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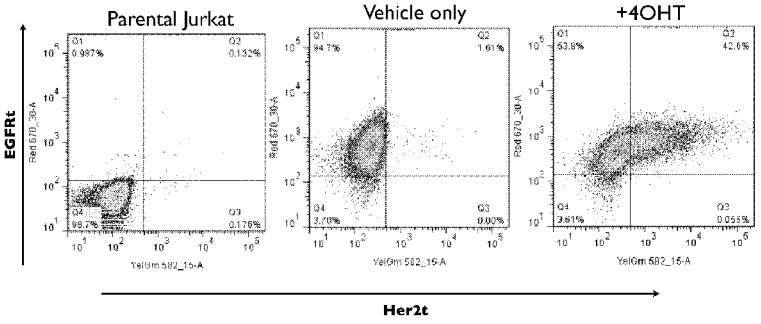
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(54) Title: DRUG RELATED TRANSGENE EXPRESSION

Fig 6A



(57) Abstract: The present invention provides nucleic acids, vectors, host cells, methods and compositions to confer and/or augment immune responses mediated by cellular immunotherapy, such as by adoptively transferring CD8+ central memory T cells or combinations of central memory T cells with CD4+ T cells that are genetically modified to express a chimeric receptor under the control of an inducible promoter. In some alternatives the genetically modified host cell comprises a nucleic acid comprising a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, a polynucleotide comprising a spacer region, a polynucleotide comprising a transmembrane domain, and a polynucleotide comprising an intracellular signaling domain under the control of a drug inducible promoter. Controlling the expression of the chimeric receptor provides for the ability to turn expression on and off depending on the status of the patient. Pharmaceutical formulations produced by the method, and methods of using the same, are also described.

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DRUG REGULATED TRANSGENE EXPRESSION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of priority to U.S. Provisional Patent Application No. 62/058,973, filed October 2, 2014, U.S. Provisional Patent Application No. 61/977,751, filed April 10, 2014, U.S. Provisional Patent Application No. 61/986,479, filed April 30, 2014, U.S. Provisional Patent Application No. 62/089,730 filed December 9, 2014, U.S. Provisional Patent Application No. 62/090845, filed December 11, 2014, and U.S. Provisional Patent Application No. 62/088,363, filed December 5, 2014. The entire disclosures of the aforementioned applications are expressly incorporated by reference in their entireties.

REFERENCE TO SEQUENCE LISTING, TABLE, OR COMPUTER PROGRAM LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled SCRI-53WO_SEQUENCE_LISTING.TXT, created April 7, 2015, which is 65kb in size. The information is the electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0003] The adoptive transfer of human T lymphocytes that are engineered by gene transfer to express chimeric antigen receptors (CARs) specific for surface molecules expressed on tumor cells has the potential to effectively treat cancer. Chimeric receptors are synthetic receptors that include an extracellular ligand binding domain, most commonly a single chain variable fragment of a monoclonal antibody (scFv) linked to intracellular signaling components, most commonly CD3ζ alone or combined with one or more costimulatory domains. Much of the research in the design of chimeric receptors has focused on defining scFvs and other ligand binding elements that target malignant cells without causing serious toxicity to essential normal tissues, and on defining the optimal composition of intracellular signaling modules to activate T cell effector functions.

[0004] Although, T cell (CAR-T) adoptive therapy clinical trials (chimeric antigen receptor expressing T cells) are demonstrating potent anti-tumor activity, it is apparent that

significant toxicities can arise, for example, engraftment-induced cytokine storm, tumor lysis syndromes and ongoing B cell cytopenias, each of which are attributable to unregulated functional outputs of constitutively expressed CARs. Such toxicities can in some context threaten to limit the applicability of CAR-T cell adoptive therapy. Clinical trials using transgene-modified adoptive T cell immunotherapies have only tested T cells that constitutively express the transgene, or are always in the "ON" state, contributing in large part to transgene associated side-effects. Suicide gene-mediated elimination of CAR-T cells can ameliorate such toxicities; however, this approach risks premature attenuation of anti-tumor activity and significantly impacts curative potential.

[0005] Current small molecule-regulated transgene expression technologies rely on a variety of drug inputs including macrolides, ecdysones and rapamycin analogs. Clinical applicability of these systems is limited due to toxic off target effects, unfavorable biodistribution and pharmacodynamics profiles, limited output dynamic range, and/or limited availability as FDA-approved commercially available pharmaceuticals. Furthermore, many of these systems use chimeric transcriptional regulators built from xenogeneic components, thus introducing the complication of immunogenicity when applying these systems to human therapeutics.

[0006] There is a need to identify methods for determining elements of chimeric receptor design that are important for therapeutic activity and cell populations to genetically modify and adoptively transfer that will provide enhanced survival and efficacy in vivo while minimizing adverse side effects. There is also a need for expression systems and methods for modulating cells for use in cell therapy, such as for modulating expression of recombinant antigen receptors such as CARs and/or other molecules expressed by such cells, such as to improve therapeutic activity, enhanced survival and/or efficacy *in vivo* and/or minimize adverse side effects.

SUMMARY OF THE INVENTION

[0007] One aspect of the disclosure includes a genetic system to deliver drug-regulated transgene expression in cells. In an alternative, regulated transgene expression is targeted to cells, such as lymphocytes designed for use in adoptive immunotherapy. This system provides rigorous safety attributes to chimeric antigen receptor (CAR) redirected adoptive therapeutic strategies without sacrificing curative intent that permits real-time clinician control of CAR expression *in vivo*. By engineering vectors that enable drug responsive transcriptional control of CAR expression, the activity of CARs and other cell

mediators can be turned "ON" and "OFF" *in vivo,* based on a clinician prescribed pharmaceutical drug input that exhibits clinically permissive pharmacokinetics, tissue distribution, and partitioning between the extracellular space and cytosol of lymphocytes. The genetic system provides for drug regulated transgene expression to enforce a functional "OFF" state in the absence of the drug and a functional "ON" state transgene expression in the presence of the drug.

[0008] One alternative of such a drug is tamoxifen. Tamoxifen is an estrogen antagonist/partial agonist that is an FDA-approved and commercially available drug. It is taken orally and can be administered on a daily basis over an extended period of time. Tamoxifen has a proven safety record, favorable pharmacokinetic profile, excellent tissue distribution and a low partition coefficient between the extracellular space and cytosol. Other drugs can be selected based on safety record, favorable pharmacokinetic profile, and excellent tissue distribution, a low partition coefficient between the extracellular space and cytosol, and/or low toxicities.

[0009] In some alternatives, the system employs a synthetic transcriptional regulator, which, in the presence of tamoxifen, binds a synthetic promoter upstream of a transgene to induce expression. The tamoxifen regulated transcription factor ("TamR-tf', also designated "HEA3") is a chimeric transcription factor composed of human subunits including the N-terminal DNA binding domain of Hepatocyte Nuclear Factor 1-alpha (HNF-1α) fused in frame to the mutant tamoxifen-specific ligand binding domain of the estrogen receptor ligand binding domain (ER-LBD), that is in turn fused to the p65 activation domain of NF-κB (p65). An exemplary amino acid sequence is provided in Table 10 and is identified as SEQ ID NO: 40. The mutant tamoxifen-specific ligand binding domain of the estrogen receptor ligand binding domain (ER-LBD) is found at amino acids 282-595 of the TamR-tf and has a mutation at position 521. The p65 activation domain of NF-κB (p65) is found at amino acids 596-862 of SEQ ID NO: 40. Further changes can be made to the transcriptional activator to increase the properties of the transcription factor including, without limitation, altering one or more amino acids in the estrogen receptor ligand binding domain to increase the affinity of the factor for estrogen analogs and altering one or more amino acids in the p65 transactivating domain.

[0010] In the absence of tamoxifen, TamR-tf is excluded from the nucleus by binding of cytosolic heat-shock protein 90 (HSP90) to the tamoxifen binding active site and transgene expression is in the "OFF" state. Nanomolar concentrations of cytosolic tamoxifen

actively out competes HSP90 for ER-LBD binding, resulting in TamR-tf translocation to the nucleus. Upon nuclear translocation, TamR-tf is readily available to bind its restricted synthetic promoter (e.g. 7xHBD/EF1αp). In the presence of tamoxifen, binding of TamR-tf to 7xHBD/EF1αp promoter induces the "ON" state of transgene expression. In some alternatives, this transcriptional regulator can be modified to provide for a varying level of control of transgene expression. Amino acid substitutions in the LBD of TamR-tf permit selective responsiveness to tamoxifen and its metabolites, where 4-hydroxy tamoxifen (4-OHT) is the most pharmacologically active metabolite, in regards to TamR-tf activity, while lacking interaction with endogenous estrogen.

[0011] In one aspect, the present disclosure relates to methods and compositions to confer and/or augment immune responses mediated by cellular immunotherapy, such as, by adoptively transferring tumor-specific, genetically modified subsets of CD8+ or CD4+ T cells alone, or in combination. The disclosure provides for chimeric receptor nucleic acids, and vectors and host cells including such nucleic acids. The nucleic acid sequence that encodes the chimeric receptor links together a number of modular components that can be excised and replaced with other components in order to customize the chimeric receptor for efficient T cell activation and recognition of a specific target molecule or an epitope on the target molecule and provide for regulated transcription as described herein.

[0012] In some alternatives, a system for inducible expression of a chimeric antigen receptor comprises: a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain; and a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the second promoter is constitutive or inducible.

[0013] In some alternatives, a system for inducible expression of a chimeric antigen receptor comprises: a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine, a polypeptide that regulates apoptosis and/or a polypeptide that modulates checkpoint signaling; and a second nucleic acid comprising a second promoter operably linked to a nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the second promoter is constitutive or inducible. In an exemplary alternative, the second nucleic acid further comprises a polynucleotide coding for a chimeric antigen receptor, under the control of a constitutive promoter. In some alternatives, the drug is tamoxifen and/or its metabolites.

[0014] In another aspect, the present disclosure provides compositions to confer and/or augment immune responses mediated by cellular immunotherapy, such as by adoptively transferring tumor-specific, subset specific genetically modified CD4+ T cells, wherein the CD4+ T cells confer and/or augment the ability of CD8+ T cells to sustain anti-tumor reactivity and increase and/or maximize tumor-specific proliferation. In some alternatives, the CD4+ cells are genetically modified to express a chimeric receptor nucleic acid and/or chimeric receptor polypeptide under the control of a regulated promoter, as described herein.

[0015] In another aspect, the present disclosure provides compositions to confer and/or augment immune responses mediated by cellular immunotherapy, such as by adoptively transferring tumor-specific, subset specific genetically modified T cells. In some alternatives, the cells are precursor T cells. In some alternatives, the cells are hematopoietic stem cells. In some alternatives, the cells are CD8+ T cells. In some alternatives, the CD8+ T cells express a chimeric receptor nucleic acid and/or chimeric receptor polypeptide under the control of a regulated promoter, as described herein.

