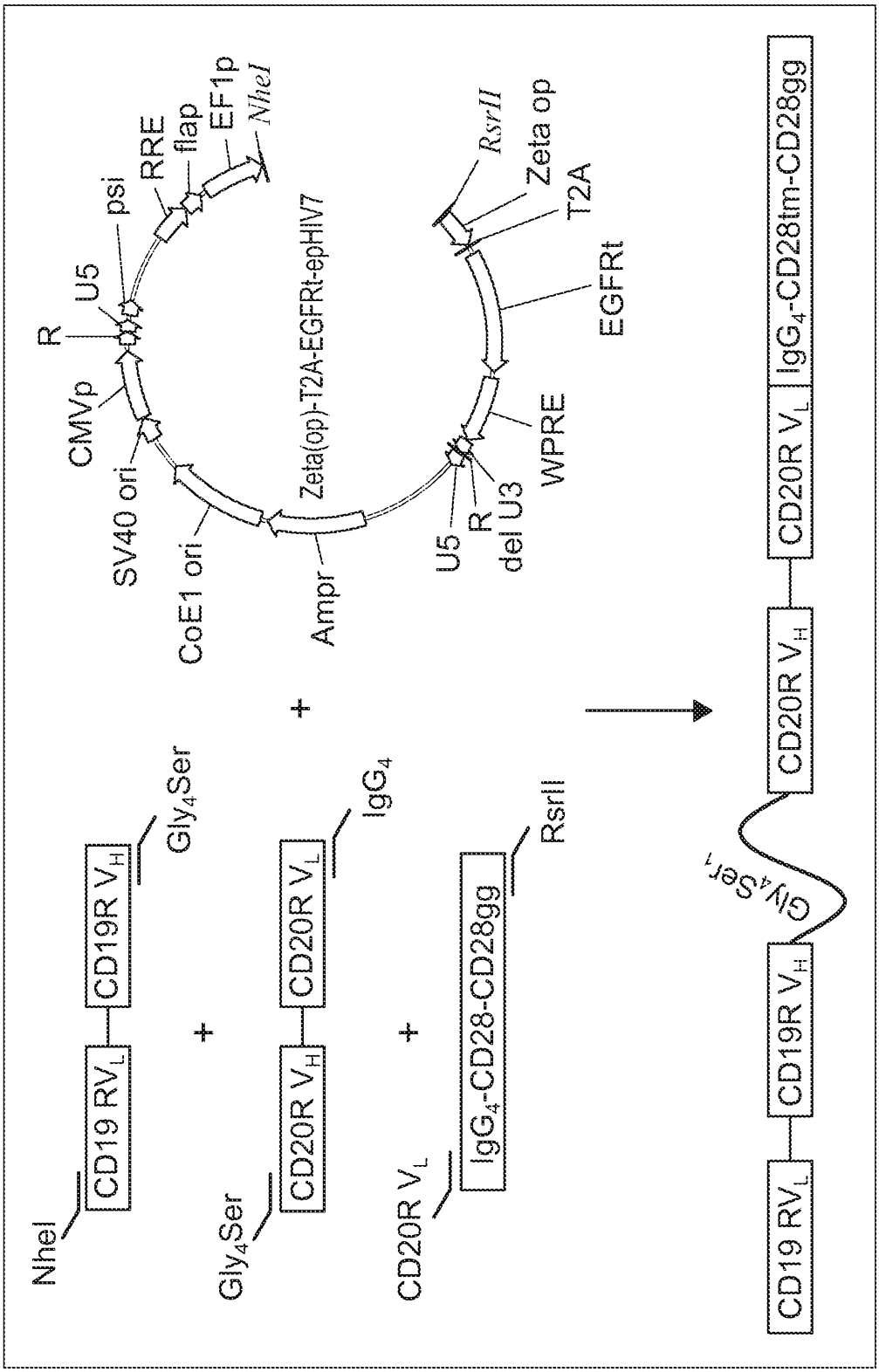
DNA: CTCCTGTATATATTCAAACAACCATTTATGAGACCAGTACAAACTACTCAA
 AA: L  L  Y  I  F  K  Q  P  F  M  R  P  V  Q  T  T  Q
DNA: GAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGT
 AA: E  E  D  G  C  S  C  R  F  P  E  E  E  E  G  G  C
DNA: GAACTGCGGGTGAAGTTCAGCAGAAGCGCCGACGCCCCTGCCTACCAGCAG
 AA: E  L  R  V  K  F  S  R  S  A  D  A  P  A  Y  Q  Q
DNA: GGCCAGAATCAGCTGTACAACGAGCTGAACCTGGGCAGAAGGGAAGAGTAC
 AA: G  Q  N  Q  L  Y  N  E  L  N  L  G  R  R  E  E  Y
DNA: GACGTCCTGGATAAGCGGAGAGGCCGGGACCCTGAGATGGGCGGCAAGCCT
 AA: D  V  L  D  K  R  R  G  R  D  P  E  M  G  G  K  P
DNA: CGGCGGAAGAACCCCCAGGAAGGCCTGTATAACGAACTGCAGAAAGACAAG
 AA: R  R  K  N  P  Q  E  G  L  Y  N  E  L  Q  K  D  K
DNA: ATGGCCGAGGCCTACAGCGAGATCGGCATGAAGGGCGAGCGGAGGCGGGGC
 AA: M  A  E  A  Y  S  E  I  G  M  K  G  E  R  R  R  G
DNA: AAGGGCCACGACGGCCTGTATCAGGGCCTGTCCACCGCCACCAAGGATACC
 AA: K  G  H  D  G  L  Y  Q  G  L  S  T  A  T  K  D  T
DNA: TACGACGCCCTGCACATGCAGGCCCTGCCCCCAAGGCTCGAGGGCGGCGGA
 AA: Y  D  A  L  H  M  Q  A  L  P  P  R  L  E  G  G  G
DNA: GAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAGAATCCCGGC
 AA: E  G  R  G  S  L  L  T  C  G  D  V  E  E  N  P  G
DNA: CCTAGGATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACAC
 AA: P  R  M  L  L  L  V  T  S  L  L  L  C  E  L  P  H
DNA: CCAGCATTCCTCCTGATCCCACGCAAAGTGTGTAACGGAATAGGTATTGGT
 AA: P  A  F  L  L  I  P  R  K  V  C  N  G  I  G  I  G
DNA: GAATTTAAAGACTCACTCTCCATAAATGCTACGAATATTAAACACTTCAAA
 AA: E  F  K  D  S  L  S  I  N  A  T  N  I  K  H  F  K
DNA: AACTGCACCTCCATCAGTGGCGATCTCCACATCCTGCCGGTGGCATTTAGG
 AA: N  C  T  S  I  S  G  D  L  H  I  L  P  V  A  F  R
DNA: GGTGACTCCTTCACACATACTCCTCCTCTGGATCCACAGGAACTGGATATT
 AA: G  D  S  F  T  H  T  P  P  L  D  P  Q  E  L  D  I
DNA: CTGAAAACCGTAAAGGAAATCACAGGGTTTTTGCTGATTCAGGCTTGGCCT
 AA: L  K  T  V  K  E  I  T  G  F  L  L  I  Q  A  W  P
DNA: GAAAACAGGACGGACCTCCATGCCTTTGAGAACCTAGAAATCATACGCGGC
 AA: E  N  R  T  D  L  H  A  F  E  N  L  E  I  I  R  G
DNA: AGGACCAAGCAACATGGTCAGTTTTCTCTTGCAGTCGTCAGCCTGAACATA
 AA: R  T  K  Q  H  G  Q  F  S  L  A  V  V  S  L  N  I
DNA: ACATCCTTGGGATTACGCTCCCTCAAGGAGATAAGTGATGGAGATGTGATA
 AA: T  S  L  G  L  R  S  L  K  E  I  S  D  G  D  V  I
DNA: ATTTCAGGAAACAAAAATTTGTGCTATGCAAATACAATAAACTGGAAAAAA
 AA: I  S  G  N  K  N  L  C  Y  A  N  T  I  N  W  K  K
DNA: CTGTTTGGGACCTCCGGTCAGAAAACCAAAATTATAAGCAACAGAGGTGAA
 AA: L  F  G  T  S  G  Q  K  T  K  I  I  S  N  R  G  E
```

```
DNA: AACAGCTGCAAGGCCACAGGCCAGGTCTGCCATGCCTTGTGCTCCCCCGAG
 AA: N  S  C  K  A  T  G  Q  V  C  H  A  L  C  S  P  E
DNA: GGCTGCTGGGGCCCGGAGCCCAGGGACTGCGTCTCTTGCCGGAATGTCAGC
 AA: G  C  W  G  P  E  P  R  D  C  V  S  C  R  N  V  S
DNA: CGAGGCAGGGAATGCGTGGACAAGTGCAACCTTCTGGAGGGTGAGCCAAGG
 AA: R  G  R  E  C  V  D  K  C  N  L  L  E  G  E  P  R
DNA: GAGTTTGTGGAGAACTCTGAGTGCATACAGTGCCACCCAGAGTGCCTGCCT
 AA: E  F  V  E  N  S  E  C  I  Q  C  H  P  E  C  L  P
DNA: CAGGCCATGAACATCACCTGCACAGGACGGGGACCAGACAACTGTATCCAG
 AA: Q  A  M  N  I  T  C  T  G  R  G  P  D  N  C  I  Q
DNA: TGTGCCCACTACATTGACGGCCCCCACTGCGTCAAGACCTGCCCGGCAGGA
 AA: C  A  H  Y  I  D  G  P  H  C  V  K  T  C  P  A  G
DNA: GTCATGGGAGAAAACAACACCCTGGTCTGGAAGTACGCAGACGCCGGCCAT
 AA: V  M  G  E  N  N  T  L  V  W  K  Y  A  D  A  G  H
DNA: GTGTGCCACCTGTGCCATCCAAACTGCACCTACGGATGCACTGGGCCAGGT
 AA: V  C  H  L  C  H  P  N  C  T  Y  G  C  T  G  P  G
DNA: CTTGAAGGCTGTCCAACGAATGGGCCTAAGATCCCGTCCATCGCCACTGGG
 AA: L  E  G  C  P  T  N  G  P  K  I  P  S  I  A  T  G
DNA: ATGGTGGGGGCCCTCCTCTTGCTGCTGGTGGTGGCCCTGGGGATCGGCCTC
 AA: M  V  G  A  L  L  L  L  L  V  V  A  L  G  I  G  L
DNA: TTCATGTGA
 AA: F  M  *
```

Figure 5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U5
psi
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Ampr
RsrII
Zeta op
T2A
EGFRt
U5
R
del U3
WPRE
NheI
CD19 RVL
CD19R VH
Gly4Ser
Gly4Ser
CD20R VH
CD20R VL
IgG4
CD20R VL
IgG4-CD28-CD28gg
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GlyxSer1
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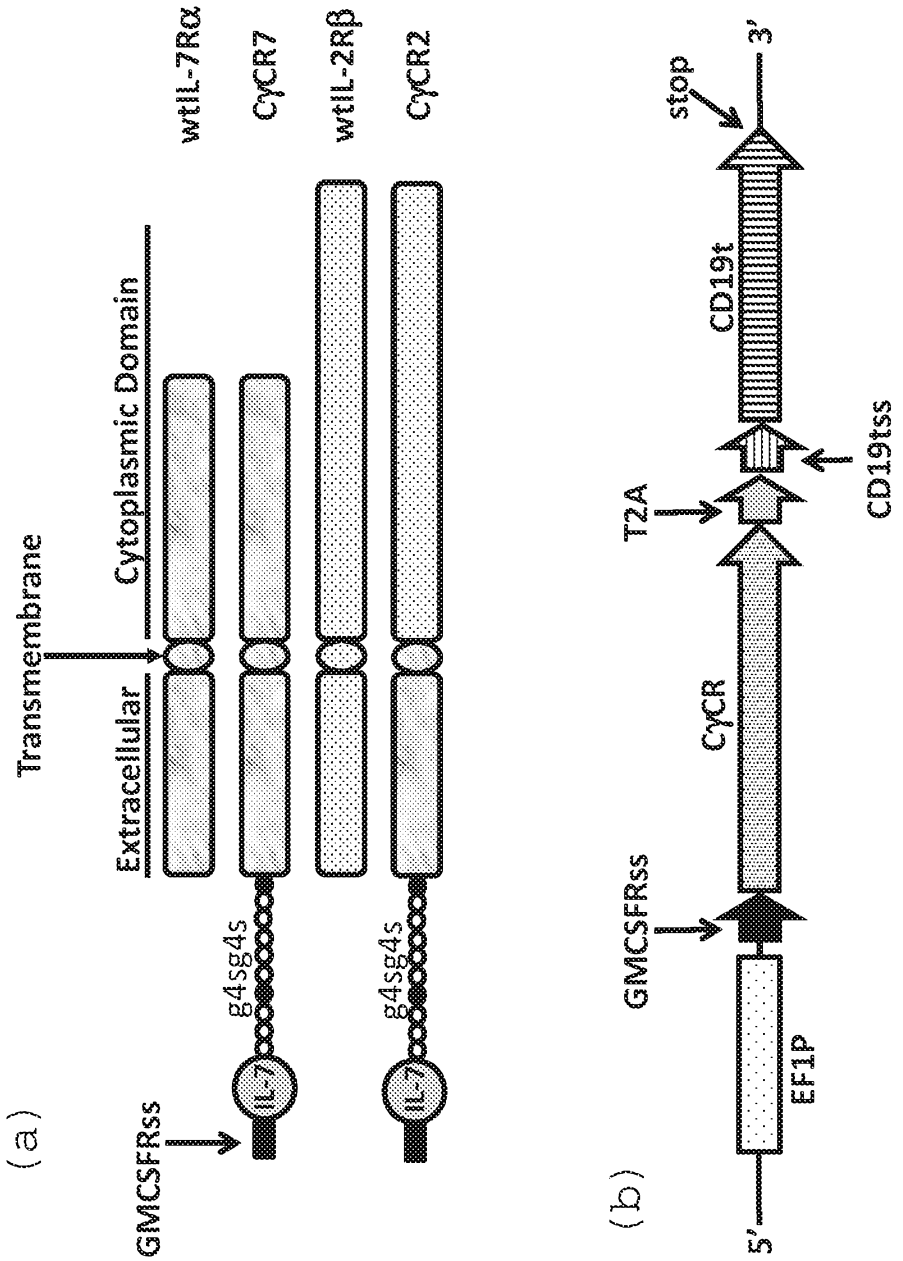
CD20R VL
IgG4-CD28tm-CD28gg
CD19 RVL
CD19R VH

Figure 6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g4sg4s
Transmembrane
Extracellular
Cytoplasmic Domain
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CγCR7
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CγCR2
(b)
5'
EF1P
GMCSFRss
CγCR
T2A
CD19tss
CD19t
stop
3'

Figure 7

IgG4hinge-CD28tm-41BB-CD3Zeta

gagagcaagtacggaccgccctgccccccttgccctatgttctgggtgctggtggtggtcggaggcgtgctggcctgctacagc
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ccaagg

DNA: GAGAGCAAGTACGGACCGCCCTGCCCCCCTTGCCCTATGTTCTGGGTGCTG
AA: E S K Y G P P C P P C P M F W V L

DNA: GTGGTGGTCGGAGGCGTGCTGGCCTGCTACAGCCTGCTGGTCACCGTGGCC
AA: V V V G G V L A C Y S L L V T V A

DNA: TTCATCATCTTTTGGGTGAAACGGGGCAGAAAGAAACTCCTGTATATATTC
AA: F I I F W V K R G R K K L L Y I F

DNA: AAACAACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGT
AA: K Q P F M R P V Q T T Q E E D G C

DNA: AGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGCGGGTGAAG
AA: S C R F P E E E E G G C E L R V K

DNA: TTCAGCAGAAGCGCCGACGCCCCTGCCTACCAGCAGGGCCAGAATCAGCTG
AA: F S R S A D A P A Y Q Q G Q N Q L

DNA: TACAACGAGCTGAACCTGGGCAGAAGGGAAGAGTACGACGTCCTGGATAAG
AA: Y N E L N L G R R E E Y D V L D K

DNA: CGGAGAGGCCGGGACCCTGAGATGGGCGGCAAGCCTCGGCGGAAGAACCCC
AA: R R G R D P E M G G K P R R K N P

DNA: CAGGAAGGCCTGTATAACGAACTGCAGAAAGACAAGATGGCCGAGGCCTAC
AA: Q E G L Y N E L Q K D K M A E A Y

DNA: AGCGAGATCGGCATGAAGGGCGAGCGGAGGCGGGGCAAGGGCCACGACGGC
AA: S E I G M K G E R R R G K G H D G

DNA: CTGTATCAGGGCCTGTCCACCGCCACCAAGGATACCTACGACGCCCTGCAC
AA: L Y Q G L S T A T K D T Y D A L H

DNA: ATGCAGGCCCTGCCCCCAAGG
AA: M Q A L P P R

Figure 8

GMCSFRss-CD19scFv-Gly4ser linker-CD20scFv-hulgG4hinge/CH2/CH3-CD28tm/CD28cyto-41BB-CD3Zeta

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acatgcaggccctgccccctaagg

Figure 9

GMCSFRss-CD19scFv-Gly4ser linker-CD20scFv-huIgG4hinge/CH2/CH3-CD28tm/CD28cyto-41BB-CD3Zeta