[0016] Some alternatives concern methods of performing cellular immunotherapy in a subject having a disease or disorder by administering to the subject a genetically modified T lymphocyte cell preparation that provides a cellular immune response and administering a drug that induces a transgene in the genetically modified T lymphocyte cells. In some alternatives, the genetically modified T lymphocyte cell preparation comprises precursor T cells. In some alternatives, the genetically modified T lymphocyte cell preparation comprises hematopoietic stem cells. In some alternatives, the genetically modified CD8+ and genetically modified CD4+ cell population are co-administered. In some alternatives, the drug is

tamoxifen and/or its metabolites. In some alternatives, the T cells are autologous or allogeneic T cells. Various modifications of the above method are possible. For example, the chimeric receptor that is expressed by the CD4+ T cell and the CD8+ T cell can be the same or different.

[0017] In some alternatives, a system for inducible expression of a chimeric antigen receptor is provided, wherein the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the second promoter is inducible. In some alternatives, the second promoter is constitutive. In some alternatives, the first promoter comprises a nucleic acid sequence of SEQ ID NO: 41. In some alternatives, the second promoter is a constitutive promoter. In some alternatives, the second promoter is the EF1αp. In some alternatives, the transcriptional activator comprises a sequence of SEQ ID NO: 40. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives, both vectors are packaged in a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the first and second nucleic acid comprise a vector. In some alternatives, the first nucleic acid further comprises a nucleic acid sequence coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a nucleic acid coding for a selectable marker. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor.

[0018] In some alternatives, a system for inducible expression of a chimeric antigen receptor is provided, wherein the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain,

wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter.. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment. In some alternatives, the ligand binding domain is single chain variable fragment. In some alternatives, the tumor specific molecule is CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, or MAGE A3 TCR or combinations thereof. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the second promoter is inducible. In some alternatives, the second promoter is constitutive.

[0019] In some alternatives, a chimeric receptor polypeptide is provided, wherein the chimeric receptor polypetide is coded for by a system. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide

spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the first promoter comprises a nucleic acid sequence of SEQ ID NO: 41. In some alternatives, the second promoter is a constitutive promoter. In some alternatives, the second promoter is the EF1αp. In some alternatives, the transcriptional activator comprises a sequence of SEQ ID NO: 40. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives, both vectors are packaged in a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the first and second nucleic acid comprise a vector. In some alternatives, the first nucleic acid further comprises a nucleic acid sequence coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a nucleic acid coding for a selectable marker. In some alternatives, the second promoter is an inducible promoter. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor.

[0020] In some alternatives, a system for inducible expression of chimeric antigen receptor is provided, wherein the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a polypeptide that modulates checkpoint signaling and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some

alternatives, the ligand binding domain is an antibody fragment, preferably a binding domain thereof. In some alternatives, the tumor specific molecule is CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, or MAGE A3 TCR or combinations thereof. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a polypeptide that modulates checkpoint signaling; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for chimeric antigen receptor comprising a ligand binding domain, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment, preferably a binding fragment thereof. In some alternatives, the ligand binding domain is single chain variable fragment. In some alternatives, the tumor specific molecule is CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, or MAGE A3 TCR or combinations thereof. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor.

[0021] In some alternatives, a host cell is provided, wherein the host cell comprises a system. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a

transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the first promoter comprises a nucleic acid sequence of SEQ ID NO: 41. In some alternatives, the second promoter is a constitutive promoter. In some alternatives, the second promoter is the EF1αp. In some alternatives, the transcriptional activator comprises a sequence of SEQ ID NO: 40. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives, both vectors are packaged in a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the first and second nucleic acid comprise a vector. In some alternatives, the first nucleic acid further comprises a nucleic acid sequence coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a nucleic acid coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment, preferably a binding fragment thereof. In some alternatives, the tumor specific molecule is CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, or MAGE A3 TCR or combinations thereof. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a polypeptide that modulates checkpoint signaling; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for chimeric antigen receptor comprising a ligand binding domain, wherein the ligand is a tumor specific molecule, viral

molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment, preferably a binding fragment thereof. In some alternatives, the ligand binding domain is single chain variable fragment. In some alternatives, the tumor specific molecule is CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, or MAGE A3 TCR or combinations thereof. In some alternatives, the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells. In some alternatives, the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the host cell is precursor T cell. In some alternatives, the precursor T cell is a hematopoietic stem cell.

[0022] In some alternatives, a composition is provided, wherein the composition comprises a host cell in a pharmaceutically acceptable excipient. In some alternatives, the host cell comprises a system. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the first promoter comprises a nucleic acid sequence of SEQ ID NO: 41. In some alternatives, the second promoter is a constitutive

promoter. In some alternatives, the second promoter is the EF1αp. In some alternatives, the transcriptional activator comprises a sequence of SEQ ID NO: 40. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives, both vectors are packaged in a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the first and second nucleic acid comprise a vector. In some alternatives, the first nucleic acid further comprises a nucleic acid sequence coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a nucleic acid coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment. In some alternatives, the tumor specific molecule is CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, or MAGE A3 TCR or combinations thereof. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a polypeptide that modulates checkpoint signaling; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment, preferably a binding

fragment thereof. In some alternatives, the ligand binding domain is single chain variable fragment. In some alternatives, the tumor specific molecule is CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, or MAGE A3 TCR or combinations thereof. In some alternatives, the host cell is precursor T cell. In some alternatives, the precursor T cell is a hematopoietic stem cell. In some alternatives, the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells. In some alternatives, the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells. In some alternatives, the composition comprises a host cell wherein the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells and further comprises another host cell wherein the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells. In some alternatives, the host cell is precursor T cell. In some alternatives, the precursor T cell is a hematopoietic stem cell. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the composition comprises a host cell wherein the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells or the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells and a second host cell, wherein the second host cell is a precursor T cell or a hematopoietic stem cell.

[0023] In some alternatives, an *in vitro* method for preparing a host cell is provided wherein the method comprises a) providing a system and b) introducing the system into a separate isolated T lymphocyte population and expanding each T lymphocyte population in vitro. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a

tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the first promoter comprises a nucleic acid sequence of SEQ ID NO: 41. In some alternatives, the second promoter is a constitutive promoter. In some alternatives, the second promoter is the EF1αp. In some alternatives, the transcriptional activator comprises a sequence of SEQ ID NO: 40. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives, both vectors are packaged in a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the first and second nucleic acid comprise a vector. In some alternatives, the first nucleic acid further comprises a nucleic acid sequence coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a nucleic acid coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment, preferably a binding fragment thereof. In some alternatives, the tumor specific molecule is CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, or MAGE A3 TCR or combinations thereof. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a

polypeptide that modulates checkpoint signaling; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for chimeric antigen receptor comprising a ligand binding domain, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment, preferably a binding fragment thereof. In some alternatives, the ligand binding domain is single chain variable fragment. In some alternatives, the tumor specific molecule is CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, or MAGE A3 TCR or combinations thereof. In some alternatives, wherein the T lymphocytes are expanded, the method further comprises culturing the cells in the presence of anti-CD3 and/or anti CD28, and at least one homeostatic cytokine until the cells expand sufficiently for use as a cell infusion. In some alternatives, the lymphocyte is CD8+ or CD4+. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the host cell is precursor T cell. In some alternatives, the precursor T cell is a hematopoietic stem cell.

[0024] In some alternatives, a use of a host cell or a composition in combination with a drug that induces expression of a transgene in the host cell or composition for the treatment of cancer or a viral infection is provided. In some alternatives, the host cell comprises a system. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a

polynucleotide coding for an intracellular signaling domain; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the first promoter comprises a nucleic acid sequence of SEQ ID NO: 41. In some alternatives, the second promoter is a constitutive promoter. In some alternatives, the second promoter is the EF1αp. In some alternatives, the transcriptional activator comprises a sequence of SEQ ID NO: 40. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives, both vectors are packaged in a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the first and second nucleic acid comprise a vector. In some alternatives, the first nucleic acid further comprises a nucleic acid sequence coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a nucleic acid coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment. In some alternatives, the tumor specific molecule is CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, or MAGE A3 TCR or combinations thereof. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a polypeptide that modulates checkpoint signaling; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for chimeric antigen receptor comprising a ligand binding domain, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell

population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment, preferably a binding fragment thereof. In some alternatives, the ligand binding domain is single chain variable fragment. In some alternatives, the tumor specific molecule is CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, or MAGE A3 TCR or combinations thereof. In some alternatives, the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells. In some alternatives, the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells. In some alternatives, the cells are precursor T cells. In some alternatives, the cells are hematopoietic stem cells. In some alternatives, the composition comprises a host cell in a pharmaceutically acceptable excipient. In some alternatives, the host cell comprises a system. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the first promoter comprises a nucleic acid sequence of SEQ ID NO: 41. In some alternatives, the second promoter is a constitutive promoter. In some alternatives, the second promoter is the EF1αp. In some alternatives, the transcriptional activator comprises a sequence of SEQ ID NO: 40. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives, both vectors are packaged in a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the first and

second nucleic acid comprise a vector. In some alternatives, the first nucleic acid further comprises a nucleic acid sequence coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a nucleic acid coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment, preferably a binding fragment thereof. In some alternatives, the tumor specific molecule is CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, or MAGE A3 TCR or combinations thereof. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a polypeptide that modulates checkpoint signaling; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment, preferably a binding fragment thereof. In some alternatives, the ligand binding domain is single chain variable fragment. In some alternatives, the tumor specific molecule is CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, or MAGE A3 TCR or combinations thereof. In some alternatives, the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central

memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells. In some alternatives, the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells. In some alternatives, the composition comprises a host cell wherein the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells and another host cell wherein the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells. In some alternatives, the cancer is a solid tumor or hematologic malignancy. In some alternatives, the solid tumor is selected from the group consisting of a breast cancer, brain cancer, lung cancer, colon cancer, renal cancer, pancreatic cancer, prostate cancer, and ovarian cancer. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the host cell is precursor T cell. In some alternatives, the precursor T cell is a hematopoietic stem cell. In some alternatives, the host cell is precursor T cell. In some alternatives, the precursor T cell is a hematopoietic stem cell. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the composition comprises a host cell wherein the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells or the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells and a second host cell, wherein the second host cell is a precursor T cell or a hematopoietic stem cell.

[0025] In some alternatives, a method of performing cellular immunotherapy in a subject having cancer or a viral infection is provided wherein the method comprises administering a composition or a host cell to the subject and administering a drug that induces expression of a transgene in the composition or the host cells. In some alternatives, the host cell comprises a system. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a

tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the first promoter comprises a nucleic acid sequence of SEQ ID NO: 41. In some alternatives, the second promoter is a constitutive promoter. In some alternatives, the second promoter is the EF1αp. In some alternatives, the transcriptional activator comprises a sequence of SEQ ID NO: 40. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives, both vectors are packaged in a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the first and second nucleic acid comprise a vector. In some alternatives, the first nucleic acid further comprises a nucleic acid sequence coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a nucleic acid coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment, preferably a binding fragment thereof. In some alternatives, the tumor specific molecule is CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, or MAGE A3 TCR or combinations thereof. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a polypeptide that modulates checkpoint signaling; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid

coding for a transcriptional activator for the inducible promoter. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment, preferably a binding fragment thereof. In some alternatives, the ligand binding domain is single chain variable fragment. In some alternatives, the tumor specific molecule is CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, or MAGE A3 TCR or combinations thereof. In some alternatives, the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells. In some alternatives, the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells. In some alternatives, the composition comprises a host cell in a pharmaceutically acceptable excipient. In some alternatives, the host cell comprises a system. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the first promoter comprises a nucleic acid sequence of SEQ ID NO: 41. In some alternatives, the second promoter is a constitutive promoter. In some alternatives, the second promoter is the EF1αp. In some alternatives, the

transcriptional activator comprises a sequence of SEQ ID NO: 40. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives, both vectors are packaged in a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the first and second nucleic acid comprise a vector. In some alternatives, the first nucleic acid further comprises a nucleic acid sequence coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a nucleic acid coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment, preferably a binding fragment thereof. In some alternatives, the tumor specific molecule is CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, or MAGE A3 TCR or combinations thereof. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a polypeptide that modulates checkpoint signaling; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment, preferably a binding fragment thereof. In some alternatives, the ligand binding domain is single chain variable fragment. In some

alternatives, the tumor specific molecule is CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, or MAGE A3 TCR or combinations thereof. In some alternatives, the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells. In some alternatives, the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells. In some alternatives, the composition comprises a host cell wherein the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells and another host cell wherein the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells. In some alternatives, the host cell is precursor T cell. In some alternatives, the precursor T cell is a hematopoietic stem cell. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the composition comprises a host cell wherein the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells or the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells and a second host cell, wherein the second host cell is a precursor T cell or a hematopoietic stem cell. In some alternatives, the cancer is selected from a solid tumor or hematologic malignancy. In some alternatives, the solid tumor is selected from the group consisting of a breast cancer, brain cancer, lung cancer, colon cancer, renal cancer, pancreatic cancer, prostate cancer, and ovarian cancer. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the host cell is a precursor T cell. In some alternatives, the precursor T cell is a hematopoietic stem cell. In some alternatives, the isolated T lymphocyte population comprises precursor T cells. In some alternatives, the precursor T cells are hematopoietic stem cells. In some alternatives, the administering of the drug is performed after administering of the composition or host cells, wherein administering is performed 1

day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 2 weeks, 4 weeks or two months, or any time in between any two values of time listed.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] **Figure 1A** shows the expression of ZsGreen and EGFRt as determined by flow cytometry in Jurkat cells transduced with a dual plasmid lentiviral construct including constructs A and B in the presence or absence of 4 hydroxy tamoxifen (4OHT). The results show the expression of EGFRt in the presence or absence of 4-OHT indicating the cells carry construct A. The results also show that the cells carry construct B as expression of ZsGreen is induced in the presence of 4-OHT. **Figure 1B** shows construct A, which comprises the constitutive promoter EF1αp linked to TamR-tf (HEA3) linked to EGFRt, and construct B comprising a synthetic promoter 7xHBD/mE1b linked to a polynucleotide coding for ZsGreen.

[0027] **Figure 2** shows the expression of ZsGreen in transduced Jurkat cells contacted with different doses of 4-OHT ranging from 50 to 1000nM.

[0028] **Figure 3** shows the on and off rate kinetics of expression of ZsGreen in transduced Jurkat cells with a single 48 hour treatment of 4-OHT followed by a washout (○) and tranduced Jurkat cells with a 24 hour treatment of 4-OHT followed by washout and then a re-stimulation with 4-OHT at day 15 (■).

[0029] **Figure 4A-D** shows the expression of ZsGreen in CD4 central memory cells transduced with dual package lentiviral constructs A and B. Cells were divided into 3 treatment groups, 4OHT alone (**Figure 4A**), 4OHT combined with CD3/CD28 bead co-treatment **(Figure 4B)** or 4OHT alone for 48 hours, followed by addition of CD3/CD28 beads (**Figure 4C**). Expression of ZsGreen was monitored over 96 hours. **Figure 4D** shows construct A, which comprises the constitutive promoter EF1αp linked to TamR-tf (HEA3) linked to EGFRt, and construct B comprising a synthetic promoter 7xHBD/mE1b linked to a polynucleotide coding for ZsGreen.

[0030] **Figures 5A-C** shows the expression of ZsGreen in CD8 central memory cells transduced with dual package lentiviral constructs A and B as shown in **Figure 4**. Cells were divided into 3 treatment groups, 4OHT alone (**Figures 5A**), 4OHT combined with CD3/CD28 bead co-treatment (**Figures 5B**) or 4OHT alone for 48 hours, followed by addition of CD3/CD28 beads (**Figures 5C**). Expression of ZsGreen was monitored over 72 hours.

[0031] **Figure 6A** shows expression of EGFRt and Her2t in Human Jurkat T cells transduced with constructs A and B as shown in **Figure 6C**. Expression of EGFRt and Her2t were monitored in the presence or absence of 4OHT. Samples were stained with EGFRt-APC antibody and Herceptin-biotin, followed by SA-PE. **Figure 6B** shows a western blot of parental Jurkat cells and transduced CD19CAR transduced cells stained with mouse antiCD247 which recognizes the intracellular CD3 zeta chain on CD19 CAR (about 48 kDA), the endogenous CD3 zeta chain has migrates at 23 kDA. The endogenous CD3 zeta chain was detected in all of the cells. The CD19 CAR CD3 zeta chain was only detected in transduced Jurkat cells exposed to 4-OHT. **Figure 6C** shows construct A, which comprises the constitutive promoter EF1αp linked to TamR-tf (HEA3) linked to EGFRt, and construct B comprising a synthetic promoter 7xHBD/mE1b linked to a polynucleotide coding for CD19CAR linked to a polynucleotide coding for Her2t.

[0032] **Figure 7A** shows expression of EGFRt and Her2t in Human Jurkat T cells transduced with TamR CD19CAR LV including an additional selective marker, DHFRdm in the presence or absence of 4OHT. Expression of EGFRt and Her2t were monitored in the presence or absence of 4OHT. Samples were stained with EGFRt-APC antibody and Herceptin-biotin, followed by SA-PE. **Figure 7B** shows a western blot of parental Jurkat cells and transduced CD19CAR transduced cells stained with mouse antiCD247 which recognizes the intracellular CD3 zeta chain on CD19 CAR (about 48 kDA), the endogenous CD3 zeta chain has migrates at 23 kDA. The endogenous CD3 zeta chain was detected in all of the cells. The CD19 CAR CD3 zeta chain was only detected in transduced Jurkat cells exposed to 4-OHT. **Figure 7C** shows construct A, which comprises the constitutive promoter EF1αp linked to TamR-tf(HEA3) linked to EGFRt, and construct B comprising a synthetic promoter 7xHBD/mE1b linked to a polynucleotide coding for CD19CAR linked to a polynucleotide coding for Her2t linked to a polynucleotide coding for DHFRdm.

[0033] **Figure 8A** shows expression of EGFRt and Her2t in Human CD4 central memory T cells transduced with TamR CD19CAR LV including an additional selective marker, DHFRdm in the presence or absence of 4OHT and antiCD3/CD28 beads. Expression of EGFRt and Her2t were monitored in the presence or absence of 4OHT and in the presence or absence of antiCD3/CD28 beads. Samples were stained with EGFRt-APC antibody and Herceptin-biotin, followed by SA-PE. **Figure 8B** shows construct A, which comprises the constitutive promoter EF1αp linked to TamR-tf(HEA3) linked to EGFRt, and construct B comprising a synthetic promoter 7xHBD/mE1b linked to a polynucleotide coding for

CD19CAR linked to a polynucleotide coding for Her2t linked to a polynucleotide coding for DHFRdm.

DETAILED DESCRIPTION

Definitions.

[0034] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains.

[0035] "About" as used herein when referring to a measurable value is meant to encompass variations of ±20% or ±10%, more preferably ±5%, even more preferably ±l%, and still more preferably ±0.1 % from the specified value.

[0036] Antigen" or "Ag" as used herein refers to a molecule that provokes an immune response. This immune response can involve either antibody production, or the activation of specific immunologically-competent cells, or both. It is readily apparent that an antigen can be generated synthesized, produced recombinantly or can be derived from a biological sample. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a biological fluid such, for example, blood, plasma or ascites fluid.

[0037] "Anti-tumor effect" as used herein, refers to a biological effect, which can be manifested by a decrease in tumor volume, a decrease in the number of tumor cells, a decrease in the number of metastases, an increase in life expectancy, or a decrease of various physiological symptoms associated with the cancerous condition. An "anti-tumor effect" can also be manifested by a decrease in recurrence or an increase in the time before recurrence.

[0038] "Chimeric receptor" as used herein refers to a synthetically designed receptor comprising a ligand binding domain of an antibody or other protein sequence that binds to a molecule associated with the disease or disorder and is linked via a spacer domain to one or more intracellular signaling domains of a T cell or other receptors, such as a costimulatory domain. Chimeric receptor can also be referred to as artificial T cell receptors, chimeric T cell receptors, chimeric immunoreceptors, and chimeric antigen receptors (CARs). These CARs are engineered receptors that can graft an arbitrary specificity onto an immune receptor cell. The term Chimeric antigen receptors or "CARs" are also considered by some investigators to include the antibody or antibody fragment, the spacer, signaling domain, and transmembrane region. However, due to the surprising effects of modifying the different components or domains of the CAR described herein, such as the epitope binding region (for

example, antibody fragment, scFv, or portion thereof), spacer, transmembrane domain, and/ or signaling domain), the components of the CAR are frequently distinguished throughout this disclosure in terms of independent elements. The variation of the different elements of the CAR can, for example, lead to stronger binding affinity for a specific epitope or antigen. "Co-stimulatory domain," as the term is used herein refers to a signaling moiety that provides to T cells a signal which, in addition to the primary signal provided by for instance the CD3 zeta chain of the TCR/CD3 complex, mediates a T cell response, including, but not limited to, activation, proliferation, differentiation, cytokine secretion, and the like. A co-stimulatory domain can include all or a portion of, but is not limited to, CD27, CD28, 4-1BB, OX40, CD30, CD40, , ICOS, lymphocyte function-associated antigen-l (LFA-l), CD2, CD7, LIGHT, NKG2C, B7-H3, or a ligand that specifically binds with CD83. In some alternatives, the co-stimulatory domain is an intracellular signaling domain that interacts with other intracellular mediators to mediate a cell response including activation, proliferation, differentiation and cytokine secretion, and the like. "Coding for" are used herein refers to the property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other macromolecules such as a defined sequence of amino acids. Thus, a gene codes for a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. A "nucleic acid sequence coding for a polypeptide" includes all nucleotide sequences that are degenerate versions of each other and that code for the same amino acid sequence.

[0039] "Conditional" or "Inducible" as used herein refer to the nucleic acid construct that includes a promoter that provides for gene expression in the presence of an inducer and does not substantially provide for gene expression in the absence of the inducer.

[0040] "Constitutive" as used herein refer to the nucleic acid construct that includes a promoter that is constitutive providing for expression of a polypeptide that is continuously produced.