```
DNA: ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAGCTGCCCCACCCCGCC
 AA: M  L  L  L  V  T  S  L  L  L  C  E  L  P  H  P  A

DNA: TTTCTGCTGATCCCCGACATCCAGATGACCCAGACCACCTCCAGCCTGAGC
 AA: F  L  L  I  P  D  I  Q  M  T  Q  T  T  S  S  L  S

DNA: GCCAGCCTGGGCGACCGGGTGACCATCAGCTGCCGGGCCAGCCAGGACATC
 AA: A  S  L  G  D  R  V  T  I  S  C  R  A  S  Q  D  I

DNA: AGCAAGTACCTGAACTGGTATCAGCAGAAGCCCGACGGCACCGTCAAGCTG
 AA: S  K  Y  L  N  W  Y  Q  Q  K  P  D  G  T  V  K  L

DNA: CTGATCTACCACACCAGCCGGCTGCACAGCGGCGTGCCCAGCCGGTTTAGC
 AA: L  I  Y  H  T  S  R  L  H  S  G  V  P  S  R  F  S

DNA: GGCAGCGGCTCCGGCACCGACTACAGCCTGACCATCTCCAACCTGGAACAG
 AA: G  S  G  S  G  T  D  Y  S  L  T  I  S  N  L  E  Q

DNA: GAAGATATCGCCACCTACTTTTGCCAGCAGGGCAACACACTGCCCTACACC
 AA: E  D  I  A  T  Y  F  C  Q  Q  G  N  T  L  P  Y  T

DNA: TTTGGCGGCGGAACAAAGCTGGAAATCACCGGCAGCACCTCCGGCAGCGGC
 AA: F  G  G  G  T  K  L  E  I  T  G  S  T  S  G  S  G

DNA: AAGCCTGGCAGCGGCGAGGGCAGCACCAAGGGCGAGGTGAAGCTGCAGGAA
 AA: K  P  G  S  G  E  G  S  T  K  G  E  V  K  L  Q  E

DNA: AGCGGCCCTGGCCTGGTGGCCCCCAGCCAGAGCCTGAGCGTGACCTGCACC
 AA: S  G  P  G  L  V  A  P  S  Q  S  L  S  V  T  C  T

DNA: GTGAGCGGCGTGAGCCTGCCCGACTACGGCGTGAGCTGGATCCGGCAGCCC
 AA: V  S  G  V  S  L  P  D  Y  G  V  S  W  I  R  Q  P

DNA: CCCAGGAAGGGCCTGGAATGGCTGGGCGTGATCTGGGGCAGCGAGACCACC
 AA: P  R  K  G  L  E  W  L  G  V  I  W  G  S  E  T  T

DNA: TACTACAACAGCGCCCTGAAGAGCCGGCTGACCATCATCAAGGACAACAGC
 AA: Y  Y  N  S  A  L  K  S  R  L  T  I  I  K  D  N  S

DNA: AAGAGCCAGGTGTTCCTGAAGATGAACAGCCTGCAGACCGACGACACCGCC
 AA: K  S  Q  V  F  L  K  M  N  S  L  Q  T  D  D  T  A

DNA: ATCTACTACTGCGCCAAGCACTACTACTACGGCGGCAGCTACGCCATGGAC
 AA: I  Y  Y  C  A  K  H  Y  Y  Y  G  G  S  Y  A  M  D

DNA: TACTGGGGCCAGGGCACCAGCGTGACCGTGAGCAGCGGAGGTGGTGGATCC
 AA: Y  W  G  Q  G  T  S  V  T  V  S  S  G  G  G  G  S

DNA: GAGGTGCAGCTGCAGCAGTCTGGGGCTGAGCTGGTGAAGCCTGGGGCCTCA
 AA: E  V  Q  L  Q  Q  S  G  A  E  L  V  K  P  G  A  S

DNA: GTGAAGATGTCCTGCAAGGCTTCTGGCTACACATTTACCAGTTACAATATG
 AA: V  K  M  S  C  K  A  S  G  Y  T  F  T  S  Y  N  M
```

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DNA: CACTGGGTAAAGCAGACACCTGGACAGGGCCTGGAATGGATTGGAGCTATT
 AA: H  W  V  K  Q  T  P  G  Q  G  L  E  W  I  G  A  I

DNA: TATCCAGGAAATGGTGATACTTCCTACAATCAGAAGTTCAAAGGCAAGGCC
 AA: Y  P  G  N  G  D  T  S  Y  N  Q  K  F  K  G  K  A

DNA: ACATTGACTGCAGACAAATCCTCCAGCACAGCCTACATGCAGCTCAGCAGC
 AA: T  L  T  A  D  K  S  S  S  T  A  Y  M  Q  L  S  S

DNA: CTGACATCTGAGGACTCTGCGGACTATTACTGTGCAAGATCTAATTATTAC
 AA: L  T  S  E  D  S  A  D  Y  Y  C  A  R  S  N  Y  Y

DNA: GGTAGTAGCTACTGGTTCTTCGATGTCTGGGGCGCAGGGACCACGGTCACC
 AA: G  S  S  Y  W  F  F  D  V  W  G  A  G  T  T  V  T

DNA: GTCTCCTCAGGCAGTACTAGCGGTGGTGGCTCCGGGGGCGGTTCCGGTGGG
 AA: V  S  S  G  S  T  S  G  G  G  S  G  G  G  S  G  G

DNA: GGCGGCAGCAGCGACATTGTGCTGACCCAATCTCCAGCTATCCTGTCTGCA
 AA: G  G  S  S  D  I  V  L  T  Q  S  P  A  I  L  S  A

DNA: TCTCCAGGGGAGAAGGTCACAATGACTTGCAGGGCCAGCTCAAGTGTAAAT
 AA: S  P  G  E  K  V  T  M  T  C  R  A  S  S  S  V  N

DNA: TACATGGACTGGTACCAGAAGAAGCCAGGATCCTCCCCCAAACCCTGGATT
 AA: Y  M  D  W  Y  Q  K  K  P  G  S  S  P  K  P  W  I

DNA: TATGCCACATCCAACCTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGT
 AA: Y  A  T  S  N  L  A  S  G  V  P  A  R  F  S  G  S

DNA: GGGTCTGGGACCTCTTACTCTCTCACAATCAGCAGAGTGGAGGCTGAAGAT
 AA: G  S  G  T  S  Y  S  L  T  I  S  R  V  E  A  E  D

DNA: GCTGCCACTTATTACTGCCAGCAGTGGAGTTTTAATCCACCCACGTTCGGA
 AA: A  A  T  Y  Y  C  Q  Q  W  S  F  N  P  P  T  F  G

DNA: GGGGGGACCAAGCTGGAAATAAAAGAGAGCAAGTACGGACCGCCCTGCCCC
 AA: G  G  T  K  L  E  I  K  E  S  K  Y  G  P  P  C  P

DNA: CCTTGCCCTGCCCCCGAGTTCCTGGGCGGACCCAGCGTGTTCCTGTTCCCC
 AA: P  C  P  A  P  E  F  L  G  G  P  S  V  F  L  F  P

DNA: CCCAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCCGAGGTGACCTGC
 AA: P  K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C

DNA: GTGGTGGTGGACGTGAGCCAGGAAGATCCCGAGGTCCAGTTCAATTGGTAC
 AA: V  V  V  D  V  S  Q  E  D  P  E  V  Q  F  N  W  Y

DNA: GTGGACGGCGTGGAAGTGCACAACGCCAAGACCAAGCCCAGAGAGGAACAG
 AA: V  D  G  V  E  V  H  N  A  K  T  K  P  R  E  E  Q

DNA: TTCAACAGCACCTACCGGGTGGTGTCTGTGCTGACCGTGCTGCACCAGGAC
 AA: F  N  S  T  Y  R  V  V  S  V  L  T  V  L  H  Q  D

DNA: TGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCCAACAAGGGCCTGCCC
 AA: W  L  N  G  K  E  Y  K  C  K  V  S  N  K  G  L  P
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DNA: AGCAGCATCGAAAAGACCATCAGCAAGGCCAAGGGCCAGCCTCGCGAGCCC
AA: S S I E K T I S K A K G Q P R E P

DNA: CAGGTGTACACCCTGCCTCCCTCCCAGGAAGAGATGACCAAGAACCAGGTG
AA: Q V Y T L P P S Q E E M T K N Q V

DNA: TCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAG
AA: S L T C L V K G F Y P S D I A V E

DNA: TGGGAGAGCAACGGCCAGCCTGAGAACAACTACAAGACCACCCCTCCCGTG
AA: W E S N G Q P E N N Y K T T P P V

DNA: CTGGACAGCGACGGCAGCTTCTTCCTGTACAGCCGGCTGACCGTGGACAAG
AA: L D S D G S F F L Y S R L T V D K

DNA: AGCCGGTGGCAGGAAGGCAACGTCTTTAGCTGCAGCGTGATGCACGAGGCC
AA: S R W Q E G N V F S C S V M H E A

DNA: CTGCACAACCACTACACCCAGAAGAGCCTGAGCCTGTCCCTGGGCAAGATG
AA: L H N H Y T Q K S L S L S L G K M

DNA: TTCTGGGTGCTGGTGGTGGTGGGCGGGGTGCTGGCCTGCTACAGCCTGCTG
AA: F W V L V V V G G V L A C Y S L L

DNA: GTGACAGTGGCCTTCATCATCTTTTGGGTGCGGAGCAAGCGGAGCAGAGGC
AA: V T V A F I I F W V R S K R S R G

DNA: GGCCACAGCGACTACATGAACATGACCCCCAGACGGCCTGGCCCCACCCGG
AA: G H S D Y M N M T P R R P G P T R

DNA: AAGCACTACCAGCCCTACGCCCCACCCAGGGACTTTGCCGCCTACAGAAGC
AA: K H Y Q P Y A P P R D F A A Y R S

DNA: AAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAACCATTTATGAGA
AA: K R G R K K L L Y I F K Q P F M R

DNA: CCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAA
AA: P V Q T T Q E E D G C S C R F P E

DNA: GAAGAAGAAGGAGGATGTGAACTGCGGGTGAAGTTCAGCAGAAGCGCCGAC
AA: E E E G G C E L R V K F S R S A D

DNA: GCCCCTGCCTACCAGCAGGGCCAGAATCAGCTGTACAACGAGCTGAACCTG
AA: A P A Y Q Q G Q N Q L Y N E L N L

DNA: GGCAGAAGGGAAGAGTACGACGTCCTGGATAAGCGGAGAGGCCGGGACCCT
AA: G R R E E Y D V L D K R R G R D P

DNA: GAGATGGGCGGCAAGCCTCGGCGGAAGAACCCCCAGGAAGGCCTGTATAAC
AA: E M G G K P R R K N P Q E G L Y N

DNA: GAACTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATCGGCATGAAG
AA: E L Q K D K M A E A Y S E I G M K

DNA: GGCGAGCGGAGGCGGGGCAAGGGCCACGACGGCCTGTATCAGGGCCTGTCC
AA: G E R R R G K G H D G L Y Q G L S

DNA: ACCGCCACCAAGGATACCTACGACGCCCTGCACATGCAGGCCCTGCCCCCA
AA: T A T K D T Y D A L H M Q A L P P

DNA: AGG
AA: R

Figure 10

GMCSFRss-CD19scFv-Gly4ser linker-CD20scFv-CD8alphaHinge-CD8alphaTM-41BB-CD3Zeta-T2A-EGFRt

atgctgctgctggtgaccagcctgctgctgtgcgagctgccccaccccgcctttctgctgatccccgacatccagatgacccaga
ccacctccagcctgagcgccagcctgggcgaccgggtgaccatcagctgccgggccagccaggacatcagcaagtacctga
actggtatcagcagaagccccgacggcaccgtcaagctgctgatctaccacaccagccggctgcacagcggcgtgccccagcc
ggtttagcggcagcggctccggcaccgactacagcctgaccatctccaacctggaacaggaagatatcgccacctacttttgcc
agcagggcaacacactgccctacaccttttggcggcggaacaaagctggaaatcaccggcagcacctccggcagcggcaagc
ctggcagcggcgagggcagcaccaagggcgaggtgaagctgcaggaaagcggccctggcctggtggccccccagccagag
cctgagcgtgacctgcaccgtgagcggcgtgagcctgccccgactacggcgtgagctggatccggcagccccccaggaaggg
cctggaatggctgggcgtgatctggggcagcgagaccacctactacaacagcgccctgaagagccggctgaccatcatcaag
gacaacagcaagagccaggtgttcctgaagatgaacagcctgcagaccgacgacaccgccatctactactgcgccaagcact
actactacggcggcagctacgccatggactactggggccagggcaccagcgtgaccgtgagcagcggaggtggtggatccg
aggtgcagctgcagcagtctggggctgagctggtgaagcctggggcctcagtgaagatgtcctgcaaggcttctggctacacat
ttaccagttacaatatgcactgggtaaagcagacacctggacagggcctggaatggattggagctatttatccaggaaatggtga
tacttcctacaatcagaagttcaaaggcaaggccacattgactgcagacaaatcctccagcacagcctacatgcagctcagcag
cctgacatctgaggactctgcggactattactgtgcaagatctaattattacggtagtagctactggttcttcgatgtctggggcgca
gggaccacggtcaccgtctcctcaggcagtactagcggtggtggctccggggggcggttccggtgggggcggcagcagcgac
attgtgctgacccaatctccagctatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaa
attacatggactggtaccagaagaagccaggatcctccccccaaaccctggatttatgccacatccaacctggcttctggagtccct
gctcgcttcagtggcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactg
ccagcagtggagttttaatccacccacgttcggaggggggaccaagctggaaataaaagagagcaagtacggaccgccctgc
ccccctttgccctaagcctaccaccaccccctgcccctagacctccaacaccccgccccaacaatcgccagccagcctctgtctctg
aggccccgaggcttgtagaccagctgctggcggagccgtgcacaccagaggactggatttcgcctgcgacatctacatctgggc
ccctctggccggcacatgtggcgtgctgctgctgagcctcgtgatcaccaagcgggggcagaaagaaactgctgtacatctttaa
gcagccccttcatgcggccccgtgcagaccacccaggaagaggacggctgctcctgcagatttccccgaggaagaagaaggcgg
ctgcgagctgagagtgaagttcagcagatccgccgacgcccctgcctaccagcagggacagaaccagctgtacaacgagctg
aacctgggcagacgggaagagtacgacgtgctggacaagcggagaggccgggaccctgagatgggcggaaagccccagaa
gaaagaaccccccaggaaggcctgtataacgaactgcagaaagacaagatggccgaggcctacagcgagatcggaatgaag
ggcgagcggagaagaggcaagggccacgatggcctgtaccagggcctgagcaccgccaccaaggacacctatgacgccct
gcacatgcaggccctgcctccaagactcgagggcggcggagagggcagaggaagtcttctaacatgcggtgacgtggagga
gaatccccggccctaggatgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatcccacgca
aagtgtgtaacggaataggtattggtgaatttaaagactcactctccataaatgctacgaatattaaacacttcaaaaactgcacctc
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aatgcgtggacaagtgcaacctctggagggtgagccaagggagtttgtggagaactctgagtgcatacagtgccacccagag
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ccactgcgtcaagacctgcccggcaggagtcatgggagaaaacaacaccctggtctggaagtacgcagacgccggccatgtg
tgccacctgtgccatccaaactgcacctacggatgcactgggccaggtcttgaaggctgtccaacgaatgggcctaagatcccg
tccatcgccactgggatggtgggggccctcctcttgctgctggtggtggccctggggatcggcctcttcatgtga

Figure 11

GMCSFRss-CD19scFv-Gly4ser linker-CD20scFv-CD8alphaHinge-CD8alphaTM-41BB-CD3Zeta-T2A-EGFRt