[0041] "Specific" or "Specificity" can refer to the characteristic of a ligand for the binding partner or alternatively, the binding partner for the ligand, and can include complementary shape, charge and hydrophobic specificity for binding. Specificity for binding can include stereospecificity, regioselectivity and chemoselectivity. In some alternatives, a method of making a nucleic acid encoding a chimeric antigen receptor is provided such that a nucleic acid encoding a chimeric antigen receptor is generated.

[0042] "Regulate" or "modulate" as described herein, refers to the act of controlling a biological process, or to exert a modifying or controlling influence on a biological or cellular process or pathway. In some alternatives, a system for inducible expression of a chimeric antigen receptor is provided, wherein the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte, a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized, a polynucleotide coding for a transmembrane domain, and d) a polynucleotide coding for an intracellular signaling domain, and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, modulation comprises modulation of cellular differentiation and apoptosis. In some alternatives, modulation comprises modulation of cellular proliferation by regulation of activity of proteins. In some alternatives, the proteins are cell cycle regulators and transcription factors. In some alternatives, the cell cycle regulators are cyclin D1, p21, p27 and/or cdc25A. In some alternatives, the transcription factors are c-Myc.

[0043] "Cytotoxic T lymphocyte "(CTL) as used herein refers to a T lymphocyte that expresses CD8 on the surface thereof (e.g., a $CD8^+$ T cell). In some alternatives such cells are preferably "memory" T cells (T_M cells) that are antigen-experienced.

"Central memory" T cell (or "T_{CM}") as used herein refers to an antigen experienced CTL that expresses CD62L, CCR-7 and/or CD45RO on the surface thereof, and does not express or has decreased expression of CD45RA, as compared to naive cells. In some alternatives, central memory cells are positive for expression of CD62L, CCR7, CD28, CD127, CD45RO, and/or CD95, and may have decreased expression of CD54RA, as compared to naïve cells. "Effector memory" T cell (or "T_{EM}") as used herein refers to an antigen experienced T cell that does not express or has decreased expression of CD62L on the surface thereof, as compared to central memory cells, and does not express or has a decreased expression of CD45RA, as compared to naïve cell. In some alternatives, effector memory cells are negative for expression of CD62L and/or CCR7, as compared to naïve cells or central memory cells, and may have variable expression of CD28 and/or CD45RA.

[0044] "Naïve " T cells as used herein refers to a non-antigen experienced T lymphocyte that expresses CD62L and/or CD45RA, and does not express CD45RO-, as compared to central or effector memory cells. In some alternatives, naïve CD8+ T lymphocytes are characterized by the expression of phenotypic markers of naïve T cells including CD62L, CCR7, CD28, CD127, and/or CD45RA.

[0045] "Effector " "T_E" T cells as used herein refers to antigen experienced cytotoxic T lymphocyte cells that do not express or have decreased expression of CD62L, CCR7, and/or CD28, and are positive for granzyme B and/or perforin, as compared to central memory or naïve T cells.

"Enriched" and "depleted" as used herein to describe amounts of cell types in a mixture refers to the subjecting of the mixture of the cells to a process or step, which results in an increase in the number of the "enriched" type and a decrease in the number of the "depleted" cells. Thus, depending upon the source of the original population of cells subjected to the enriching process, a mixture or composition may contain 60, 70, 80, 90, 95, or 99 percent or more (in number or count) of the "enriched" cells and/or 40, 30, 20, 10, 5 or 1 percent or less (in number or count) of the "depleted" cells.

[0046] "Epitope" as used herein refers to a part of an antigen or molecule that is recognized by the immune system including antibodies, T cells, and/ or B cells. Epitopes usually have at least 7 amino acids and can be linear or conformational.

"Isolated," when used to describe the various polypeptides disclosed herein, means polypeptide or nucleic acid that has been identified and separated and/or recovered from a component of its natural environment. Preferably, the isolated polypeptide or nucleic acid is free of association with all components with which it is naturally associated. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide or nucleic acid, and can include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes.

[0047] "Intracellular signaling domain" as used herein refers to all or a portion of one or more domains of a molecule (here the chimeric receptor molecule) that provides for activation of a lymphocyte. Intracellular domains of such molecules mediate a signal by interacting with cellular mediators to result in proliferation, differentiation, activation and other effector functions. In some alternatives, such molecules include all or portions of CD28, CD3, or 4-1BB, or combinations thereof.

[0048] "Ligand" as used herein refers to a substance that binds specifically to another substance to form a complex. Examples of ligands include epitopes on antigens, molecules that bind to receptors, substrates, inhibitors, hormones, and/or activators. "Ligand binding domain" as used herein refers to substance or portion of a substance that binds to a ligand. Examples of ligand binding domains include antigen binding portions of antibodies, extracellular domains of receptors, and/or active sites of enzymes. "Operably linked" as used herein refers to functional linkage between a regulatory sequence and a heterologous nucleic acid sequence resulting in expression of the latter. For example, a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein coding regions, in the same reading frame.

[0049] "Percent (%) amino acid sequence identity" with respect to the chimeric receptor polypeptide sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference sequence for each of the ligand binding domain, spacer, transmembrane domain, and/or the lymphocyte activating domain, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, ALIGN-2 or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared. For example, % amino acid sequence identity values generated using the WU-BLAST-2 computer program [Altschul et al., Methods in Enzymology, 266:460-480 (1996)] uses several search parameters, most of which are set to the default values. Those that are not set to default values (i.e., the adjustable parameters) are set with the following values: overlap span=1, overlap fraction=0.125, word threshold (T) =11 and scoring matrix=BLOSUM62. A % amino acid sequence identity value is determined by dividing (a) the number of matching identical amino acid residues between the each or all of the polypeptide amino acid sequence of the reference chimeric receptor sequence provided in Table 2 and the comparison amino

acid sequence of interest as determined by WU-BLAST-2 by (b) the total number of amino acid residues of the polypeptide of interest. In some alternatives, the percent sequence identity of amino acids or nucleic acids are determined by computer software.

[0050] "Chimeric receptor variant polynucleotide" or "chimeric receptor variant nucleic acid sequence" as used herein refers to a polypeptide-encoding nucleic acid molecule as defined below having at least 80%, 85%, 90%, or 95% nucleic acid sequence identity (or a percentage nucleic acid sequence identity within a range defined by any two of the aforementioned percentages) with the polynucleotide acid sequence shown in **Table 1** or a specifically derived fragment thereof, such as polynucleotide coding for an antigen binding domain, a polynucleotide encoding a spacer domain, a polynucleotide coding for a transmembrane domain and/ or a polynucleotide coding for a lymphocyte stimulatory domain. Ordinarily, a chimeric receptor variant of polynucleotide or fragment thereof will have at least 80% nucleic acid sequence identity, more preferably at least 81% nucleic acid sequence identity, more preferably at least 82% nucleic acid sequence identity, more preferably at least 83% nucleic acid sequence identity, more preferably at least 84% nucleic acid sequence identity, more preferably at least 85% nucleic acid sequence identity, more preferably at least 86% nucleic acid sequence identity, more preferably at least 87% nucleic acid sequence identity, more preferably at least 88% nucleic acid sequence identity, more preferably at least 89% nucleic acid sequence identity, more preferably at least 90% nucleic acid sequence identity, more preferably at least 91% nucleic acid sequence identity, more preferably at least 92% nucleic acid sequence identity, more preferably at least 93% nucleic acid sequence identity, more preferably at least 94% nucleic acid sequence identity, more preferably at least 95% nucleic acid sequence identity, more preferably at least 96% nucleic acid sequence identity, more preferably at least 97% nucleic acid sequence identity, more preferably at least 98% nucleic acid sequence identity and yet more preferably at least 99% nucleic acid sequence identity with the nucleic acid sequence as shown in Table or a derived fragment thereof. Variants do not encompass the native nucleotide sequence. In this regard, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of chimeric receptor variant polynucleotides having at least 80% nucleic acid sequence identity to the nucleotide sequence of **Table 1** will encode a polypeptide having an amino acid sequence which is identical to the amino acid sequence of **Table 2**.

[0051] "Substantially purified" refers to a molecule that has 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% or less other molecule types or other cell types. A substantially

purified cell also refers to a cell, which has been separated from other cell types with which it is normally associated in its naturally occurring state. In some instances, a population of substantially purified cells refers to a homogenous population of cells.

[0052] "Not substantially found" when used in reference the presence of a tumor antigen or other molecules on normal cells refers to the percentage of a normal cell type that has the antigen or molecule, and / or the density of the antigen on the cells. In some alternatives, not substantially found means that the antigen or molecule is found on less than 50% of normal cell type and/or at a 50% less density as compared to the amount of cells or antigen found on a tumor cell or other diseased cell.

[0053] "T cells" or "T lymphocytes" as used herein can be from any mammalian, preferably primate, species, including monkeys, dogs, and humans. In some alternatives the T cells are allogeneic (from the same species but different donor) as the recipient subject; in some alternatives the T cells are autologous (the donor and the recipient are the same); in some alternatives the T cells arc syngeneic (the donor and the recipients are different but are identical twins).

[0054] "Vector" or "construct" is a nucleic acid used to introduce heterologous nucleic acids into a cell that has regulatory elements to provide expression of the heterologous nucleic acids in the cell. Vectors include but are not limited to plasmid, minicircles, yeast, and viral genomes. In some alternatives, the vectors are plasmid, minicircles, yeast, or viral genomes.

[0055] "Apoptosis" as described herein, refers to the process of programmed cell death (PCD) that can occur in multicellular organisms. Biochemical events lead to characteristic cell changes (morphology) and death. These changes include blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation. In apoptosis, a cell initiates intracellular apoptotic signaling in response to a stress, which can bring about cell suicide. The binding of nuclear receptors by glucocorticoids, heat, radiation, nutrient deprivation, viral infection, hypoxia and increased intracellular calcium concentration, for example, by damage to the membrane, can all trigger the release of intracellular apoptotic signals by a damaged cell. A number of cellular components, such as poly ADP ribose polymerase, can also help regulate apoptosis.

[0056] Before the actual process of cell death is precipitated by enzymes, apoptotic signals must cause regulatory proteins to initiate the apoptosis pathway. This step allows apoptotic signals to cause cell death, or the process to be stopped, should the cell no

longer need to die. Several proteins are involved, but two main methods of regulation have been identified: targeting mitochondria functionality, or directly transducing the signal via adaptor proteins to the apoptotic mechanisms. Another extrinsic pathway for initiation identified in several toxin studies is an increase in calcium concentration within a cell caused by drug activity, which also can cause apoptosis via a calcium binding protease calpain.

[0057] Apoptosis can be regulated by many factors. These factors can include but are not limited to genes that can express IL-2, IL-15, Chemokine receptors, Bcl2, CA-Akt, dn-TGFbetaRIII, dn-SHP1/2, and/or PD-1CD28 chimeras. IL-15 regulates T and natural killer cell activation and proliferation. In rodent lymphocytes, IL15 was shown to prevent apoptosis by inducing an apoptosis inhibitor, BCL2L1/BCL-X(L). In humans with celiac disease, IL-15 similarly suppresses apoptosis in T-lymphocytes by inducing Bcl-2 and/or BCL-xL. Bcl-2 (B-cell lymphoma 2), encoded in humans by the BCL2 gene, is the founding member of the Bcl-2 family of regulator proteins that regulate cell death (apoptosis), by either inducing (pro-apoptotic) it or inhibiting it (anti-apoptotic). Bcl-2 is specifically considered as an important anti-apoptotic protein and is, thus classified as an oncogene. Protein kinase B (PKB), also known as Akt, is a serine/threonine-specific protein kinase that plays a key role in multiple cellular processes such as glucose metabolism, apoptosis, cell proliferation, transcription and cell migration. In some alternatives, a system for inducible expression of chimeric antigen receptor is provided, wherein the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a polypeptide that modulates checkpoint signaling and b) a second nucleic acid comprising a second constitutive or inducible promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the polypeptide that regulates apoptosis or modulates checkpoint signaling comprises IL-2, IL-15, Chemokine receptors, Bcl2, CA-Akt, dn-TGFbetaRIII, dn-SHP1/2 or PD-1CD28 chimeras.

[0058] "Checkpoint signaling" as described herein, blocks the cell cycle at specific transition points, checkpoints to ensure that the events of the cell cycle take please in the correct order. Checkpoint signaling can also be activated. By way of example and not of limitation, checkpoint signaling can occur by damage to the DNA so that the cell cycle does not have to proceed until the damage is repaired. "Cell cycle checkpoints" are control mechanisms in eukaryotic cells which ensure proper division of the cell. Each checkpoint serves as a potential halting point along the cell cycle, during which the conditions of the cell

are assessed, with progression through the various phases of the cell cycle occurring when favorable conditions are met. Currently, there are three known checkpoints: the G1 checkpoint, also known as the restriction or start checkpoint; the G2/M checkpoint; and the metaphase checkpoint, also known as the spindle checkpoint. The biochemical pathways that restrain cell cycle transition and/or induce cell death after stress are known as cell cycle checkpoints. These checkpoints maintain the fidelity of DNA replication, repair, and division. Polypeptides that can regulate checkpoint signaling can include but are not limited to p53, p107, p130, and transcriptional repressor Rb.

[0059] "Negative checkpoint regulators" as described herein, refers to factors that can restrict the ability of T-cell responses to effectively attack tumors. They are also referred to as negative checkpoint signaling. In some alternatives, a system for inducible expression of chimeric antigen receptor is provided, wherein the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that inhibits regulates apoptosis, or a polypeptide that modulates checkpoint signaling and b) a second nucleic acid comprising a second constitutive or inducible promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the polypeptide that modulates checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises VISTA, LAG-3 and/or TIM3.

[0060] In another example, cell cycle inhibitors mediating the growth inhibitory cues of upstream signaling pathways, the cyclin-CDK inhibitors of the Cip/Kip family p21Cip1, p27Kip1, and p57Kip2 have emerged as multifaceted proteins with functions beyond cell cycle regulation. In addition to regulating the cell cycle, Cip/Kip proteins can also play important roles in apoptosis, transcriptional regulation, cell fate determination, cell migration and cytoskeletal dynamics. A complex phosphorylation network modulates Cip/Kip protein functions by altering their subcellular localization, protein-protein interactions, and stability. These functions are essential for the maintenance of normal cell and tissue homeostasis, in processes ranging from embryonic development to tumor suppression. In some alternatives, a system for inducible expression of chimeric antigen receptor is provided, wherein the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a polypeptide that

modulates checkpoint signaling and b) a second nucleic acid comprising a second constitutive or inducible promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the polypeptide modulates checkpoint signaling. In some alternatives, the polypeptide that modulates checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises VISTA, LAG-3 and/or TIM3.

[0061] "T cell precursors" as described herein refers to lymphoid precursor cells that can migrate to the thymus and become T cell precursors, which do not express a T cell receptor. All T cells originate from hematopoietic stem cells in the bone marrow. Hematopoietic progenitors (lymphoid progenitor cells) from hematopoietic stem cells populate the thymus and expand by cell division to generate a large population of immature thymocytes. The earliest thymocytes express neither CD4 nor CD8, and are therefore classed as double-negative ($CD4^-CD8^-$) cells. As they progress through their development, they become double-positive thymocytes ($CD4^+CD8^+$), and finally mature to single-positive ($CD4^+CD8^-$ or $CD4^-CD8^+$) thymocytes that are then released from the thymus to peripheral tissues.

[0062] About 98% of thymocytes die during the development processes in the thymus by failing either positive selection or negative selection, whereas the other 2% survive and leave the thymus to become mature immunocompetent T cells.

[0063] The double negative (DN) stage of the precursor T cell is focused on producing a functional β-chain whereas the double positive (DP) stage is focused on producing a functional α-chain, ultimately producing a functional αβ T cell receptor. As the developing thymocyte progresses through the four DN stages (DN1, DN2, DN3, and DN4), the T cell expresses an invariant α-chain but rearranges the β-chain locus. If the rearranged β-chain successfully pairs with the invariant α-chain, signals are produced which cease rearrangement of the β-chain (and silence the alternate allele) and result in proliferation of the cell. Although these signals require this pre-TCR at the cell surface, they are dependent on ligand binding to the pre-TCR. These thymocytes will then express both CD4 and CD8 and progresses to the double positive (DP) stage where selection of the α-chain takes place. If a rearranged β-chain does not lead to any signaling (e.g. as a result of an inability to pair with the invariant α-chain), the cell may die by neglect (lack of signaling).

[0064] "Hematopoietic stem cells" or "HSC" as described herein, are precursor cells that can give rise to myeloid cells such as, for example, macrophages, monocytes,

macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, dendritic cells and lymphoid lineages (such as, for example, T-cells, B-cells, NK-cells). HSCs have a heterogeneous population in which three classes of stem cells exist, which are distinguished by their ratio of lymphoid to myeloid progeny in the blood (L/M).

[0065] This disclosure provides for a system that has an inducible component for expression of transgenes and a constitutive component for expression of transgenes. The system can be tailored to provide for regulated expression of one or more transgenes to provide for functional characteristics in the transduced cells.

[0066] In some alternatives, a transgene under the control of the inducible promoter is a chimeric antigen receptor (CAR). The inducible promoter provides for the capacity to terminate CAR expression in cells while providing for reactivation of the cells at a later date (e.g. in the case of relapse). In addition, the cycling of CAR T cells through on and off periods can minimize exhaustion and/or anergy due to chronic stimulation of the T cell receptors.

[0067] The design of the vectors also provides for additional transgenes that can enhance one or more functional characteristics of transduced cells, such as enhanced tumor potency, survival and proliferation of transduced cells. In some alternatives, these transgenes are under the control of an inducible promoter. Such transgenes include, without limitation, genes that promote survival and proliferation, genes that prevent apoptosis, and genes that that regulate checkpoint signaling. Such genes include genes encoding IL-2, IL-15, Chemokine receptors, Bcl2, CA-Akt, dn-TGFbetaRIII, dn-SHP1/2, and/or PD-1CD28 chimeras. In some alternatives, the transgenes are genes encoding IL-2, IL-15, Chemokine receptors, Bcl2, CA-Akt, dn-TGFbetaRIII, dn-SHP1/2, and/or PD-1CD28 chimeras. In some alternatives, the gene that modulates checkpoint signaling, encodes a polypeptide that inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises VISTA, LAG-3 and/or TIM3.

[0068] The disclosure provides for a system comprising first and second nucleic acids, and vectors and host cells including such nucleic acids. Each of the first and second nucleic acids comprise a number of modular components that can be excised and replaced with other components in order to customize the system for a specific target cell. In some alternatives, the first nucleic acid includes an inducible promoter for control of the expression of the genes (e.g polynucleotide coding for a chimeric antigen receptor) in an on and off manner as needed. In other alternatives, the second nucleic acid comprises a constitutive

promoter that provides for expression of a transcriptional activator. In some alternatives, the gene encodes for a chimeric antigen receptor.

Inducible System.

[0069] The disclosure provides a system useful for providing regulated expression of transgenes in cells. Such transgenes include, without limitation, T cell receptors, affinity matured T cell receptors, chimeric antigen receptors, chemokine receptors, cytokines, genes that inhibit apoptosis, and/or genes that modulate checkpoint signaling. In some alternatives, the polypeptide that modulates checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises VISTA, LAG-3 and/or TIM3. In some alternatives, the system contains a number of modular components that provide for easy substitution of elements of the nucleic acid. In some alternatives of the system, the system provides regulation of expression of transgenes in cells. In some alternatives, the transgenes code for T cell receptors, affinity matured T cell receptors, chimeric antigen receptors, chemokine receptors, cytokines, genes that regulate apoptosis, and/or genes that modulate checkpoint signaling. In some alternatives, the gene that modulates checkpoint signaling encodes a polypeptide inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises VISTA, LAG-3 and/or TIM3.

[0070] In some alternatives, a system for inducible expression of chimeric antigen receptor comprises: a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a chimeric antigen receptor, the chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; and d) a polynucleotide coding for an intracellular signaling domain; and a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional modulator for the inducible promoter. In some alternatives, the second promoter is constitutive or inducible.

[0071] In some alternatives, a polynucleotide coding for a chimeric antigen receptor comprises a polynucleotide coding for a ligand binding domain, wherein the target

molecule is a tumor specific antigen, a polynucleotide coding for a polypeptide spacer wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and a polynucleotide coding for an intracellular signaling domain. In some alternatives, an expression vector comprises a first and/or second nucleic acid, as described herein. Polypeptides encoded by all of or a portion of the chimeric receptor nucleic acids are also included herein.

[0072] In other alternatives, a first nucleic acid comprises a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a gene that promotes cell survival and proliferation, a gene that regulates apoptosis, and/or a gene that modulates checkpoint signaling. Such genes include genes encoding IL-2, IL-15, Chemokine receptors, Bcl2, CA-Akt, dn-TGFbetaRIII, dn-SHP1/2, and/or PD-1CD28 chimeras. In some alternatives, the gene that modulates checkpoint signaling encodes a polypeptide that inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises VISTA, LAG-3 and/or TIM3.

Inducible Promoters.

[0073] A system comprises a first nucleic acid comprising a first promoter inducible by a drug. By utilizing an inducible promoter, transgene expression can be turned on and off in order to avoid toxic side effects and/or to allow the cells to rest during remission. Although several inducible promoter systems are known, clinical applicability of these systems is limited due to toxic off target effects, unfavorable biodistribution and pharmacodynamics profiles, limited output dynamic range, and/or limited availability as FDA-approved commercially available pharmaceuticals. Furthermore, many of these systems use chimeric transcriptional regulators built from xenogeneic components, thus introducing the complication of immunogenicity when applying these systems to human therapeutics.

[0074] In some alternatives, a first promoter is inducible by a drug. The drug is selected based on safety record, favorable pharmacokinetic profile, tissue distribution, a low partition coefficient between the extracellular space and cytosol, low immunogenicity, low toxicities, and/or high expression in lymphocytes. In a specific alternative, a drug is selected that is FDA approved, provides for transgene expression in lymphocytes, does not activate other undesirable gene expression, and induces a promoter that does not contain any xenogeneic components. In some alternatives, the inducible promoter is activated by a

transcriptional activator that interacts with a drug. The transcriptional activator is activated or able to bind to and activate the inducible promoter in the presence of the drug.