```
DNA: ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAGCTGCCCCACCCCGCC
 AA: M  L  L  L  V  T  S  L  L  L  C  E  L  P  H  P  A

DNA: TTTCTGCTGATCCCCGACATCCAGATGACCCAGACCACCTCCAGCCTGAGC
 AA: F  L  L  I  P  D  I  Q  M  T  Q  T  T  S  S  L  S

DNA: GCCAGCCTGGGCGACCGGGTGACCATCAGCTGCCGGGCCAGCCAGGACATC
 AA: A  S  L  G  D  R  V  T  I  S  C  R  A  S  Q  D  I

DNA: AGCAAGTACCTGAACTGGTATCAGCAGAAGCCCGACGGCACCGTCAAGCTG
 AA: S  K  Y  L  N  W  Y  Q  Q  K  P  D  G  T  V  K  L

DNA: CTGATCTACCACACCAGCCGGCTGCACAGCGGCGTGCCCAGCCGGTTTAGC
 AA: L  I  Y  H  T  S  R  L  H  S  G  V  P  S  R  F  S

DNA: GGCAGCGGCTCCGGCACCGACTACAGCCTGACCATCTCCAACCTGGAACAG
 AA: G  S  G  S  G  T  D  Y  S  L  T  I  S  N  L  E  Q

DNA: GAAGATATCGCCACCTACTTTTGCCAGCAGGGCAACACACTGCCCTACACC
 AA: E  D  I  A  T  Y  F  C  Q  Q  G  N  T  L  P  Y  T

DNA: TTTGGCGGCGGAACAAAGCTGGAAATCACCGGCAGCACCTCCGGCAGCGGC
 AA: F  G  G  G  T  K  L  E  I  T  G  S  T  S  G  S  G

DNA: AAGCCTGGCAGCGGCGAGGGCAGCACCAAGGGCGAGGTGAAGCTGCAGGAA
 AA: K  P  G  S  G  E  G  S  T  K  G  E  V  K  L  Q  E

DNA: AGCGGCCCTGGCCTGGTGGCCCCCAGCCAGAGCCTGAGCGTGACCTGCACC
 AA: S  G  P  G  L  V  A  P  S  Q  S  L  S  V  T  C  T

DNA: GTGAGCGGCGTGAGCCTGCCCGACTACGGCGTGAGCTGGATCCGGCAGCCC
 AA: V  S  G  V  S  L  P  D  Y  G  V  S  W  I  R  Q  P

DNA: CCCAGGAAGGGCCTGGAATGGCTGGGCGTGATCTGGGGCAGCGAGACCACC
 AA: P  R  K  G  L  E  W  L  G  V  I  W  G  S  E  T  T

DNA: TACTACAACAGCGCCCTGAAGAGCCGGCTGACCATCATCAAGGACAACAGC
 AA: Y  Y  N  S  A  L  K  S  R  L  T  I  I  K  D  N  S

DNA: AAGAGCCAGGTGTTCCTGAAGATGAACAGCCTGCAGACCGACGACACCGCC
 AA: K  S  Q  V  F  L  K  M  N  S  L  Q  T  D  D  T  A

DNA: ATCTACTACTGCGCCAAGCACTACTACTACGGCGGCAGCTACGCCATGGAC
 AA: I  Y  Y  C  A  K  H  Y  Y  Y  G  G  S  Y  A  M  D

DNA: TACTGGGGCCAGGGCACCAGCGTGACCGTGAGCAGCGGAGGTGGTGGATCC
 AA: Y  W  G  Q  G  T  S  V  T  V  S  S  G  G  G  G  S

DNA: GAGGTGCAGCTGCAGCAGTCTGGGGCTGAGCTGGTGAAGCCTGGGGCCTCA
 AA: E  V  Q  L  Q  Q  S  G  A  E  L  V  K  P  G  A  S

DNA: GTGAAGATGTCCTGCAAGGCTTCTGGCTACACATTTACCAGTTACAATATG
 AA: V  K  M  S  C  K  A  S  G  Y  T  F  T  S  Y  N  M
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```
DNA: CACTGGGTAAAGCAGACACCTGGACAGGGCCTGGAATGGATTGGAGCTATT
 AA: H  W  V  K  Q  T  P  G  Q  G  L  E  W  I  G  A  I

DNA: TATCCAGGAAATGGTGATACTTCCTACAATCAGAAGTTCAAAGGCAAGGCC
 AA: Y  P  G  N  G  D  T  S  Y  N  Q  K  F  K  G  K  A

DNA: ACATTGACTGCAGACAAATCCTCCAGCACAGCCTACATGCAGCTCAGCAGC
 AA: T  L  T  A  D  K  S  S  S  T  A  Y  M  Q  L  S  S

DNA: CTGACATCTGAGGACTCTGCGGACTATTACTGTGCAAGATCTAATTATTAC
 AA: L  T  S  E  D  S  A  D  Y  Y  C  A  R  S  N  Y  Y

DNA: GGTAGTAGCTACTGGTTCTTCGATGTCTGGGGCGCAGGGACCACGGTCACC
 AA: G  S  S  Y  W  F  F  D  V  W  G  A  G  T  T  V  T

DNA: GTCTCCTCAGGCAGTACTAGCGGTGGTGGCTCCGGGGGCGGTTCCGGTGGG
 AA: V  S  S  G  S  T  S  G  G  G  S  G  G  G  S  G  G

DNA: GGCGGCAGCAGCGACATTGTGCTGACCCAATCTCCAGCTATCCTGTCTGCA
 AA: G  G  S  S  D  I  V  L  T  Q  S  P  A  I  L  S  A

DNA: TCTCCAGGGGAGAAGGTCACAATGACTTGCAGGGCCAGCTCAAGTGTAAAT
 AA: S  P  G  E  K  V  T  M  T  C  R  A  S  S  S  V  N

DNA: TACATGGACTGGTACCAGAAGAAGCCAGGATCCTCCCCCAAACCCTGGATT
 AA: Y  M  D  W  Y  Q  K  K  P  G  S  S  P  K  P  W  I

DNA: TATGCCACATCCAACCTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGT
 AA: Y  A  T  S  N  L  A  S  G  V  P  A  R  F  S  G  S

DNA: GGGTCTGGGACCTCTTACTCTCTCACAATCAGCAGAGTGGAGGCTGAAGAT
 AA: G  S  G  T  S  Y  S  L  T  I  S  R  V  E  A  E  D

DNA: GCTGCCACTTATTACTGCCAGCAGTGGAGTTTTAATCCACCCACGTTCGGA
 AA: A  A  T  Y  Y  C  Q  Q  W  S  F  N  P  P  T  F  G

DNA: GGGGGGACCAAGCTGGAAATAAAAGAGAGCAAGTACGGACCGCCCTGCCCC
 AA: G  G  T  K  L  E  I  K  E  S  K  Y  G  P  P  C  P

DNA: CCTTGCCCTAAGCCTACCACCACCCCCTGCCCCTAGACCTCCAACACCCGCC
 AA: P  C  P  K  P  T  T  T  P  A  P  R  P  P  T  P  A

DNA: CCAACAATCGCCAGCCAGCCTCTGTCTCTGAGGCCCGAGGCTTGTAGACCA
 AA: P  T  I  A  S  Q  P  L  S  L  R  P  E  A  C  R  P

DNA: GCTGCTGGCGGAGCCGTGCACACCAGAGGACTGGATTTCGCCTGCGACATC
 AA: A  A  G  G  A  V  H  T  R  G  L  D  F  A  C  D  I

DNA: TACATCTGGGCCCCTCTGGCCGGCACATGTGGCGTGCTGCTGCTGAGCCTC
 AA: Y  I  W  A  P  L  A  G  T  C  G  V  L  L  L  S  L

DNA: GTGATCACCAAGCGGGGCAGAAAGAAACTGCTGTACATCTTTAAGCAGCCC
 AA: V  I  T  K  R  G  R  K  K  L  L  Y  I  F  K  Q  P

DNA: TTCATGCGGCCCGTGCAGACCACCCAGGAAGAGGACGGCTGCTCCTGCAGA
 AA: F  M  R  P  V  Q  T  T  Q  E  E  D  G  C  S  C  R
```

DNA: TTCCCCGAGGAAGAAGAAGGCGGCTGCGAGCTGAGAGTGAAGTTCAGCAGA
AA: F P E E E E G G C E L R V K F S R

DNA: TCCGCCGACGCCCCTGCCTACCAGCAGGGACAGAACCAGCTGTACAACGAG
AA: S A D A P A Y Q Q G Q N Q L Y N E

DNA: CTGAACCTGGGCAGACGGGAAGAGTACGACGTGCTGGACAAGCGGAGAGGC
AA: L N L G R R E E Y D V L D K R R G

DNA: CGGGACCCTGAGATGGGCGGAAAGCCCAGAAGAAAGAACCCCCAGGAAGGC
AA: R D P E M G G K P R R K N P Q E G

DNA: CTGTATAACGAACTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATC
AA: L Y N E L Q K D K M A E A Y S E I

DNA: GGAATGAAGGGCGAGCGGAGAAGAGGCAAGGGCCACGATGGCCTGTACCAG
AA: G M K G E R R R G K G H D G L Y Q

DNA: GGCCTGAGCACCGCCACCAAGGACACCTATGACGCCCTGCACATGCAGGCC
AA: G L S T A T K D T Y D A L H M Q A

DNA: CTGCCTCCAAGACTCGAGGGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACA
AA: L P P R L E G G G E G R G S L L T

DNA: TGCGGTGACGTGGAGGAGAATCCCGGCCCTAGGATGCTTCTCCTGGTGACA
AA: C G D V E E N P G P R M L L L V T

DNA: AGCCTTCTGCTCTGTGAGTTACCACACCCAGCATTCCTCCTGATCCCACGC
AA: S L L L C E L P H P A F L L I P R

DNA: AAAGTGTGTAACGGAATAGGTATTGGTGAATTTAAAGACTCACTCTCCATA
AA: K V C N G I G I G E F K D S L S I

DNA: AATGCTACGAATATTAAACACTTCAAAAACTGCACCTCCATCAGTGGCGAT
AA: N A T N I K H F K N C T S I S G D

DNA: CTCCACATCCTGCCGGTGGCATTTAGGGGTGACTCCTTCACACATACTCCT
AA: L H I L P V A F R G D S F T H T P

DNA: CCTCTGGATCCACAGGAACTGGATATTCTGAAAACCGTAAAGGAAATCACA
AA: P L D P Q E L D I L K T V K E I T

DNA: GGGTTTTTGCTGATTCAGGCTTGGCCTGAAAACAGGACGGACCTCCATGCC
AA: G F L L I Q A W P E N R T D L H A

DNA: TTTGAGAACCTAGAAATCATACGCGGCAGGACCAAGCAACATGGTCAGTTT
AA: F E N L E I I R G R T K Q H G Q F

DNA: TCTCTTGCAGTCGTCAGCCTGAACATAACATCCTTGGGATTACGCTCCCTC
AA: S L A V V S L N I T S L G L R S L

DNA: AAGGAGATAAGTGATGGAGATGTGATAATTTCAGGAAACAAAAATTTGTGC
AA: K E I S D G D V I I S G N K N L C

DNA: TATGCAAATACAATAAACTGGAAAAAACTGTTTGGGACCTCCGGTCAGAAA
AA: Y A N T I N W K K L F G T S G Q K