[0075] A specific alternative of a drug is a drug that binds to an estrogen receptor ligand binding domain of a transcriptional activator. In some alternatives, the drug includes tamoxifen, its metabolites, analogs, and pharmaceutically acceptable salts and/or hydrates or solvates thereof.

[0076] Tamoxifen, CAS RN: 10540-29-1, is also known as 2-(4-((1Z)-1,2-diphenyl-1-butenyl)phenoxy)-N,N-dimethyl- ethanamine, or (Z)-2-(para-(1,2-Diphenyl-1-butenyl)phenoxy)-N,N-dimethylamine (IUPAC), and has a molecular formula of $C_{26}H_{29}NO$, M.W. 371.52. Tamoxifen is a Selective Estrogen Receptor Modulator with tissue-specific activities. Tamoxifen acts as an anti-estrogen (inhibiting agent) agent in the mammary tissue, but as an estrogen (stimulating agent) in cholesterol metabolism, bone density, and cell proliferation in the endometrium. Tamoxifen is frequently administered orally as a pharmaceutically acceptable salt. For example, Tamoxifen citrate (RN 54965-24-1, M.W. 563.643) is indicated for treatment of metastatic breast cancer, and as an adjuvant for the treatment of breast cancer in women following mastectomy axillary dissection, and breast irradiation. Tamoxifen citrate is also indicated to reduce incidence of breast cancer in women at high risk for breast cancer.

[0077] Metabolites of tamoxifen in rat, mouse and human breast cancer patients, including major metabolites N-desmethyltamoxifen (RN 31750-48-8, M.W. 357.494) and 4-hydroxytamoxifen (4-OHT) (RN 68392-35-8, M.W. 387.52, Afimoxifene), are disclosed in Robinson et al., Metabolites, pharmacodynamics, and pharmacokinetics of tamoxifen in rats and mice compared to the breast cancer patient. Drug Metab Dispos January 1991 19:36-43, which is incorporated by reference herein in its entirety. Additional cytochrome P-450 metabolites are disclosed in Crewe et al., 2002, including cis-4-hydroxytamoxifen (RN 174592, M.W. 387.52; Afimoxifene, E-isomer), and 4'-hydroxytamoxifen ((Z)-4-(1-(4-(2-(dimethylamino)ethoxy)phenyl)-1-phenylbut-1-en-2-yl)phenol). See Crewe et al., 2002, Metabolism of Tamoxifen by recombinant human cytochrome P-450 enzymes: Formation of the 4-hydroxy, 4'-hydroxy and N-desmethyl metabolites and isomerization of trans-4-hydroxytamoxifen, Drug Metab Dispos, 30(8): 869-874, FIG. 1, which is incorporated herein by reference.

[0078] Compounds with structural similarity to tamoxifen include, but are not limited to, cis-tamoxifen (RN 13002-65-8, M.W. 371.521), 4-methyltamoxifen (RN 73717-

95-5, M.W. 385.548), N-desmethyltamoxifen (RN 31750-48-8, M.W. 357.494), (Z)-desethyl methyl tamoxifen (RN 15917-50-7, M.W. 357.494), (E)-desethyl methyl tamoxifen (RN 31750-45-5, M.W. 357.494), trans-4-hydoxytamoxifen (RN 68047-06-3, M.W. 387.52), Afimoxifene (RN 68392-35-8, M.W. 387.52, 4-hydroxytamoxifen), Afimoxifene, E-isomer (RN 174592-47-3, M.W. 387.52), 4-chlorotamoxifen (RN 77588-46-6, M.W. 405.966), 4-fluorotamoxifen (RN 73617-96-6, M.W. 389.511), Toremifene (RN 89778-26-7, M.W. 405.966), desethyl tamoxifen (RN 19957-51-8, M.W. 343.47), (E)-desethyl tamoxifen (RN 97151-10-5, M.W. 343.47), (Z)-desethyl tamoxifen (RN 97151-11-6, M.W. 343.47), Miproxifene (RN 129612-87-9, M.W. 429.6), 2-(p-(beta-ethyl-alpha-phenylstyryl)phenoxy)triethylamine (RN 749-86-0, M.W. 399.575), Droloxifene (RN 82413-20-5, M.W. 387.52), 4-iodo-tamoxifen (RN 116057-68-2, M.W. 497.413), dihydrotamoxifen (RN 109640-20-2, M.W. 373.537), (E)-N,N-dimethyl-2-(4-(1-(2-methylphenyl)-2-phenyl-1-butenyl)phenoxy)ethanamine (RN 97150-96-4, M.W. 385.548), or 4-hydroxytoremifene (RN 110503-62-3, M.W. 421.965); and/or pharmaceutically acceptable salts and/or hydrates or solvates thereof.

[0079] For example, citrate salts of tamoxifen, or citrate salts of compounds with structural similarity to tamoxifen, include, but are not limited to tamoxifen citrate (RN 54965-24-1, M.W. 563.64), 2-(p-(1,2-diphenyl-1-butenyl)phenoxy)-N,N-dimethylethylamine citrate (RN 7244-97-5, 563.64), (E)-tamoxifen citrate (RN 76487-65-5, M.W. 563.64), Toremifene citrate (RN 89778-27-8, M.W. 598.088), Droloxifene citrate (RN 97752-20-0, M.W. 579.64), 2-(p-(1,2-bis(p-methoxyphenyl)-1-butenyl)phenoxy)triethylamine citrate (RN 42920-39-8, M.W. 651.748), 2-(4-(1,2-diphenylethenyl)phenoxy)-N,N-diethyl-ethanamine 2-hydroxy-1,2,3-propanetricarboxylate (RN 40297-42-5, M.W. 563.643), 2-(p-(alpha-phenylstyryl)phenoxy)triethylamine citrate (RN 102433-95-4, M.W. 563.64), 2-(p-(2-(p-methoxyphenyl)-1-phenyl-1-butenyl)phenoxy)triethylamine citrate (1:1) (RN 42824-34-0, M.W. 637.72), 2-(p-(1-(p-methoxyphenyl)-2-phenylpropenyl)phenoxy)triethylamine citrate (RN 13554-24-0, M.W. 607.696), 2-(p-(alpha-(p-methoxyphenyl)styryl)phenoxy)triethylamine citrate monohydrate (RN 13542-71-7, M.W. 593.669), 2-(p-(p-methoxy-alpha-phenylphenethyl) phenoxy)triethylamine citrate (RN 16421-72-0, M.W. 595.685), alpha-(p-(2-(diethylamino)ethoxy)phenyl)-beta-ethyl-p-methoxy-alpha-phenylphenethyl alcohol citrate (1:1) (RN 35263-93-5, M.W. 639.737), 1-(p-(2-(diethylamino)ethoxy)phenyl)-2-(p-methoxyphenyl)-1-phenylethanol citrate (M.W. 611.68), alpha-p-(2-(diethylamino)ethoxy)phenyl)-beta-ethyl-alpha-(p-hydroxyphenyl)-p-

methoxyphenethyl alcohol citrate (RN 35263-96-8, M.W. 655.737), and/or 2-(p-(p-methoxy-alpha-methylphenethyl)phenoxy)-triethylamine citrate (RN 15624-34-7, M.W. 533.614).

[0080] In some alternatives, an affective amount of the drug for inducing expression is an amount that provides for an increase in transgene expression over uninduced and/or basal level of expression. In some alternatives, this amount can be readily determined using known dosages and pharmacokinetic profile of the drug.

[0081] In some alternatives, the inducible promoter has a low level of basal activity. When a lentiviral vector is used, the level of basal activity in uninduced cells is 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% or less, as compared to when cells are induced to express the gene. The level of basal activity can be determined by measuring the amount of the expression of the transgene (e.g. marker gene) in the absence of the inducer (e.g. drug) using flow cytometry.

[0082] In some alternatives, the inducible promoter provides for a high level of induced activity, as compared to uninduced or basal activity. In some alternatives, the level of activity in the induced state is 2, 4, 6, 8, or 10 fold or greater than the activity level in the uninduced state. In some alternatives, transgene expression under control of the inducible promoter is turned off in the absence of a transactivator in less than 10, 8, 6, 4, 2, or 1 days excluding 0 days.

[0083] In some alternatives, an inducible promoter can be designed and/or modified to provide for a low level of basal activity, a high level of inducibility, and/or a short time for reversibility. In some alternatives, the inducible promoter is the 7xHBD/mE1b promoter. An exemplary sequence for the promoter is found in **Table 12** (SEQ ID NO: 41) For example, in the 7xHBD/mE1b promoter, mutations can be made to enhance the binding of the transcriptional activator.

[0084] In some alternatives, the system employs a synthetic transcriptional activator which, in the presence of the drug (e.g.tamoxifen), binds a synthetic promoter upstream of a transgene to induce expression. In some alternatives, the transcriptional activator is TamR-tf (HEA3). The tamoxifen regulated transcription factor ("TamR-tf", also designated "HEA3") is a chimeric transcription factor composed of human subunits including the N-terminal DNA binding domain of Hepatocyte Nuclear Factor 1-alpha (HNF-1α)(e.g. amino acids 1-281 of SEQ ID NO: 40) fused in frame to the mutant tamoxifen-specific ligand binding domain of the estrogen receptor ligand binding domain (ER-LBD), that is in turn fused to the p65 activation domain of NF-κB (p65). An exemplary amino acid sequence is

provided in **Table 10** and is identified as SEQ ID NO: 40. The mutant tamoxifen-specific ligand binding domain of the estrogen receptor ligand binding domain (ER-LBD) is found at amino acids 282-595 of the TamR-tf and has a mutation at position 521. The p65 activation domain of NF-κB (p65 or TAD) is found at amino acids 596 to 862.

[0085] In some alternatives, a system for inducible expression of a chimeric antigen receptor is provided, wherein the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the first promoter comprises a nucleic acid sequence of SEQ ID NO: 41. In some alternatives, the second promoter is a constitutive promoter. In some alternatives, the second promoter is the EF1αp. In some alternatives, the transcriptional activator comprises a sequence of SEQ ID NO: 40. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives, both vectors are packaged in a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the first and second nucleic acid comprise a vector. In some alternatives, the first nucleic acid further comprises a nucleic acid sequence coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a nucleic acid coding for a selectable marker. In some alternatives, a system for inducible expression of chimeric antigen receptor is provided, wherein the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a polypeptide that modulates checkpoint signaling; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the polypeptide that modulates checkpoint signaling inhibits negative

checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises VISTA, LAG-3 and/or TIM3. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment, preferably a binding fragment thereof. In some alternatives, the ligand binding domain is single chain variable fragment. In some alternatives, the tumor specific molecule is CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, or MAGE A3 TCR or combinations thereof. In some alternatives, the system employs a synthetic transcriptional activator which, in the presence of a drug binds a synthetic promoter upstream of a transgene to induce expression. In some alternatives, the transcriptional activator is TamR-tf (HEA3). In some alternatives, the drug is tamoxifen.

[0086] Additional changes can be made to the transcriptional activator to increase the properties of the transcription factor including, without limitation, altering one or more amino acids in the estrogen receptor ligand binding domain and/or altering one or more amino acids in the p65 transactivating domain. Altering amino acids in the estrogen receptor binding domain can provide for more specific binding of the drug to the transcriptional activator. An example of a transcriptional activator with altered amino acid sequence in the ER-LBD is shown in **Table 11** (SEQ ID NO:43). Mutations are made at amino acid position 400, 543, and 544 of SEQ ID NO: 40. The transcriptional activator with altered sequence has increased affinity for tamoxifen or 4-OHT. Altering amino acids in the p65 transactivating domain can provide for increased expression of the transgene in the absence of activation of the transduced cells.

[0087] In the absence of tamoxifen, TamR-tf is excluded from the nucleus by binding of cytosolic heat-shock protein 90 (HSP90) to the tamoxifen binding active site and transgene expression is in the "OFF" state. Nanomolar concentrations of cytosolic tamoxifen actively outcompete HSP90 for ER-LBD binding, resulting in TamR-tf translocation to the

nucleus. Upon nuclear translocation, TamR-tf is readily available to bind its restricted synthetic promoter. In the presence of tamoxifen, binding of TamR-tf to 7xHBD/EF1αp promoter induces the "ON" state of transgene expression. In some alternatives, this transcriptional regulator can be modified to provide for varying level of control of transgene expression. Amino acid substitutions in the LBD of TamR-tf (HEA-3) permit selective responsiveness to tamoxifen and its metabolites, where 4-hydroxy tamoxifen (4-OHT) is the most pharmacologically active metabolite, in regards to TamR-tf (HEA-3) activity, while lacking interaction with endogenous estrogen.

[0088] In some alternatives, the inducible promoter functions in a lentiviral construct and/or in lymphocytes.

Chimeric Antigen Receptors.

[0089] A system for expression of chimeric antigen receptor comprises: a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a chimeric antigen receptor, the chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain. In other alternatives, another polynucleotide coding for a chimeric antigen receptor is under the control of a constitutive promoter. In some alternatives, the drug is tamoxifen.

Ligand binding domain.

[0090] In some alternatives, the chimeric receptor nucleic acid comprises a polynucleotide coding for a ligand binding domain. In some alternatives, the ligand binding domain specifically binds to a tumor or viral specific antigen and said ligand binding domain may be humanized. In some alternatives, a ligand binding domain, includes without limitation, receptors or portions thereof, small peptides, peptidomimetics, substrates, cytokines, and the like. In some alternatives, the ligand binding domain is an antibody or fragment thereof, preferably a binding fragment thereof, any of which may be humanized. A nucleic acid sequence coding for an antibody or antibody fragment can readily be determined. In a specific alternative, the polynucleotide codes for a single chain Fv that specifically binds

CD19. In other specific alternatives, the polynucleotide codes for a single chain Fv that specifically binds HER2, CE7, hB7H3, or EGFR and, optionally, said polynucleotide encodes a humanized version thereof. The sequences of these antibodies and binding domains thereof are known to or can readily be determined by those of skill in the art.

[0091] Tumor antigens are proteins that are produced by tumor cells that elicit an immune response. The selection of the ligand binding domain of the invention will depend on the type of cancer to be treated, and can target tumor antigens or other tumor cell surface molecules. A tumor sample from a subject can be characterized for the presence of certain biomarkers or cell surface markers. For example, breast cancer cells from a subject can be positive or negative for each of Her2Neu, Estrogen receptor, and/or the Progesterone receptor. A tumor antigen or cell surface molecule is selected that is found on the individual subject's tumor cells. Tumor antigens and cell surface molecules are well known in the art and include, for example, carcinoembryonic antigen (CEA), prostate specific antigen, PSMA, Her2/neu, estrogen receptor, progesterone receptor, ephrinB2, CD19, CD20, CD22, CD23, CD123, CS-1, CE7, hB7H3, ROR1, mesothelin, c-Met, GD-2, and/or MAGE A3 TCR. In some alternatives, a target molecule is a cell surface molecule that is found on tumor cells and is not substantially found on normal tissues, or restricted in its expression to non-vital normal tissues.

[0092] In one alternative, the target molecule on the tumor comprises one or more epitopes associated with a malignant tumor. Malignant tumors express a number of proteins that can serve as target antigens for T cell receptor or chimeric receptor mediated recognition. Other target molecules belong to the group of cell transformation-related molecules such as the oncogene HER-2/Neu/ErbB2. In some alternatives, the tumor antigen is selectively expressed or overexpressed on the tumor cells as compared to control cells of the same tissue type. In other alternatives, the tumor antigen is a cell surface polypeptide.

[0093] Once a tumor cell surface molecule that might be targeted with a chimeric receptor is identified, an epitope of the target molecule is selected and characterized. Antibodies that specifically bind a tumor cell surface molecule can be prepared using methods of obtaining monoclonal antibodies, methods of phage display, methods to generate human or humanized antibodies, or methods using a transgenic animal or plant engineered to produce human antibodies. Phage display libraries of partially or fully synthetic antibodies are available and can be screened for an antibody or fragment thereof that can bind to the target molecule. Phage display libraries of human antibodies are also available. In some alternatives,

antibodies specifically bind to a tumor cell surface molecule and do not cross react with nonspecific components such as bovine serum albumin or other unrelated antigens. Once identified, the amino acid sequence or polynucleotide sequence coding for the antibody can be isolated and/or determined.

[0094] Antibodies or antigen binding fragments include all or a portion of polyclonal antibodies, a monoclonal antibody, a human antibody, a humanized antibody, a synthetic antibody, a chimeric antibody, a bispecific antibody, a minibody, and a linear antibody. "Antibody fragments" comprise a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody and can readily be prepared. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments. In some alternatives the antibody fragments are Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; or multispecific antibodies formed from antibody fragments. Any of such aforementioned antibodies or antibody fragments can be humanized and used with the compositions and methods described herein.

[0095] In some alternatives, a number of different antibodies that bind to a particular tumor cell surface molecules can be isolated and characterized. In some alternatives, the antibodies are characterized based on epitope specificity of the targeted molecule. In addition, in some cases, antibodies that bind to the same epitope can be selected based on the affinity of the antibody for that epitope. In some alternatives, an antibody has an affinity of at least 1 mM, and preferably <50 nM. In some alternatives, the antibody has an affinity of 50 nM, 100 nM, 200 nM, 300nM 400 nM, 500 nM, 1uM, 100uM, 200uM, 300uM, 400uM, 500uM, 600uM, 700 uM, 800uM, 900uM or 1mM or an affinity within a range defined by any two of the aforementioned values. In some alternatives, an antibody is selected that has a higher affinity for the epitope, as compared to other antibodies. For example, an antibody is selected that has at least a 2 fold, at least a 5 fold, at least a 10 fold, at least a 20 fold, at least a 30 fold, at least a 40 fold, or at least a 50 fold greater affinity than a reference antibody that binds to the same epitope or an affinity that is greater than a reference antibody within a range defined by any two of the aforementioned values.

[0096] In some alternatives, target molecules are CD19, CD20, CD22, CD23, CE7, hB7H3, EGFR, CD123, CS-1, ROR1, mesothelin, Her2, c-Met, PSMA, GD-2, or

MAGE A3 TCR or combinations thereof. In some alternatives, the antibody or binding fragment there of specific for these target molecules is humanized.

[0097] In specific alternatives, the target antigen is CD19. A number of antibodies specific for CD19 are known to those of skill in the art and can be readily characterized for sequence, epitope binding, and affinity. In a specific alternative, the chimeric receptor construct includes a scFV sequence from a FMC63 antibody. In other alternatives, the scFV is a human or humanized ScFv comprising a variable light chain comprising a CDRL1 sequence of RASQDISKYLN (SEQ ID NO: 88) , CDRL2 sequence of SRLHSGV(SEQ ID NO: 89), and a CDRL3 sequence of GNTLPYTFG (SEQ ID NO: 90). In other alternatives, the scFV is a human or humanized ScFv comprising a variable heavy chain comprising CDRH1 sequence of DYGVS (SEQ ID NO: 91), CDRH2 sequence of VIWGSETTYYNSALKS (SEQ ID NO: 92), and a CDRH3 sequence of YAMDYWG (SEQ ID NO: 93). The disclosure also contemplates variable regions that have at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity to that of the scFv for FMC63 and that have at least the same affinity for CD19.

[0098] In some alternatives, CDR regions are found within antibody regions as numbered by Kabat as follows: for the light chain; CDRL1 amino acids 24-34;CDRL2 amino acids 50-56; CDRL3 at amino acids 89-97; for the heavy chain at CDRH1 at amino acids 31-35; CDRH2 at amino acids 50-65; and for CDRH3 at amino acids 95-102. CDR regions in antibodies can be readily determined.

[0099] In specific alternatives, the target antigen is Her2. A number of antibodies specific for Her2 are known to those of skill in the art and can be readily characterized for sequence, epitope binding, and affinity. In a specific alternative, the chimeric receptor construct includes a scFV sequence from a Herceptin antibody. In other alternatives, the scFV is a human or humanized ScFv comprising a variable light chain comprising a CDRL1 sequence, CDRL2 sequence and a CDRL3 sequence of the Herceptin antibody. In other alternatives, the scFV is a human or humanized ScFv comprising a variable heavy chain comprising CDRH1 sequence, CDRH2, and a CDRH3 sequence of Herceptin. The CDR sequences can readily be determined from the amino acid sequence of Herceptin. The disclosure also contemplates variable regions that have at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity to that of the scFv for Herceptin and that have at least the same affinity for Her2.

[0100] "Humanized antibodies," as described herein, refers to antibodies from non-human species whose protein sequences have been modified to increase their similarity to antibody variants produced naturally in humans. The process of "humanization" can be applied to monoclonal antibodies developed for administration to humans (for example, antibodies developed as anti-cancer drugs). Humanization can be desirable when the process of developing a specific antibody involves utilization of a non-human immune system (such as that in mice). The protein sequences of antibodies produced in this way are partially distinct from homologous antibodies occurring naturally in humans, and are therefore potentially immunogenic when administered to human patients. Humanized antibodies are distinct from chimeric antibodies, in that they have the protein sequences made more similar to human antibodies but can carry a larger stretch of non-human protein. A derivative of a humanized antibody can refer to a segment of an antibody or sequence that is derived from a humanized antibody. In some alternatives, the ligand binding domain comprises a humanized antibody or portion thereof. In some alternatives, the ligand binding domain comprises a scFv. In some alternatives, the scFv is a humanized scFv.

[0101] Humanization can be desirable in some alternatives for reducing the immunogenicity of monoclonal antibodies that are derived from xenogeneic sources, such as, for example, rodents. Humanization is also desirable in some alternatives so as to improve the interaction of the antibody or a fragment thereof with the human immune system. Due to the development of hybridoma technology, a large number of xenogeneic antibodies are highly immunogenic in humans, which can ultimately limit their clinical applications especially when administration may need to be repeated. Additionally, they can be rapidly removed from the circulation and can cause systemic inflammatory effects as well. Therefore humanization strategies are desirable in some alternatives to circumvent these situations. Techniques for antibody humanization are known to those skilled in the art.