```
DNA: ACCAAAATTATAAGCAACAGAGGTGAAAACAGCTGCAAGGCCACAGGCCAG
 AA: T  K  I  I  S  N  R  G  E  N  S  C  K  A  T  G  Q

DNA: GTCTGCCATGCCTTGTGCTCCCCCGAGGGCTGCTGGGGCCCGGAGCCCAGG
 AA: V  C  H  A  L  C  S  P  E  G  C  W  G  P  E  P  R

DNA: GACTGCGTCTCTTGCCGGAATGTCAGCCGAGGCAGGGAATGCGTGGACAAG
 AA: D  C  V  S  C  R  N  V  S  R  G  R  E  C  V  D  K

DNA: TGCAACCTTCTGGAGGGTGAGCCAAGGGAGTTTGTGGAGAACTCTGAGTGC
 AA: C  N  L  L  E  G  E  P  R  E  F  V  E  N  S  E  C

DNA: ATACAGTGCCACCCAGAGTGCCTGCCTCAGGCCATGAACATCACCTGCACA
 AA: I  Q  C  H  P  E  C  L  P  Q  A  M  N  I  T  C  T

DNA: GGACGGGGACCAGACAACTGTATCCAGTGTGCCCACTACATTGACGGCCCC
 AA: G  R  G  P  D  N  C  I  Q  C  A  H  Y  I  D  G  P

DNA: CACTGCGTCAAGACCTGCCCGGCAGGAGTCATGGGAGAAAACAACACCCTG
 AA: H  C  V  K  T  C  P  A  G  V  M  G  E  N  N  T  L

DNA: GTCTGGAAGTACGCAGACGCCGGCCATGTGTGCCACCTGTGCCATCCAAAC
 AA: V  W  K  Y  A  D  A  G  H  V  C  H  L  C  H  P  N

DNA: TGCACCTACGGATGCACTGGGCCAGGTCTTGAAGGCTGTCCAACGAATGGG
 AA: C  T  Y  G  C  T  G  P  G  L  E  G  C  P  T  N  G

DNA: CCTAAGATCCCGTCCATCGCCACTGGGATGGTGGGGGCCCTCCTCTTGCTG
 AA: P  K  I  P  S  I  A  T  G  M  V  G  A  L  L  L  L

DNA: CTGGTGGTGGCCCTGGGGATCGGCCTCTTCATGTGA
 AA: L  V  V  A  L  G  I  G  L  F  M  *
```

Figure 12

T2A-EGFRt

ctcgagggcggcggagagggcagaggaagtcttctaacatgcggtgacgtggaggagaatcccggccctaggatgcttctcc
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Figure 13

T2A-EGFRt

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DNA: CTCGAGGGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTG
 AA: L  E  G  G  G  E  G  R  G  S  L  L  T  C  G  D  V

DNA: GAGGAGAATCCCGGCCCTAGGATGCTTCTCCTGGTGACAAGCCTTCTGCTC
 AA: E  E  N  P  G  P  R  M  L  L  L  V  T  S  L  L  L

DNA: TGTGAGTTACCACACCCAGCATTCCTCCTGATCCCACGCAAAGTGTGTAAC
 AA: C  E  L  P  H  P  A  F  L  L  I  P  R  K  V  C  N

DNA: GGAATAGGTATTGGTGAATTTAAAGACTCACTCTCCATAAATGCTACGAAT
 AA: G  I  G  I  G  E  F  K  D  S  L  S  I  N  A  T  N

DNA: ATTAAACACTTCAAAAACTGCACCTCCATCAGTGGCGATCTCCACATCCTG
 AA: I  K  H  F  K  N  C  T  S  I  S  G  D  L  H  I  L

DNA: CCGGTGGCATTTAGGGGTGACTCCTTCACACATACTCCTCCTCTGGATCCA
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DNA: CAGGAACTGGATATTCTGAAAACCGTAAAGGAAATCACAGGGTTTTTGCTG
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DNA: GAAATCATACGCGGCAGGACCAAGCAACATGGTCAGTTTTCTCTTGCAGTC
 AA: E  I  I  R  G  R  T  K  Q  H  G  Q  F  S  L  A  V

DNA: GTCAGCCTGAACATAACATCCTTGGGATTACGCTCCCTCAAGGAGATAAGT
 AA: V  S  L  N  I  T  S  L  G  L  R  S  L  K  E  I  S

DNA: GATGGAGATGTGATAATTTCAGGAAACAAAAATTTGTGCTATGCAAATACA
 AA: D  G  D  V  I  I  S  G  N  K  N  L  C  Y  A  N  T

DNA: ATAAACTGGAAAAAACTGTTTGGGACCTCCGGTCAGAAAACCAAAATTATA
 AA: I  N  W  K  K  L  F  G  T  S  G  Q  K  T  K  I  I

DNA: AGCAACAGAGGTGAAAACAGCTGCAAGGCCACAGGCCAGGTCTGCCATGCC
 AA: S  N  R  G  E  N  S  C  K  A  T  G  Q  V  C  H  A

DNA: TTGTGCTCCCCCGAGGGCTGCTGGGGCCCGGAGCCCAGGGACTGCGTCTCT
 AA: L  C  S  P  E  G  C  W  G  P  E  P  R  D  C  V  S

DNA: TGCCGGAATGTCAGCCGAGGCAGGGAATGCGTGGACAAGTGCAACCTTCTG
 AA: C  R  N  V  S  R  G  R  E  C  V  D  K  C  N  L  L

DNA: GAGGGTGAGCCAAGGGAGTTTGTGGAGAACTCTGAGTGCATACAGTGCCAC
 AA: E  G  E  P  R  E  F  V  E  N  S  E  C  I  Q  C  H

DNA: CCAGAGTGCCTGCCTCAGGCCATGAACATCACCTGCACAGGACGGGGACCA
 AA: P  E  C  L  P  Q  A  M  N  I  T  C  T  G  R  G  P

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DNA: TGCACTGGGCCAGGTCTTGAAGGCTGTCCAACGAATGGGCCTAAGATCCCG
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DNA: TCCATCGCCACTGGGATGGTGGGGGCCCTCCTCTTGCTGCTGGTGGTGGCC
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Ala Phe Leu Leu Ile Pro Met Thr Gln Thr Thr Ser Ser Leu Ser Ala
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agc ctg ggc gac cgg gtg acc atc agc tgc cgg gcc agc cag gac atc         144
Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile
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agc aag tac ctg aac tgg tat cag cag aag ccc gac ggc acc gtc aag         192
Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys
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ctg ctg atc tac cac acc agc cgg ctg cac agc ggc gtg ccc agc cgg         240
Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg
65                  70                  75                  80

ttt agc ggc agc ggc tcc ggc acc gac tac agc ctg acc atc tcc aac         288
Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn
                85                  90                  95

ctg gaa cag gaa gat atc gcc acc tac ttt tgc cag cag ggc aac aca         336
Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr
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ctg ccc tac acc ttt ggc ggc gga aca aag ctg gaa atc acc ggc agc         384
Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr Gly Ser
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acc tcc ggc agc ggc aag cct ggc agc ggc gag ggc agc acc aag ggc         432
Thr Ser Gly Ser Gly Lys Pro Gly Ser Gly Glu Gly Ser Thr Lys Gly
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gag gtg aag ctg cag gaa agc ggc cct ggc ctg gtg gcc ccc agc cag         480
Glu Val Lys Leu Gln Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln
145                 150                 155                 160

agc ctg agc gtg acc tgc acc gtg agc ggc gtg agc ctg ccc gac tac         528
Ser Leu Ser Val Thr Cys Thr Val Ser Gly Val Ser Leu Pro Asp Tyr
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ggc gtg agc tgg atc cgg cag ccc ccc agg aag ggc ctg gaa tgg ctg         576
Gly Val Ser Trp Ile Arg Gln Pro Pro Arg Lys Gly Leu Glu Trp Leu
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225                 230                 235                 240

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Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser Val Lys
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atg tcc tgc aag gct tct ggc tac aca ttt acc agt tac aat atg cac        912
Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Asn Met His
    290                 295                 300

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Trp Val Lys Gln Thr Pro Gly Gln Gly Leu Glu Trp Ile Gly Ala Ile
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tat cca gga aat ggt gat act tcc tac aat cag aag ttc aaa ggc aag       1008
Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe Lys Gly Lys
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Ser Ser Leu Thr Ser Glu Asp Ser Ala Asp Tyr Tyr Cys Ala Arg Ser
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aat tat tac ggt agt agc tac tgg ttc ttc gat gtc tgg ggc gca ggg       1152
Asn Tyr Tyr Gly Ser Ser Tyr Trp Phe Phe Asp Val Trp Gly Ala Gly
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cca gct atc ctg tct gca tct cca ggg gag aag gtc aca atg act tgc       1296
Pro Ala Ile Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys
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agg gcc agc tca agt gta aat tac atg gac tgg tac cag aag aag cca       1344
Arg Ala Ser Ser Ser Val Asn Tyr Met Asp Trp Tyr Gln Lys Lys Pro
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gga tcc tcc ccc aaa ccc tgg att tat gcc aca tcc aac ctg gct tct       1392
Gly Ser Ser Pro Lys Pro Trp Ile Tyr Ala Thr Ser Asn Leu Ala Ser
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Gln Gln Trp Ser Phe Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu
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gaa ata aaa gag agc aag tac gga ccg ccc tgc ccc cct tgc cct atg       1584
Glu Ile Lys Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Met
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Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu
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Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Lys Arg Gly Arg Lys
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aaa ctc ctg tat ata ttc aaa caa cca ttt atg aga cca gta caa act       1728
Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr
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act caa gag gaa gat ggc tgt agc tgc cga ttt cca gaa gaa gaa gaa       1776
Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu
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Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro
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Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly
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aga agg gaa gag tac gac gtc ctg gat aag cgg aga ggc cgg gac cct       1920
Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro
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gag atg ggc ggc aag cct cgg cgg aag aac ccc cag gaa ggc ctg tat       1968
Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr
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aac gaa ctg cag aaa gac aag atg gcc gag gcc tac agc gag atc ggc       2016
Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly
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Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln
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Ala Leu Pro Pro Arg Leu Glu Gly Gly Gly Glu Gly Arg Gly Ser Leu
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Pro Glu Asn Arg Thr Asp Leu His Ala Phe Glu Asn Leu Glu Ile Ile
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Arg Gly Arg Thr Lys Gln His Gly Gln Phe Ser Leu Ala Val Val Ser
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His Ala Leu Cys Ser Pro Glu Gly Cys Trp Gly Pro Glu Pro Arg Asp
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tgc gtc tct tgc cgg aat gtc agc cga ggc agg gaa tgc gtg gac aag       2880
Cys Val Ser Cys Arg Asn Val Ser Arg Gly Arg Glu Cys Val Asp Lys
945                 950                 955                 960

tgc aac ctt ctg gag ggt gag cca agg gag ttt gtg gag aac tct gag       2928
Cys Asn Leu Leu Glu Gly Glu Pro Arg Glu Phe Val Glu Asn Ser Glu
                965                 970                 975

tgc ata cag tgc cac cca gag tgc ctg cct cag gcc atg aac atc acc       2976
Cys Ile Gln Cys His Pro Glu Cys Leu Pro Gln Ala Met Asn Ile Thr
            980                 985                 990

tgc aca gga cgg gga cca gac aac  tgt atc cag tgt gcc  cac tac att     3024
Cys Thr Gly Arg Gly Pro Asp Asn  Cys Ile Gln Cys Ala  His Tyr Ile
        995                 1000                1005

gac ggc  ccc cac tgc gtc aag  acc tgc ccg gca gga  gtc atg gga        3069
Asp Gly  Pro His Cys Val Lys  Thr Cys Pro Ala Gly  Val Met Gly
```

```
    1010                1015                1020

gaa aac  aac acc ctg gtc tgg  aag tac gca gac gcc  ggc cat gtg        3114
Glu Asn  Asn Thr Leu Val Trp  Lys Tyr Ala Asp Ala  Gly His Val
    1025                1030                1035

tgc cac  ctg tgc cat cca aac  tgc acc tac gga tgc  act ggg cca        3159
Cys His  Leu Cys His Pro Asn  Cys Thr Tyr Gly Cys  Thr Gly Pro
    1040                1045                1050

ggt ctt  gaa ggc tgt cca acg  aat ggg cct aag atc  ccg tcc atc        3204
Gly Leu  Glu Gly Cys Pro Thr  Asn Gly Pro Lys Ile  Pro Ser Ile
    1055                1060                1065

gcc act  ggg atg gtg ggg gcc  ctc ctc ttg ctg ctg  gtg gtg gcc        3249
Ala Thr  Gly Met Val Gly Ala  Leu Leu Leu Leu Leu  Val Val Ala
    1070                1075                1080

ctg ggg  atc ggc ctc ttc atg  tga                                   3273
Leu Gly  Ile Gly Leu Phe Met
    1085                1090
```

<210> 3
<211> 1090
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 3

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Met Leu Leu Leu Val Thr Ser Leu Leu Leu Cys Glu Leu Pro His Pro
1               5                   10                  15


Ala Phe Leu Leu Ile Pro Met Thr Gln Thr Thr Ser Ser Leu Ser Ala
            20                  25                  30


Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile
        35                  40                  45


Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys
    50                  55                  60


Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg
65                  70                  75                  80


Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn
                85                  90                  95


Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr
            100                 105                 110


Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr Gly Ser
        115                 120                 125


Thr Ser Gly Ser Gly Lys Pro Gly Ser Gly Glu Gly Ser Thr Lys Gly
    130                 135                 140
```

```
Glu Val Lys Leu Gln Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln
145                 150                 155                 160

Ser Leu Ser Val Thr Cys Thr Val Ser Gly Val Ser Leu Pro Asp Tyr
                165                 170                 175

Gly Val Ser Trp Ile Arg Gln Pro Pro Arg Lys Gly Leu Glu Trp Leu
            180                 185                 190

Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr Tyr Asn Ser Ala Leu Lys
        195                 200                 205

Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser Lys Ser Gln Val Phe Leu
    210                 215                 220

Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Ile Tyr Tyr Cys Ala
225                 230                 235                 240

Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala Met Asp Tyr Trp Gly Gln
                245                 250                 255

Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser Glu Val Gln
            260                 265                 270

Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser Val Lys
        275                 280                 285

Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Asn Met His
    290                 295                 300

Trp Val Lys Gln Thr Pro Gly Gln Gly Leu Glu Trp Ile Gly Ala Ile
305                 310                 315                 320

Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe Lys Gly Lys
                325                 330                 335

Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu
            340                 345                 350

Ser Ser Leu Thr Ser Glu Asp Ser Ala Asp Tyr Tyr Cys Ala Arg Ser
        355                 360                 365

Asn Tyr Tyr Gly Ser Ser Tyr Trp Phe Phe Asp Val Trp Gly Ala Gly
    370                 375                 380

Thr Thr Val Thr Val Ser Ser Gly Ser Thr Ser Gly Gly Gly Ser Gly
385                 390                 395                 400

Gly Gly Ser Gly Gly Gly Gly Ser Ser Asp Ile Val Leu Thr Gln Ser
                405                 410                 415
```

```
Pro Ala Ile Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys
            420                 425                 430

Arg Ala Ser Ser Ser Val Asn Tyr Met Asp Trp Tyr Gln Lys Lys Pro
        435                 440                 445

Gly Ser Ser Pro Lys Pro Trp Ile Tyr Ala Thr Ser Asn Leu Ala Ser
    450                 455                 460

Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser
465                 470                 475                 480

Leu Thr Ile Ser Arg Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys
                485                 490                 495

Gln Gln Trp Ser Phe Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu
            500                 505                 510

Glu Ile Lys Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Met
        515                 520                 525

Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu
    530                 535                 540

Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Lys Arg Gly Arg Lys
545                 550                 555                 560

Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr
                565                 570                 575

Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu
            580                 585                 590

Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro
        595                 600                 605

Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly
    610                 615                 620

Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro
625                 630                 635                 640

Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr
                645                 650                 655

Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly
            660                 665                 670

Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln
        675                 680                 685
```

```
Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln
    690                 695                 700

Ala Leu Pro Pro Arg Leu Glu Gly Gly Gly Glu Gly Arg Gly Ser Leu
705                 710                 715                 720

Leu Thr Cys Gly Asp Val Glu Glu Asn Pro Gly Pro Arg Met Leu Leu
                725                 730                 735

Leu Val Thr Ser Leu Leu Leu Cys Glu Leu Pro His Pro Ala Phe Leu
            740                 745                 750

Leu Ile Pro Arg Lys Val Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys
        755                 760                 765

Asp Ser Leu Ser Ile Asn Ala Thr Asn Ile Lys His Phe Lys Asn Cys
    770                 775                 780

Thr Ser Ile Ser Gly Asp Leu His Ile Leu Pro Val Ala Phe Arg Gly
785                 790                 795                 800

Asp Ser Phe Thr His Thr Pro Pro Leu Asp Pro Gln Glu Leu Asp Ile
                805                 810                 815

Leu Lys Thr Val Lys Glu Ile Thr Gly Phe Leu Leu Ile Gln Ala Trp
            820                 825                 830

Pro Glu Asn Arg Thr Asp Leu His Ala Phe Glu Asn Leu Glu Ile Ile
        835                 840                 845

Arg Gly Arg Thr Lys Gln His Gly Gln Phe Ser Leu Ala Val Val Ser
    850                 855                 860

Leu Asn Ile Thr Ser Leu Gly Leu Arg Ser Leu Lys Glu Ile Ser Asp
865                 870                 875                 880

Gly Asp Val Ile Ile Ser Gly Asn Lys Asn Leu Cys Tyr Ala Asn Thr
                885                 890                 895

Ile Asn Trp Lys Lys Leu Phe Gly Thr Ser Gly Gln Lys Thr Lys Ile
            900                 905                 910

Ile Ser Asn Arg Gly Glu Asn Ser Cys Lys Ala Thr Gly Gln Val Cys
        915                 920                 925

His Ala Leu Cys Ser Pro Glu Gly Cys Trp Gly Pro Glu Pro Arg Asp
    930                 935                 940

Cys Val Ser Cys Arg Asn Val Ser Arg Gly Arg Glu Cys Val Asp Lys
945                 950                 955                 960
```

```
Cys Asn Leu Leu Glu Gly Glu Pro Arg Glu Phe Val Glu Asn Ser Glu
                965                 970                 975

Cys Ile Gln Cys His Pro Glu Cys Leu Pro Gln Ala Met Asn Ile Thr
            980                 985                 990

Cys Thr Gly Arg Gly Pro Asp Asn  Cys Ile Gln Cys Ala  His Tyr Ile
        995                 1000                1005

Asp Gly  Pro His Cys Val Lys  Thr Cys Pro Ala Gly  Val Met Gly
    1010                1015                1020

Glu Asn  Asn Thr Leu Val Trp  Lys Tyr Ala Asp Ala  Gly His Val
    1025                1030                1035

Cys His  Leu Cys His Pro Asn  Cys Thr Tyr Gly Cys  Thr Gly Pro
    1040                1045                1050

Gly Leu  Glu Gly Cys Pro Thr  Asn Gly Pro Lys Ile  Pro Ser Ile
    1055                1060                1065

Ala Thr  Gly Met Val Gly Ala  Leu Leu Leu Leu Leu  Val Val Ala
    1070                1075                1080

Leu Gly  Ile Gly Leu Phe Met
    1085                1090
```

<210> 4
<211> 582
<212> DNA
<213> Artificial Sequence

<220>
<223> IgG4hinge-CD28tm-41BB-CD3Zeta

<400> 4
gagagcaagt acggaccgcc ctgcccccct tgccctatgt tctgggtgct ggtggtggtc 60

ggaggcgtgc tggcctgcta cagcctgctg gtcaccgtgg ccttcatcat cttttgggtg 120

aaacggggca gaaagaaact cctgtatata ttcaaacaac catttatgag accagtacaa 180

actactcaag aggaagatgg ctgtagctgc cgatttccag aagaagaaga aggaggatgt 240

gaactgcggg tgaagttcag cagaagcgcc gacgcccctg cctaccagca gggccagaat 300

cagctgtaca acgagctgaa cctgggcaga agggaagagt acgacgtcct ggataagcgg 360

agaggccggg accctgagat gggcggcaag cctcggcgga agaaccccca ggaaggcctg 420

tataacgaac tgcagaaaga caagatggcc gaggcctaca gcgagatcgg catgaagggc 480

gagcggaggc ggggcaaggg ccacgacggc ctgtatcagg gcctgtccac cgccaccaag 540

gatacctacg acgccctgca catgcaggcc ctgccccaa gg   582

<210> 5

<211> 582
<212> DNA
<213> Artificial Sequence

<220>
<223> IgG4hinge-CD28tm-41BB-CD3Zeta

<220>
<221> CDS
<222> (1)..(582)

<400> 5

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gag agc aag tac gga ccg ccc tgc ccc cct tgc cct atg ttc tgg gtg          48
Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Met Phe Trp Val
1               5                   10                  15

ctg gtg gtg gtc gga ggc gtg ctg gcc tgc tac agc ctg ctg gtc acc          96
Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val Thr
            20                  25                  30

gtg gcc ttc atc atc ttt tgg gtg aaa cgg ggc aga aag aaa ctc ctg         144
Val Ala Phe Ile Ile Phe Trp Val Lys Arg Gly Arg Lys Lys Leu Leu
        35                  40                  45

tat ata ttc aaa caa cca ttt atg aga cca gta caa act act caa gag         192
Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu
    50                  55                  60

gaa gat ggc tgt agc tgc cga ttt cca gaa gaa gaa gaa gga gga tgt         240
Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys
65                  70                  75                  80

gaa ctg cgg gtg aag ttc agc aga agc gcc gac gcc cct gcc tac cag         288
Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln
                85                  90                  95

cag ggc cag aat cag ctg tac aac gag ctg aac ctg ggc aga agg gaa         336
Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu
            100                 105                 110

gag tac gac gtc ctg gat aag cgg aga ggc cgg gac cct gag atg ggc         384
Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly
        115                 120                 125

ggc aag cct cgg cgg aag aac ccc cag gaa ggc ctg tat aac gaa ctg         432
Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu
    130                 135                 140

cag aaa gac aag atg gcc gag gcc tac agc gag atc ggc atg aag ggc         480
Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly
145                 150                 155                 160

gag cgg agg cgg ggc aag ggc cac gac ggc ctg tat cag ggc ctg tcc         528
Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser
                165                 170                 175

acc gcc acc aag gat acc tac gac gcc ctg cac atg cag gcc ctg ccc         576
Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro
            180                 185                 190

cca agg                                                                 582
Pro Arg
```

<210> 6
<211> 194

<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 6

```
Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Met Phe Trp Val
1               5                  10                  15

Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val Thr
            20                  25                  30

Val Ala Phe Ile Ile Phe Trp Val Lys Arg Gly Arg Lys Lys Leu Leu
        35                  40                  45

Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu
    50                  55                  60

Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys
65                  70                  75                  80

Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln
                85                  90                  95

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

Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly
        115                 120                 125

Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu
    130                 135                 140

Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly
145                 150                 155                 160

Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser
                165                 170                 175

Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro
            180                 185                 190

Pro Arg
```

<210> 7
<211> 2910
<212> DNA
<213> Artificial Sequence

<220>
<223> GMCSFRss-CD19scFv-Gly4serlinker-CD20scFv-huIgG4hinge/CH2/CH3-CD28 tm/CD28cyto-41BB-CD3Zeta

<400> 7
atgctgctgc tggtgaccag cctgctgctg tgcgagctgc cccaccccgc ctttctgctg 60

atccccgaca tccagatgac ccagaccacc tccagcctga gcgccagcct gggcgaccgg 120

gtgaccatca gctgccgggc cagccaggac atcagcaagt acctgaactg gtatcagcag 180

aagcccgacg gcaccgtcaa gctgctgatc taccacacca gccggctgca cagcggcgtg 240

cccagccggt ttagcggcag cggctccggc accgactaca gcctgaccat ctccaacctg 300

gaacaggaag atatcgccac ctacttttgc cagcagggca acacactgcc ctacaccttt 360

ggcggcggaa caaagctgga aatcaccggc agcacctccg gcagcggcaa gcctggcagc 420

ggcgagggca gcaccaaggg cgaggtgaag ctgcaggaaa gcggccctgg cctggtggcc 480

cccagccaga gcctgagcgt gacctgcacc gtgagcggcg tgagcctgcc cgactacggc 540

gtgagctgga tccggcagcc ccccaggaag ggcctggaat ggctgggcgt gatctggggc 600

agcgagacca cctactacaa cagcgccctg aagagccggc tgaccatcat caaggacaac 660

agcaagagcc aggtgttcct gaagatgaac agcctgcaga ccgacgacac cgccatctac 720

tactgcgcca agcactacta ctacggcggc agctacgcca tggactactg gggccagggc 780

accagcgtga ccgtgagcag cggaggtggt ggatccgagg tgcagctgca gcagtctggg 840

gctgagctgg tgaagcctgg ggcctcagtg aagatgtcct gcaaggcttc tggctacaca 900

tttaccagtt acaatatgca ctgggtaaag cagacacctg gacagggcct ggaatggatt 960

ggagctattt atccaggaaa tggtgatact tcctacaatc agaagttcaa aggcaaggcc 1020

acattgactg cagacaaatc ctccagcaca gcctacatgc agctcagcag cctgacatct 1080

gaggactctg cggactatta ctgtgcaaga tctaattatt acggtagtag ctactggttc 1140

ttcgatgtct ggggcgcagg gaccacggtc accgtctcct caggcagtac tagcggtggt 1200

ggctccgggg gcggttccgg tgggggcggc agcagcgaca ttgtgctgac ccaatctcca 1260

gctatcctgt ctgcatctcc aggggagaag gtcacaatga cttgcagggc cagctcaagt 1320

gtaaattaca tggactggta ccagaagaag ccaggatcct cccccaaacc ctggatttat 1380

gccacatcca acctggcttc tggagtccct gctcgcttca gtggcagtgg gtctgggacc 1440

tcttactctc tcacaatcag cagagtggag gctgaagatg ctgccactta ttactgccag 1500

cagtggagtt ttaatccacc cacgttcgga gggggggacca agctggaaat aaaagagagc 1560

aagtacggac cgccctgccc cccttgccct gcccccgagt tcctgggcgg acccagcgtg 1620

ttcctgttcc cccccaagcc caaggacacc ctgatgatca gccggacccc cgaggtgacc 1680

tgcgtggtgg tggacgtgag ccaggaagat cccgaggtcc agttcaattg gtacgtggac 1740

ggcgtggaag tgcacaacgc caagaccaag cccagagagg aacagttcaa cagcacctac 1800

cgggtggtgt ctgtgctgac cgtgctgcac caggactggc tgaacggcaa agaatacaag 1860

tgcaaggtgt ccaacaaggg cctgcccagc agcatcgaaa agaccatcag caaggccaag 1920

ggccagcctc gcgagcccca ggtgtacacc ctgcctccct cccaggaaga gatgaccaag 1980

aaccaggtgt ccctgacctg cctggtgaag ggcttctacc ccagcgacat cgccgtggag 2040

tgggagagca acggccagcc tgagaacaac tacaagacca cccctcccgt gctggacagc 2100

gacggcagct tcttcctgta cagccggctg accgtggaca agagccggtg gcaggaaggc 2160

aacgtcttta gctgcagcgt gatgcacgag gccctgcaca accactacac ccagaagagc 2220

ctgagcctgt ccctgggcaa gatgttctgg gtgctggtgg tggtgggcgg ggtgctggcc 2280

tgctacagcc tgctggtgac agtggccttc atcatctttt gggtgcggag caagcggagc 2340

agaggcggcc acagcgacta catgaacatg acccccagac ggcctggccc cacccggaag 2400

cactaccagc cctacgcccc acccagggac tttgccgcct acagaagcaa acggggcaga 2460

aagaaactcc tgtatatatt caaacaacca tttatgagac cagtacaaac tactcaagag 2520

gaagatggct gtagctgccg atttccagaa gaagaagaag gaggatgtga actgcgggtg 2580

aagttcagca gaagcgccga cgcccctgcc taccagcagg gccagaatca gctgtacaac 2640

gagctgaacc tgggcagaag ggaagagtac gacgtcctgg ataagcggag aggccgggac 2700

cctgagatgg gcggcaagcc tcggcggaag aacccccagg aaggcctgta taacgaactg 2760

cagaaagaca agatggccga ggcctacagc gagatcggca tgaagggcga gcggaggcgg 2820

ggcaagggcc acgacggcct gtatcagggc ctgtccaccg ccaccaagga tacctacgac 2880

gccctgcaca tgcaggccct gccccaagg 2910

<210> 8
<211> 2910
<212> DNA
<213> Artificial Sequence

<220>
<223> GMCSFR-ssCD19scFv-Gly4serlinker-CD20scFv-huIgG4hinge/CH2/CH3-CD28tm/CD28cyto-41BB-CD3Zeta

<220>
<221> CDS
<222> (1)..(2910)

<400> 8
atg ctg ctg ctg gtg acc agc ctg ctg ctg tgc gag ctg ccc cac ccc 48
Met Leu Leu Leu Val Thr Ser Leu Leu Leu Cys Glu Leu Pro His Pro
1 5 10 15

gcc ttt ctg ctg atc ccc gac atc cag atg acc cag acc acc tcc agc 96
Ala Phe Leu Leu Ile Pro Asp Ile Gln Met Thr Gln Thr Thr Ser Ser
20 25 30

ctg agc gcc agc ctg ggc gac cgg gtg acc atc agc tgc cgg gcc agc 144
Leu Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser
35 40 45

cag gac atc agc aag tac ctg aac tgg tat cag cag aag ccc gac ggc 192
Gln Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly
50 55 60

acc gtc aag ctg ctg atc tac cac acc agc cgg ctg cac agc ggc gtg 240
Thr Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val
65 70 75 80

```
ccc agc cgg ttt agc ggc agc ggc tcc ggc acc gac tac agc ctg acc        288
Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr
                85                  90                  95

atc tcc aac ctg gaa cag gaa gat atc gcc acc tac ttt tgc cag cag        336
Ile Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln
            100                 105                 110

ggc aac aca ctg ccc tac acc ttt ggc ggc gga aca aag ctg gaa atc        384
Gly Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
        115                 120                 125

acc ggc agc acc tcc ggc agc ggc aag cct ggc agc ggc gag ggc agc        432
Thr Gly Ser Thr Ser Gly Ser Gly Lys Pro Gly Ser Gly Glu Gly Ser
    130                 135                 140

acc aag ggc gag gtg aag ctg cag gaa agc ggc cct ggc ctg gtg gcc        480
Thr Lys Gly Glu Val Lys Leu Gln Glu Ser Gly Pro Gly Leu Val Ala
145                 150                 155                 160

ccc agc cag agc ctg agc gtg acc tgc acc gtg agc ggc gtg agc ctg        528
Pro Ser Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly Val Ser Leu
                165                 170                 175

ccc gac tac ggc gtg agc tgg atc cgg cag ccc ccc agg aag ggc ctg        576
Pro Asp Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg Lys Gly Leu
            180                 185                 190

gaa tgg ctg ggc gtg atc tgg ggc agc gag acc acc tac tac aac agc        624
Glu Trp Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr Tyr Asn Ser
        195                 200                 205

gcc ctg aag agc cgg ctg acc atc atc aag gac aac agc aag agc cag        672
Ala Leu Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser Lys Ser Gln
    210                 215                 220

gtg ttc ctg aag atg aac agc ctg cag acc gac gac acc gcc atc tac        720
Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Ile Tyr
225                 230                 235                 240

tac tgc gcc aag cac tac tac tac ggc ggc agc tac gcc atg gac tac        768
Tyr Cys Ala Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala Met Asp Tyr
                245                 250                 255

tgg ggc cag ggc acc agc gtg acc gtg agc agc gga ggt ggt gga tcc        816
Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser
            260                 265                 270

gag gtg cag ctg cag cag tct ggg gct gag ctg gtg aag cct ggg gcc        864
Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
        275                 280                 285

tca gtg aag atg tcc tgc aag gct tct ggc tac aca ttt acc agt tac        912
Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
    290                 295                 300

aat atg cac tgg gta aag cag aca cct gga cag ggc ctg gaa tgg att        960
Asn Met His Trp Val Lys Gln Thr Pro Gly Gln Gly Leu Glu Trp Ile
305                 310                 315                 320

gga gct att tat cca gga aat ggt gat act tcc tac aat cag aag ttc       1008
Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
                325                 330                 335

aaa ggc aag gcc aca ttg act gca gac aaa tcc tcc agc aca gcc tac       1056
Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
            340                 345                 350
```

```
atg cag ctc agc agc ctg aca tct gag gac tct gcg gac tat tac tgt       1104
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Asp Tyr Tyr Cys
        355                 360                 365

gca aga tct aat tat tac ggt agt agc tac tgg ttc ttc gat gtc tgg       1152
Ala Arg Ser Asn Tyr Tyr Gly Ser Ser Tyr Trp Phe Phe Asp Val Trp
    370                 375                 380

ggc gca ggg acc acg gtc acc gtc tcc tca ggc agt act agc ggt ggt       1200
Gly Ala Gly Thr Thr Val Thr Val Ser Ser Gly Ser Thr Ser Gly Gly
385                 390                 395                 400

ggc tcc ggg ggc ggt tcc ggt ggg ggc ggc agc agc gac att gtg ctg       1248
Gly Ser Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Asp Ile Val Leu
                405                 410                 415

acc caa tct cca gct atc ctg tct gca tct cca ggg gag aag gtc aca       1296
Thr Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly Glu Lys Val Thr
            420                 425                 430

atg act tgc agg gcc agc tca agt gta aat tac atg gac tgg tac cag       1344
Met Thr Cys Arg Ala Ser Ser Ser Val Asn Tyr Met Asp Trp Tyr Gln
        435                 440                 445

aag aag cca gga tcc tcc ccc aaa ccc tgg att tat gcc aca tcc aac       1392
Lys Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr Ala Thr Ser Asn
    450                 455                 460

ctg gct tct gga gtc cct gct cgc ttc agt ggc agt ggg tct ggg acc       1440
Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr
465                 470                 475                 480

tct tac tct ctc aca atc agc aga gtg gag gct gaa gat gct gcc act       1488
Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala Glu Asp Ala Ala Thr
                485                 490                 495

tat tac tgc cag cag tgg agt ttt aat cca ccc acg ttc gga ggg ggg       1536
Tyr Tyr Cys Gln Gln Trp Ser Phe Asn Pro Pro Thr Phe Gly Gly Gly
            500                 505                 510

acc aag ctg gaa ata aaa gag agc aag tac gga ccg ccc tgc ccc cct       1584
Thr Lys Leu Glu Ile Lys Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro
        515                 520                 525

tgc cct gcc ccc gag ttc ctg ggc gga ccc agc gtg ttc ctg ttc ccc       1632
Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
    530                 535                 540

ccc aag ccc aag gac acc ctg atg atc agc cgg acc ccc gag gtg acc       1680
Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
545                 550                 555                 560

tgc gtg gtg gtg gac gtg agc cag gaa gat ccc gag gtc cag ttc aat       1728
Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn
                565                 570                 575

tgg tac gtg gac ggc gtg gaa gtg cac aac gcc aag acc aag ccc aga       1776
Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
            580                 585                 590

gag gaa cag ttc aac agc acc tac cgg gtg gtg tct gtg ctg acc gtg       1824
Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
        595                 600                 605

ctg cac cag gac tgg ctg aac ggc aaa gaa tac aag tgc aag gtg tcc       1872
Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
    610                 615                 620
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aac aag ggc ctg ccc agc agc atc gaa aag acc atc agc aag gcc aag       1920
Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys
625                 630                 635                 640

ggc cag cct cgc gag ccc cag gtg tac acc ctg cct ccc tcc cag gaa       1968
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu
                645                 650                 655

gag atg acc aag aac cag gtg tcc ctg acc tgc ctg gtg aag ggc ttc       2016
Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
            660                 665                 670

tac ccc agc gac atc gcc gtg gag tgg gag agc aac ggc cag cct gag       2064
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
        675                 680                 685

aac aac tac aag acc acc cct ccc gtg ctg gac agc gac ggc agc ttc       2112
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
    690                 695                 700

ttc ctg tac agc cgg ctg acc gtg gac aag agc cgg tgg cag gaa ggc       2160
Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly
705                 710                 715                 720

aac gtc ttt agc tgc agc gtg atg cac gag gcc ctg cac aac cac tac       2208
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
                725                 730                 735

acc cag aag agc ctg agc ctg tcc ctg ggc aag atg ttc tgg gtg ctg       2256
Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys Met Phe Trp Val Leu
            740                 745                 750

gtg gtg gtg ggc ggg gtg ctg gcc tgc tac agc ctg ctg gtg aca gtg       2304
Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val Thr Val
        755                 760                 765

gcc ttc atc atc ttt tgg gtg cgg agc aag cgg agc aga ggc ggc cac       2352
Ala Phe Ile Ile Phe Trp Val Arg Ser Lys Arg Ser Arg Gly Gly His
    770                 775                 780

agc gac tac atg aac atg acc ccc aga cgg cct ggc ccc acc cgg aag       2400
Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys
785                 790                 795                 800

cac tac cag ccc tac gcc cca ccc agg gac ttt gcc gcc tac aga agc       2448
His Tyr Gln Pro Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser
                805                 810                 815

aaa cgg ggc aga aag aaa ctc ctg tat ata ttc aaa caa cca ttt atg       2496
Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met
            820                 825                 830

aga cca gta caa act act caa gag gaa gat ggc tgt agc tgc cga ttt       2544
Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe
        835                 840                 845

cca gaa gaa gaa gaa gga gga tgt gaa ctg cgg gtg aag ttc agc aga       2592
Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg
    850                 855                 860

agc gcc gac gcc cct gcc tac cag cag ggc cag aat cag ctg tac aac       2640
Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn
865                 870                 875                 880

gag ctg aac ctg ggc aga agg gaa gag tac gac gtc ctg gat aag cgg       2688
Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg
                885                 890                 895
```

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aga ggc cgg gac cct gag atg ggc ggc aag cct cgg cgg aag aac ccc       2736
Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro
            900                 905                 910

cag gaa ggc ctg tat aac gaa ctg cag aaa gac aag atg gcc gag gcc       2784
Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala
        915                 920                 925

tac agc gag atc ggc atg aag ggc gag cgg agg cgg ggc aag ggc cac       2832
Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His
    930                 935                 940

gac ggc ctg tat cag ggc ctg tcc acc gcc acc aag gat acc tac gac       2880
Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp
945                 950                 955                 960

gcc ctg cac atg cag gcc ctg ccc cca agg                               2910
Ala Leu His Met Gln Ala Leu Pro Pro Arg
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            20                  25                  30

Leu Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser
        35                  40                  45

Gln Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly
    50                  55                  60

Thr Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val
65                  70                  75                  80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr
                85                  90                  95

Ile Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln
            100                 105                 110

Gly Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
        115                 120                 125

Thr Gly Ser Thr Ser Gly Ser Gly Lys Pro Gly Ser Gly Glu Gly Ser
    130                 135                 140

Thr Lys Gly Glu Val Lys Leu Gln Glu Ser Gly Pro Gly Leu Val Ala
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145                 150                 155                 160

Pro Ser Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly Val Ser Leu
                165                 170                 175

Pro Asp Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg Lys Gly Leu
            180                 185                 190

Glu Trp Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr Tyr Asn Ser
        195                 200                 205

Ala Leu Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser Lys Ser Gln
    210                 215                 220

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

Tyr Cys Ala Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala Met Asp Tyr
                245                 250                 255

Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser
            260                 265                 270

Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
        275                 280                 285

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
    290                 295                 300

Asn Met His Trp Val Lys Gln Thr Pro Gly Gln Gly Leu Glu Trp Ile
305                 310                 315                 320

Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
                325                 330                 335

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
            340                 345                 350

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Asp Tyr Tyr Cys
        355                 360                 365

Ala Arg Ser Asn Tyr Tyr Gly Ser Ser Tyr Trp Phe Phe Asp Val Trp
    370                 375                 380

Gly Ala Gly Thr Thr Val Thr Val Ser Ser Gly Ser Thr Ser Gly Gly
385                 390                 395                 400

Gly Ser Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Asp Ile Val Leu
                405                 410                 415

Thr Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly Glu Lys Val Thr
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            420                 425                 430

Met Thr Cys Arg Ala Ser Ser Ser Val Asn Tyr Met Asp Trp Tyr Gln
        435                 440                 445

Lys Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr Ala Thr Ser Asn
    450                 455                 460

Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr
465                 470                 475                 480

Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala Glu Asp Ala Ala Thr
                485                 490                 495

Tyr Tyr Cys Gln Gln Trp Ser Phe Asn Pro Pro Thr Phe Gly Gly Gly
            500                 505                 510

Thr Lys Leu Glu Ile Lys Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro
        515                 520                 525

Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
    530                 535                 540

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
545                 550                 555                 560

Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn
                565                 570                 575

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
            580                 585                 590

Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
        595                 600                 605

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
    610                 615                 620

Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys
625                 630                 635                 640

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu
                645                 650                 655

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
            660                 665                 670

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
        675                 680                 685

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
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690 695 700

Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly
705 710 715 720

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
725 730 735

Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys Met Phe Trp Val Leu
740 745 750

Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val Thr Val
755 760 765

Ala Phe Ile Ile Phe Trp Val Arg Ser Lys Arg Ser Arg Gly Gly His
770 775 780

Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys
785 790 795 800

His Tyr Gln Pro Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser
805 810 815

Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met
820 825 830

Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe
835 840 845

Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg
850 855 860

Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn
865 870 875 880

Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg
885 890 895

Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro
900 905 910

Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala
915 920 925

Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His
930 935 940

Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp
945 950 955 960

Ala Leu His Met Gln Ala Leu Pro Pro Arg

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-41BB-CD3Zeta-T2A-EGFRt

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1               5                   10                  15

gcc ttt ctg ctg atc ccc gac atc cag atg acc cag acc acc tcc agc          96
Ala Phe Leu Leu Ile Pro Asp Ile Gln Met Thr Gln Thr Thr Ser Ser
            20                  25                  30

ctg agc gcc agc ctg ggc gac cgg gtg acc atc agc tgc cgg gcc agc         144
Leu Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser
        35                  40                  45

cag gac atc agc aag tac ctg aac tgg tat cag cag aag ccc gac ggc         192
Gln Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly
    50                  55                  60

acc gtc aag ctg ctg atc tac cac acc agc cgg ctg cac agc ggc gtg         240
Thr Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val
65                  70                  75                  80

ccc agc cgg ttt agc ggc agc ggc tcc ggc acc gac tac agc ctg acc         288
Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr
                85                  90                  95

atc tcc aac ctg gaa cag gaa gat atc gcc acc tac ttt tgc cag cag         336
Ile Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln
            100                 105                 110

ggc aac aca ctg ccc tac acc ttt ggc ggc gga aca aag ctg gaa atc         384
Gly Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
        115                 120                 125

acc ggc agc acc tcc ggc agc ggc aag cct ggc agc ggc gag ggc agc         432
Thr Gly Ser Thr Ser Gly Ser Gly Lys Pro Gly Ser Gly Glu Gly Ser
    130                 135                 140

acc aag ggc gag gtg aag ctg cag gaa agc ggc cct ggc ctg gtg gcc         480
Thr Lys Gly Glu Val Lys Leu Gln Glu Ser Gly Pro Gly Leu Val Ala
145                 150                 155                 160

ccc agc cag agc ctg agc gtg acc tgc acc gtg agc ggc gtg agc ctg         528
Pro Ser Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly Val Ser Leu
                165                 170                 175

ccc gac tac ggc gtg agc tgg atc cgg cag ccc ccc agg aag ggc ctg         576
Pro Asp Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg Lys Gly Leu
            180                 185                 190

gaa tgg ctg ggc gtg atc tgg ggc agc gag acc acc tac tac aac agc         624
Glu Trp Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr Tyr Asn Ser
        195                 200                 205

gcc ctg aag agc cgg ctg acc atc atc aag gac aac agc aag agc cag         672
Ala Leu Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser Lys Ser Gln
    210                 215                 220

gtg ttc ctg aag atg aac agc ctg cag acc gac gac acc gcc atc tac         720
Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Ile Tyr
225                 230                 235                 240

tac tgc gcc aag cac tac tac tac ggc ggc agc tac gcc atg gac tac         768
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Tyr Cys Ala Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala Met Asp Tyr
                245                 250                 255

tgg ggc cag ggc acc agc gtg acc gtg agc agc gga ggt ggt gga tcc  816
Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser
            260                 265                 270

gag gtg cag ctg cag cag tct ggg gct gag ctg gtg aag cct ggg gcc  864
Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
        275                 280                 285

tca gtg aag atg tcc tgc aag gct tct ggc tac aca ttt acc agt tac  912
Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
    290                 295                 300

aat atg cac tgg gta aag cag aca cct gga cag ggc ctg gaa tgg att  960
Asn Met His Trp Val Lys Gln Thr Pro Gly Gln Gly Leu Glu Trp Ile
305                 310                 315                 320

gga gct att tat cca gga aat ggt gat act tcc tac aat cag aag ttc 1008
Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
                325                 330                 335

aaa ggc aag gcc aca ttg act gca gac aaa tcc tcc agc aca gcc tac 1056
Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
            340                 345                 350

atg cag ctc agc agc ctg aca tct gag gac tct gcg gac tat tac tgt 1104
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Asp Tyr Tyr Cys
        355                 360                 365

gca aga tct aat tat tac ggt agt agc tac tgg ttc ttc gat gtc tgg 1152
Ala Arg Ser Asn Tyr Tyr Gly Ser Ser Tyr Trp Phe Phe Asp Val Trp
    370                 375                 380

ggc gca ggg acc acg gtc acc gtc tcc tca ggc agt act agc ggt ggt 1200
Gly Ala Gly Thr Thr Val Thr Val Ser Ser Gly Ser Thr Ser Gly Gly
385                 390                 395                 400

ggc tcc ggg ggc ggt tcc ggt ggg ggc ggc agc agc gac att gtg ctg 1248
Gly Ser Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Asp Ile Val Leu
                405                 410                 415

acc caa tct cca gct atc ctg tct gca tct cca ggg gag aag gtc aca 1296
Thr Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly Glu Lys Val Thr
            420                 425                 430

atg act tgc agg gcc agc tca agt gta aat tac atg gac tgg tac cag 1344
Met Thr Cys Arg Ala Ser Ser Ser Val Asn Tyr Met Asp Trp Tyr Gln
        435                 440                 445

aag aag cca gga tcc tcc ccc aaa ccc tgg att tat gcc aca tcc aac 1392
Lys Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr Ala Thr Ser Asn
    450                 455                 460

ctg gct tct gga gtc cct gct cgc ttc agt ggc agt ggg tct ggg acc 1440
Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr
465                 470                 475                 480

tct tac tct ctc aca atc agc aga gtg gag gct gaa gat gct gcc act 1488
Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala Glu Asp Ala Ala Thr
                485                 490                 495

tat tac tgc cag cag tgg agt ttt aat cca ccc acg ttc gga ggg ggg 1536
Tyr Tyr Cys Gln Gln Trp Ser Phe Asn Pro Pro Thr Phe Gly Gly Gly
            500                 505                 510

acc aag ctg gaa ata aaa gag agc aag tac gga ccg ccc tgc ccc cct 1584
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Thr Lys Leu Glu Ile Lys Glu Ser Lys Tyr Gly Pro Cys Pro Pro
        515                 520                 525

tgc cct aag cct acc acc acc cct gcc cct aga cct cca aca ccc gcc       1632
Cys Pro Lys Pro Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala
    530                 535                 540

cca aca atc gcc agc cag cct ctg tct ctg agg ccc gag gct tgt aga       1680
Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg
545                 550                 555                 560

cca gct gct ggc gga gcc gtg cac acc aga gga ctg gat ttc gcc tgc       1728
Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys
                565                 570                 575

gac atc tac atc tgg gcc cct ctg gcc ggc aca tgt ggc gtg ctg ctg       1776
Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu
            580                 585                 590

ctg agc ctc gtg atc acc aag cgg ggc aga aag aaa ctg ctg tac atc       1824
Leu Ser Leu Val Ile Thr Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile
        595                 600                 605

ttt aag cag ccc ttc atg cgg ccc gtg cag acc acc cag gaa gag gac       1872
Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp
    610                 615                 620

ggc tgc tcc tgc aga ttc ccc gag gaa gaa gaa ggc ggc tgc gag ctg       1920
Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu
625                 630                 635                 640

aga gtg aag ttc agc aga tcc gcc gac gcc cct gcc tac cag cag gga       1968
Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly
                645                 650                 655

cag aac cag ctg tac aac gag ctg aac ctg ggc aga cgg gaa gag tac       2016
Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr
            660                 665                 670

gac gtg ctg gac aag cgg aga ggc cgg gac cct gag atg ggc gga aag       2064
Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys
        675                 680                 685

ccc aga aga aag aac ccc cag gaa ggc ctg tat aac gaa ctg cag aaa       2112
Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys
    690                 695                 700

gac aag atg gcc gag gcc tac agc gag atc gga atg aag ggc gag cgg       2160
Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg
705                 710                 715                 720

aga aga ggc aag ggc cac gat ggc ctg tac cag ggc ctg agc acc gcc       2208
Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala
                725                 730                 735

acc aag gac acc tat gac gcc ctg cac atg cag gcc ctg cct cca aga       2256
Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
            740                 745                 750

ctc gag ggc ggc gga gag ggc aga gga agt ctt cta aca tgc ggt gac       2304
Leu Glu Gly Gly Gly Glu Gly Arg Gly Ser Leu Leu Thr Cys Gly Asp
        755                 760                 765

gtg gag gag aat ccc ggc cct agg atg ctt ctc ctg gtg aca agc ctt       2352
Val Glu Glu Asn Pro Gly Pro Arg Met Leu Leu Leu Val Thr Ser Leu
    770                 775                 780

ctg ctc tgt gag tta cca cac cca gca ttc ctc ctg atc cca cgc aaa       2400
```

```
Leu Leu Cys Glu Leu Pro His Pro Ala Phe Leu Leu Ile Pro Arg Lys
785                 790                 795                 800

gtg tgt aac gga ata ggt att ggt gaa ttt aaa gac tca ctc tcc ata       2448
Val Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys Asp Ser Leu Ser Ile
                805                 810                 815

aat gct acg aat att aaa cac ttc aaa aac tgc acc tcc atc agt ggc       2496
Asn Ala Thr Asn Ile Lys His Phe Lys Asn Cys Thr Ser Ile Ser Gly
            820                 825                 830

gat ctc cac atc ctg ccg gtg gca ttt agg ggt gac tcc ttc aca cat       2544
Asp Leu His Ile Leu Pro Val Ala Phe Arg Gly Asp Ser Phe Thr His
        835                 840                 845

act cct cct ctg gat cca cag gaa ctg gat att ctg aaa acc gta aag       2592
Thr Pro Pro Leu Asp Pro Gln Glu Leu Asp Ile Leu Lys Thr Val Lys
    850                 855                 860

gaa atc aca ggg ttt ttg ctg att cag gct tgg cct gaa aac agg acg       2640
Glu Ile Thr Gly Phe Leu Leu Ile Gln Ala Trp Pro Glu Asn Arg Thr
865                 870                 875                 880

gac ctc cat gcc ttt gag aac cta gaa atc ata cgc ggc agg acc aag       2688
Asp Leu His Ala Phe Glu Asn Leu Glu Ile Ile Arg Gly Arg Thr Lys
                885                 890                 895

caa cat ggt cag ttt tct ctt gca gtc gtc agc ctg aac ata aca tcc       2736
Gln His Gly Gln Phe Ser Leu Ala Val Val Ser Leu Asn Ile Thr Ser
            900                 905                 910

ttg gga tta cgc tcc ctc aag gag ata agt gat gga gat gtg ata att       2784
Leu Gly Leu Arg Ser Leu Lys Glu Ile Ser Asp Gly Asp Val Ile Ile
        915                 920                 925

tca gga aac aaa aat ttg tgc tat gca aat aca ata aac tgg aaa aaa       2832
Ser Gly Asn Lys Asn Leu Cys Tyr Ala Asn Thr Ile Asn Trp Lys Lys
    930                 935                 940

ctg ttt ggg acc tcc ggt cag aaa acc aaa att ata agc aac aga ggt       2880
Leu Phe Gly Thr Ser Gly Gln Lys Thr Lys Ile Ile Ser Asn Arg Gly
945                 950                 955                 960

gaa aac agc tgc aag gcc aca ggc cag gtc tgc cat gcc ttg tgc tcc       2928
Glu Asn Ser Cys Lys Ala Thr Gly Gln Val Cys His Ala Leu Cys Ser
                965                 970                 975

ccc gag ggc tgc tgg ggc ccg gag ccc agg gac tgc gtc tct tgc cgg       2976
Pro Glu Gly Cys Trp Gly Pro Glu Pro Arg Asp Cys Val Ser Cys Arg
            980                 985                 990

aat gtc agc cga ggc agg gaa tgc  gtg gac aag tgc aac  ctt ctg gag     3024
Asn Val Ser Arg Gly Arg Glu Cys  Val Asp Lys Cys Asn  Leu Leu Glu
        995                 1000                1005

ggt gag  cca agg gag ttt gtg  gag aac tct gag tgc  ata cag tgc        3069
Gly Glu  Pro Arg Glu Phe Val  Glu Asn Ser Glu Cys  Ile Gln Cys
    1010                1015                1020

cac cca  gag tgc ctg cct cag  gcc atg aac atc acc  tgc aca gga        3114
His Pro  Glu Cys Leu Pro Gln  Ala Met Asn Ile Thr  Cys Thr Gly
    1025                1030                1035

cgg gga  cca gac aac tgt atc  cag tgt gcc cac tac  att gac ggc        3159
Arg Gly  Pro Asp Asn Cys Ile  Gln Cys Ala His Tyr  Ile Asp Gly
    1040                1045                1050

ccc cac  tgc gtc aag acc tgc  ccg gca gga gtc atg  gga gaa aac        3204
```

```
Pro His  Cys Val Lys Thr Cys  Pro Ala Gly Val Met  Gly Glu Asn
    1055                1060                1065

aac acc  ctg gtc tgg aag tac  gca gac gcc ggc cat  gtg tgc cac        3249
Asn Thr  Leu Val Trp Lys Tyr  Ala Asp Ala Gly His  Val Cys His
    1070                1075                1080

ctg tgc  cat cca aac tgc acc  tac gga tgc act ggg  cca ggt ctt        3294
Leu Cys  His Pro Asn Cys Thr  Tyr Gly Cys Thr Gly  Pro Gly Leu
    1085                1090                1095

gaa ggc  tgt cca acg aat ggg  cct aag atc ccg tcc  atc gcc act        3339
Glu Gly  Cys Pro Thr Asn Gly  Pro Lys Ile Pro Ser  Ile Ala Thr
    1100                1105                1110

ggg atg  gtg ggg gcc ctc ctc  ttg ctg ctg gtg gtg  gcc ctg ggg        3384
Gly Met  Val Gly Ala Leu Leu  Leu Leu Leu Val Val  Ala Leu Gly
    1115                1120                1125

atc ggc  ctc ttc atg tga                                          3402
Ile Gly  Leu Phe Met
    1130
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<400> 12

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Met Leu Leu Leu Val Thr Ser Leu Leu Leu Cys Glu Leu Pro His Pro
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Ala Phe Leu Leu Ile Pro Asp Ile Gln Met Thr Gln Thr Thr Ser Ser
            20                  25                  30

Leu Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser
        35                  40                  45

Gln Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly
    50                  55                  60

Thr Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val
65                  70                  75                  80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr
                85                  90                  95

Ile Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln
            100                 105                 110

Gly Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
        115                 120                 125

Thr Gly Ser Thr Ser Gly Ser Gly Lys Pro Gly Ser Gly Glu Gly Ser
    130                 135                 140
```

```
Thr Lys Gly Glu Val Lys Leu Gln Glu Ser Gly Pro Gly Leu Val Ala
145                 150                 155                 160

Pro Ser Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly Val Ser Leu
                165                 170                 175

Pro Asp Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg Lys Gly Leu
            180                 185                 190

Glu Trp Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr Tyr Asn Ser
        195                 200                 205

Ala Leu Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser Lys Ser Gln
    210                 215                 220

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

Tyr Cys Ala Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala Met Asp Tyr
                245                 250                 255

Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser
            260                 265                 270

Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
        275                 280                 285

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
    290                 295                 300

Asn Met His Trp Val Lys Gln Thr Pro Gly Gln Gly Leu Glu Trp Ile
305                 310                 315                 320

Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
                325                 330                 335

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
            340                 345                 350

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Asp Tyr Tyr Cys
        355                 360                 365

Ala Arg Ser Asn Tyr Tyr Gly Ser Ser Tyr Trp Phe Phe Asp Val Trp
    370                 375                 380

Gly Ala Gly Thr Thr Val Thr Val Ser Ser Gly Ser Thr Ser Gly Gly
385                 390                 395                 400

Gly Ser Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Asp Ile Val Leu
                405                 410                 415
```

```
Thr Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly Glu Lys Val Thr
            420                 425                 430

Met Thr Cys Arg Ala Ser Ser Ser Val Asn Tyr Met Asp Trp Tyr Gln
        435                 440                 445

Lys Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr Ala Thr Ser Asn
    450                 455                 460

Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr
465                 470                 475                 480

Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala Glu Asp Ala Ala Thr
                485                 490                 495

Tyr Tyr Cys Gln Gln Trp Ser Phe Asn Pro Pro Thr Phe Gly Gly Gly
            500                 505                 510

Thr Lys Leu Glu Ile Lys Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro
        515                 520                 525

Cys Pro Lys Pro Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala
    530                 535                 540

Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg
545                 550                 555                 560

Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys
                565                 570                 575

Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu
            580                 585                 590

Leu Ser Leu Val Ile Thr Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile
        595                 600                 605

Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp
    610                 615                 620

Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu
625                 630                 635                 640

Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly
                645                 650                 655

Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr
            660                 665                 670

Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys
        675                 680                 685
```

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Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys
    690                 695                 700

Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg
705                 710                 715                 720

Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala
                725                 730                 735

Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
            740                 745                 750

Leu Glu Gly Gly Gly Glu Gly Arg Gly Ser Leu Leu Thr Cys Gly Asp
        755                 760                 765

Val Glu Glu Asn Pro Gly Pro Arg Met Leu Leu Leu Val Thr Ser Leu
    770                 775                 780

Leu Leu Cys Glu Leu Pro His Pro Ala Phe Leu Leu Ile Pro Arg Lys
785                 790                 795                 800

Val Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys Asp Ser Leu Ser Ile
                805                 810                 815

Asn Ala Thr Asn Ile Lys His Phe Lys Asn Cys Thr Ser Ile Ser Gly
            820                 825                 830

Asp Leu His Ile Leu Pro Val Ala Phe Arg Gly Asp Ser Phe Thr His
        835                 840                 845

Thr Pro Pro Leu Asp Pro Gln Glu Leu Asp Ile Leu Lys Thr Val Lys
    850                 855                 860

Glu Ile Thr Gly Phe Leu Leu Ile Gln Ala Trp Pro Glu Asn Arg Thr
865                 870                 875                 880

Asp Leu His Ala Phe Glu Asn Leu Glu Ile Ile Arg Gly Arg Thr Lys
                885                 890                 895

Gln His Gly Gln Phe Ser Leu Ala Val Val Ser Leu Asn Ile Thr Ser
            900                 905                 910

Leu Gly Leu Arg Ser Leu Lys Glu Ile Ser Asp Gly Asp Val Ile Ile
        915                 920                 925

Ser Gly Asn Lys Asn Leu Cys Tyr Ala Asn Thr Ile Asn Trp Lys Lys
    930                 935                 940

Leu Phe Gly Thr Ser Gly Gln Lys Thr Lys Ile Ile Ser Asn Arg Gly
945                 950                 955                 960
```

Glu Asn Ser Cys Lys Ala Thr Gly Gln Val Cys His Ala Leu Cys Ser
965 970 975

Pro Glu Gly Cys Trp Gly Pro Glu Pro Arg Asp Cys Val Ser Cys Arg
980 985 990

Asn Val Ser Arg Gly Arg Glu Cys Val Asp Lys Cys Asn Leu Leu Glu
995 1000 1005

Gly Glu Pro Arg Glu Phe Val Glu Asn Ser Glu Cys Ile Gln Cys
1010 1015 1020

His Pro Glu Cys Leu Pro Gln Ala Met Asn Ile Thr Cys Thr Gly
1025 1030 1035

Arg Gly Pro Asp Asn Cys Ile Gln Cys Ala His Tyr Ile Asp Gly
1040 1045 1050

Pro His Cys Val Lys Thr Cys Pro Ala Gly Val Met Gly Glu Asn
1055 1060 1065

Asn Thr Leu Val Trp Lys Tyr Ala Asp Ala Gly His Val Cys His
1070 1075 1080

Leu Cys His Pro Asn Cys Thr Tyr Gly Cys Thr Gly Pro Gly Leu
1085 1090 1095

Glu Gly Cys Pro Thr Asn Gly Pro Lys Ile Pro Ser Ile Ala Thr
1100 1105 1110

Gly Met Val Gly Ala Leu Leu Leu Leu Leu Val Val Ala Leu Gly
1115 1120 1125

Ile Gly Leu Phe Met
1130

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<211> 1146
<212> DNA
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<223> T2A-EGFRt

<400> 13
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cccggcccta ggatgcttct cctggtgaca agccttctgc tctgtgagtt accacaccca 120

gcattcctcc tgatcccacg caaagtgtgt aacggaatag gtattggtga atttaaagac 180

tcactctcca taaatgctac gaatattaaa cacttcaaaa actgcacctc catcagtggc 240

gatctccaca tcctgccggt ggcatttagg ggtgactcct tcacacatac tcctcctctg 300

gatccacagg aactggatat tctgaaaacc gtaaaggaaa tcacagggtt tttgctgatt 360

caggcttggc ctgaaaacag gacggacctc catgcctttg agaacctaga aatcatacgc 420

ggcaggacca agcaacatgg tcagttttct cttgcagtcg tcagcctgaa cataacatcc 480

ttgggattac gctccctcaa ggagataagt gatggagatg tgataatttc aggaaacaaa 540

aatttgtgct atgcaaatac aataaactgg aaaaaactgt ttgggacctc cggtcagaaa 600

accaaaatta taagcaacag aggtgaaaac agctgcaagg ccacaggcca ggtctgccat 660

gccttgtgct cccccgaggg ctgctggggc ccggagccca gggactgcgt ctcttgccgg 720

aatgtcagcc gaggcaggga atgcgtggac aagtgcaacc ttctggaggg tgagccaagg 780

gagtttgtgg agaactctga gtgcatacag tgccacccag agtgcctgcc tcaggccatg 840

aacatcacct gcacaggacg gggaccagac aactgtatcc agtgtgccca ctacattgac 900

ggcccccact gcgtcaagac ctgcccggca ggagtcatgg gagaaaacaa caccctggtc 960

tggaagtacg cagacgccgg ccatgtgtgc cacctgtgcc atccaaactg cacctacgga 1020

tgcactgggc caggtcttga aggctgtcca acgaatgggc ctaagatccc gtccatcgcc 1080

actgggatgg tgggggccct cctcttgctg ctggtggtgg ccctggggat cggcctcttc 1140

atgtga 1146

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Leu Glu Gly Gly Gly Glu Gly Arg Gly Ser Leu Leu Thr Cys Gly Asp
1 5 10 15

gtg gag gag aat ccc ggc cct agg atg ctt ctc ctg gtg aca agc ctt 96
Val Glu Glu Asn Pro Gly Pro Arg Met Leu Leu Leu Val Thr Ser Leu
20 25 30

ctg ctc tgt gag tta cca cac cca gca ttc ctc ctg atc cca cgc aaa 144
Leu Leu Cys Glu Leu Pro His Pro Ala Phe Leu Leu Ile Pro Arg Lys
35 40 45

gtg tgt aac gga ata ggt att ggt gaa ttt aaa gac tca ctc tcc ata 192
Val Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys Asp Ser Leu Ser Ile
50 55 60

aat gct acg aat att aaa cac ttc aaa aac tgc acc tcc atc agt ggc 240
Asn Ala Thr Asn Ile Lys His Phe Lys Asn Cys Thr Ser Ile Ser Gly
65 70 75 80

gat ctc cac atc ctg ccg gtg gca ttt agg ggt gac tcc ttc aca cat 288
Asp Leu His Ile Leu Pro Val Ala Phe Arg Gly Asp Ser Phe Thr His

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                85                  90                  95

act cct cct ctg gat cca cag gaa ctg gat att ctg aaa acc gta aag        336
Thr Pro Pro Leu Asp Pro Gln Glu Leu Asp Ile Leu Lys Thr Val Lys
            100                 105                 110

gaa atc aca ggg ttt ttg ctg att cag gct tgg cct gaa aac agg acg        384
Glu Ile Thr Gly Phe Leu Leu Ile Gln Ala Trp Pro Glu Asn Arg Thr
        115                 120                 125

gac ctc cat gcc ttt gag aac cta gaa atc ata cgc ggc agg acc aag        432
Asp Leu His Ala Phe Glu Asn Leu Glu Ile Ile Arg Gly Arg Thr Lys
    130                 135                 140

caa cat ggt cag ttt tct ctt gca gtc gtc agc ctg aac ata aca tcc        480
Gln His Gly Gln Phe Ser Leu Ala Val Val Ser Leu Asn Ile Thr Ser
145                 150                 155                 160

ttg gga tta cgc tcc ctc aag gag ata agt gat gga gat gtg ata att        528
Leu Gly Leu Arg Ser Leu Lys Glu Ile Ser Asp Gly Asp Val Ile Ile
                165                 170                 175

tca gga aac aaa aat ttg tgc tat gca aat aca ata aac tgg aaa aaa        576
Ser Gly Asn Lys Asn Leu Cys Tyr Ala Asn Thr Ile Asn Trp Lys Lys
            180                 185                 190

ctg ttt ggg acc tcc ggt cag aaa acc aaa att ata agc aac aga ggt        624
Leu Phe Gly Thr Ser Gly Gln Lys Thr Lys Ile Ile Ser Asn Arg Gly
        195                 200                 205

gaa aac agc tgc aag gcc aca ggc cag gtc tgc cat gcc ttg tgc tcc        672
Glu Asn Ser Cys Lys Ala Thr Gly Gln Val Cys His Ala Leu Cys Ser
    210                 215                 220

ccc gag ggc tgc tgg ggc ccg gag ccc agg gac tgc gtc tct tgc cgg        720
Pro Glu Gly Cys Trp Gly Pro Glu Pro Arg Asp Cys Val Ser Cys Arg
225                 230                 235                 240

aat gtc agc cga ggc agg gaa tgc gtg gac aag tgc aac ctt ctg gag        768
Asn Val Ser Arg Gly Arg Glu Cys Val Asp Lys Cys Asn Leu Leu Glu
                245                 250                 255

ggt gag cca agg gag ttt gtg gag aac tct gag tgc ata cag tgc cac        816
Gly Glu Pro Arg Glu Phe Val Glu Asn Ser Glu Cys Ile Gln Cys His
            260                 265                 270

cca gag tgc ctg cct cag gcc atg aac atc acc tgc aca gga cgg gga        864
Pro Glu Cys Leu Pro Gln Ala Met Asn Ile Thr Cys Thr Gly Arg Gly
        275                 280                 285

cca gac aac tgt atc cag tgt gcc cac tac att gac ggc ccc cac tgc        912
Pro Asp Asn Cys Ile Gln Cys Ala His Tyr Ile Asp Gly Pro His Cys
    290                 295                 300

gtc aag acc tgc ccg gca gga gtc atg gga gaa aac aac acc ctg gtc        960
Val Lys Thr Cys Pro Ala Gly Val Met Gly Glu Asn Asn Thr Leu Val
305                 310                 315                 320

tgg aag tac gca gac gcc ggc cat gtg tgc cac ctg tgc cat cca aac       1008
Trp Lys Tyr Ala Asp Ala Gly His Val Cys His Leu Cys His Pro Asn
                325                 330                 335

tgc acc tac gga tgc act ggg cca ggt ctt gaa ggc tgt cca acg aat       1056
Cys Thr Tyr Gly Cys Thr Gly Pro Gly Leu Glu Gly Cys Pro Thr Asn
            340                 345                 350

ggg cct aag atc ccg tcc atc gcc act ggg atg gtg ggg gcc ctc ctc       1104
Gly Pro Lys Ile Pro Ser Ile Ala Thr Gly Met Val Gly Ala Leu Leu
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355 360 365

ttg ctg ctg gtg gtg gcc ctg ggg atc ggc ctc ttc atg tga 1146
Leu Leu Leu Val Val Ala Leu Gly Ile Gly Leu Phe Met
370 375 380

<210> 15
<211> 381
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 15

Leu Glu Gly Gly Gly Glu Gly Arg Gly Ser Leu Leu Thr Cys Gly Asp
1 5 10 15

Val Glu Glu Asn Pro Gly Pro Arg Met Leu Leu Leu Val Thr Ser Leu
20 25 30

Leu Leu Cys Glu Leu Pro His Pro Ala Phe Leu Leu Ile Pro Arg Lys
35 40 45

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

Asn Ala Thr Asn Ile Lys His Phe Lys Asn Cys Thr Ser Ile Ser Gly
65 70 75 80

Asp Leu His Ile Leu Pro Val Ala Phe Arg Gly Asp Ser Phe Thr His
85 90 95

Thr Pro Pro Leu Asp Pro Gln Glu Leu Asp Ile Leu Lys Thr Val Lys
100 105 110

Glu Ile Thr Gly Phe Leu Leu Ile Gln Ala Trp Pro Glu Asn Arg Thr
115 120 125

Asp Leu His Ala Phe Glu Asn Leu Glu Ile Ile Arg Gly Arg Thr Lys
130 135 140

Gln His Gly Gln Phe Ser Leu Ala Val Val Ser Leu Asn Ile Thr Ser
145 150 155 160

Leu Gly Leu Arg Ser Leu Lys Glu Ile Ser Asp Gly Asp Val Ile Ile
165 170 175

Ser Gly Asn Lys Asn Leu Cys Tyr Ala Asn Thr Ile Asn Trp Lys Lys
180 185 190

Leu Phe Gly Thr Ser Gly Gln Lys Thr Lys Ile Ile Ser Asn Arg Gly
195 200 205

```
Glu Asn Ser Cys Lys Ala Thr Gly Gln Val Cys His Ala Leu Cys Ser
    210                 215                 220

Pro Glu Gly Cys Trp Gly Pro Glu Pro Arg Asp Cys Val Ser Cys Arg
225                 230                 235                 240

Asn Val Ser Arg Gly Arg Glu Cys Val Asp Lys Cys Asn Leu Leu Glu
                245                 250                 255

Gly Glu Pro Arg Glu Phe Val Glu Asn Ser Glu Cys Ile Gln Cys His
            260                 265                 270

Pro Glu Cys Leu Pro Gln Ala Met Asn Ile Thr Cys Thr Gly Arg Gly
        275                 280                 285

Pro Asp Asn Cys Ile Gln Cys Ala His Tyr Ile Asp Gly Pro His Cys
    290                 295                 300

Val Lys Thr Cys Pro Ala Gly Val Met Gly Glu Asn Asn Thr Leu Val
305                 310                 315                 320

Trp Lys Tyr Ala Asp Ala Gly His Val Cys His Leu Cys His Pro Asn
                325                 330                 335

Cys Thr Tyr Gly Cys Thr Gly Pro Gly Leu Glu Gly Cys Pro Thr Asn
            340                 345                 350

Gly Pro Lys Ile Pro Ser Ile Ala Thr Gly Met Val Gly Ala Leu Leu
        355                 360                 365

Leu Leu Leu Val Val Ala Leu Gly Ile Gly Leu Phe Met
    370                 375                 380
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