[0102] In some alternatives, a polynucleotide coding for a ligand binding domain is operably linked to a polynucleotide coding for a spacer region. In some alternatives, the polynucleotide coding for a ligand binding domain can also have one or more restriction enzyme sites at the 5' and/or 3' ends of the coding sequence in order to provide for easy excision and replacement of the polynucleotide with another polynucleotide coding for a ligand binding domain coding for a different antigen or that has different binding characteristics. For example, a restriction site, NheI, is encoded upstream of the leader sequence; and a 3' RsrII located within the hinge region allows subcloning of any desirable

scFv into a chimeric receptor vector. In some alternatives, the polynucleotide is codon optimized for expression in mammalian cells.

[0103] In some alternatives, the polynucleotide coding for a ligand binding domain is operably linked to a signal peptide. In some alternatives, the signal peptide is a signal peptide for granulocyte colony stimulating factor. Polynucleotides coding for other signal peptides such as CD8 alpha can be utilized. In some alternatives, the polynucleotide codes for CD8 alpha.

[0104] In some alternatives, the polynucleotide coding for a ligand binding domain is operably linked to a promoter. A promoter is selected that provides for expression of the chimeric antigen receptor in a mammalian cell. In a specific alternative, the promoter is an inducible promoter.

[0105] A specific alternative of a polynucleotide coding for a ligand binding domain is shown in **Table 1** as the scFv from an antibody that specifically binds CD19, such as FMC63. A polynucleotide encoding for a flexible linker including the amino acids GSTSGSGKPGSGEGSTKG (SEQ ID NO: 94) separates the VH and VL chains in the scFV. The amino acid sequence of the scFv including the linker is shown in **Table 2**. (SEQ ID NO:11) Other CD19-targeting antibodies such as SJ25C1 and HD37 are known. (SJ25C1: Bejcek et al. Cancer Res 2005, PMID 7538901; HD37: Pezutto et al. JI 1987, PMID 2437199).

Spacer.

[0106] In some alternatives, the chimeric receptor nucleic acid comprises a polynucleotide coding for a spacer region. Typically a spacer region is found between the ligand binding domain and the transmembrane domain of the chimeric receptor. In some alternatives, a spacer region provides for flexibility of the ligand binding domain and allows for high expression levels in lymphocytes. A CD19-specific chimeric receptor having a spacer domain of 229 amino acids had less antitumor activity than a CD19-specific chimeric receptor with a short spacer region comprised of the modified IgG4 hinge only. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor.

[0107] In some alternatives, a spacer region has at least 10 to 229 amino acids, 10 to 200 amino acids, 10 to 175 amino acids, 10 to 150 amino acids, 10 to 125 amino acids, 10 to 100 amino acids, 10 to 75 amino acids, 10 to 50 amino acids, 10 to 40 amino acids, 10 to

30 amino acids, 10 to 20 amino acids, or 10 to 15 amino acids, or a length within a range defined by any two of the aforementioned lengths. In some alternatives, a spacer region has 12 amino acids or less, 119 amino acids or less, or 229 amino acids or less but greater than 1 or 2 amino acids. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor.

[0108] In some alternatives, the spacer region is derived from a hinge region of an immunoglobulin like molecule. In some alternatives, a spacer region comprises all or a portion of the hinge region from a human IgG1, human IgG2, a human IgG3, or a human IgG4, and can contain one or more amino acid substitutions. Exemplary sequences of the hinge regions are provided in **Table 8**. In some alternatives, a portion of the hinge region includes the upper hinge amino acids found between the variable heavy chain and the core, and the core hinge amino acids including a polyproline region. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor.

[0109] In some alternatives, hinge region sequences can be modified in one or more amino acids in order to avoid undesirable structural interactions such as dimerization. In a specific alternative, the spacer region comprises a portion of a modified human hinge region from IgG4, for example, as shown in **Table 2** or **Table 8** (SEQ ID NO: 21). A representative of a polynucleotide coding for a portion of a modified IgG4 hinge region is provided in **Table 1.** (SEQ ID NO: 4). In some alternatives, a hinge region can have at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with a hinge region amino acid sequence identified in **Table 2** or **Table 8**, or any other percent sequence identity between any two of the percent sequence identities listed. In a specific alternative, a portion of a human hinge region from IgG4 has an amino acid substitution in the core amino acids from CPSP to CPPC.

[0110] In some alternatives, all or a portion of the hinge region is combined with one or more domains of a constant region of an immunoglobulin. For example, a portion of a hinge region can be combined with all or a portion of a CH2 or CH3 domain or variant thereof. In some alternatives, the spacer region does not include the 47-48 amino acid hinge region sequence from CD8apha or the spacer region consisting of an extracellular portion of the CD28 molecule.

[0111] In some alternatives, a short spacer region has 12 amino acids or less and comprises all or a portion of a IgG4 hinge region sequence or variant thereof, an intermediate spacer region has 119 amino acids or less and comprises all or a portion of a IgG4 hinge region sequence and a CH3 region or variant thereof, and a long spacer has 229 amino acids or less and comprises all or a portion of a IgG4 hinge region sequence, a CH2 region, and a CH3 region or variant thereof. In some alternatives, a short spacer region has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 amino acids or a size within a range defined by any two of the aforementioned amino acid lengths. In some alternatives, a medium spacer region has 13, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 or 119 amino acids or a size within a range defined by any two of the aforementioned amino acid lengths. In some alternatives, a spacer region has 120, 130, 140, 150, 160, 170, 180, 190, 200, 210 or 219 amino acids or a size within a range defined by any two of the aforementioned amino acid lengths.

[0112] A polynucleotide coding for a spacer region can be readily prepared by synthetic or recombinant methods from the amino acid sequence. In some alternatives, a polynucleotide coding for a spacer region is operably linked to a polynucleotide coding for a transmembrane region. In some alternatives, the polynucleotide coding for the spacer region can also have one or more restriction enzyme sites at the 5' and/or 3' ends of the coding sequence in order to provide for easy excision and replacement of the polynucleotide with another polynucleotide coding for a different spacer region. In some alternatives, the polynucleotide coding for the spacer region is codon optimized for expression in mammalian cells.

[0113] In an alternative, the spacer region is a hinge region sequence from IgG1, IgG2, IgG3, or IgG4 or a portion thereof, a hinge region sequence from IgG1, IgG2, IgG3, or IgG4 in combination with all or a portion of a CH2 region or variant thereof, a hinge region sequence from IgG1, IgG2, IgG3, or IgG4 in combination with all or a portion of a CH3 region or variant thereof, and a hinge region sequence from IgG1, IgG2, IgG3, or IgG4 in combination with all or a portion of a CH2 region or variant thereof, and/or a CH3 region or variant thereof. In some alternatives, a short spacer region is a modified IgG4 hinge sequence (SEQ ID NO: 4) having 12 amino acids or less but greater than one or two amino acids, an intermediate sequence is a IgG4 hinge sequence with a CH3 sequence having 119 amino acids or less but greater than one or two amino acids (SEQ ID NO: 62); or a IgG4 hinge sequence with a CH2 and CH3 region having 229 amino acids or less but greater than one or two amino acids (SEQ ID NO: 50).

Transmembrane domain.

[0114] In some alternatives, the chimeric receptor nucleic acid comprises a polynucleotide coding for a transmembrane domain. The transmembrane domain provides for anchoring of the chimeric receptor in the membrane.

[0115] In an alternative, the transmembrane domain that naturally is associated with one of the domains in the chimeric receptor is used. In some cases, the transmembrane domain can be selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex.

[0116] The transmembrane domain can be derived either from a natural or a synthetic source. When the source is natural, the domain can be derived from any membrane-bound or transmembrane protein.

[0117] Transmembrane regions comprise at least the transmembrane region(s) of) the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3, CD45, CD4, CD8, CD9, CD16, CD22; CD33, CD37, CD64, CD80, CD86, CD134, CD137 and/or CD154. In a specific alternative, the transmembrane domain comprises the amino acid sequence of the CD28 transmembrane domain as shown in **Table 2**. A representative polynucleotide sequence coding for the CD28 transmembrane domain is shown in **Table 1** (SEQ ID NO: 5).

[0118] A transmembrane domain can be synthetic or a variant of a naturally occurring transmembrane domain. In some alternatives, synthetic or variant transmembrane domains comprise predominantly hydrophobic residues such as leucine and valine. In some alternatives, a transmembrane domain can have at least 80%, 85%, 90%, 95%, or 100% amino acid sequence identity with a transmembrane domain as shown in **Table 2** or **Table 6** or an amino acid sequence identity within a range defined by any two of the aforementioned values. Variant transmembrane domains preferably have a hydrophobic score of at least 50 as calculated by Kyte Doolittle.

[0119] A polynucleotide coding for a transmembrane domain can be readily prepared by synthetic or recombinant methods. In some alternatives, a polynucleotide coding for a transmembrane domain is operably linked to a polynucleotide coding for an intracellular signaling region. In some alternatives, the polynucleotide coding for a transmembrane domain can also have one or more restriction enzyme sites at the 5' and/or 3' ends of the coding sequence in order to provide for easy excision and replacement of the polynucleotide coding

for a transmembrane domain with another polynucleotide coding for a different transmembrane domain. In some alternatives, the polynucleotide coding for a transmembrane domain is codon optimized for expression in mammalian cells. In some alternatives, the mammalian cells are human cells.

Intracellular signaling domain.

[0120] In some alternatives, the chimeric receptor nucleic acid comprises a polynucleotide coding for an intracellular signaling domain. The intracellular signaling domain provides for activation of one function of the transduced cell expressing the chimeric receptor upon binding to the ligand expressed on tumor cells. In some alternatives, the intracellular signaling domain contains one or more intracellular signaling domains. In some alternatives, the intracellular signaling domain is a portion of and/or a variant of an intracellular signaling domain that provides for activation of at least one function of the transduced cell.

[0121] Examples of intracellular signaling domains for use in a chimeric receptor of the disclosure include the cytoplasmic sequences of the CD3 zeta chain, and/or co-receptors that act in concert to initiate signal transduction following chimeric receptor engagement, as well as any derivative or variant of these sequences and any synthetic sequence that has the same functional capability. T cell activation can be said to be mediated by two distinct classes of cytoplasmic signaling sequence: those that initiate antigen-dependent primary activation and provide a T cell receptor like signal (primary cytoplasmic signaling sequences) and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal (secondary cytoplasmic signaling sequences). Primary cytoplasmic signaling sequences that act in a stimulatory manner can contain signaling motifs which are known as receptor tyrosine-based activation motifs or ITAMs. Examples of ITAM containing primary cytoplasmic signaling sequences include those derived from CD3 zeta, FcR gamma, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and/or CD66d. In some alternatives, the primary signaling intracellular domain can have at least 80%, 85%, 90%, or 95% sequence identity to CD3zeta having a sequence provided in **Table 2** or at least a percent sequence identity that is within a range defined by any two of the percent sequence identities listed. In some alternatives of the variants, of CD3 zeta retain at least one, two, three or all ITAM regions as shown in **Table 7.**

[0122] In a preferred alternative, the intracellular signaling domain of the chimeric receptor can be designed to comprise the CD3-zeta signaling domain by itself or combined with any other desired cytoplasmic domain(s). For example, the intracellular signaling domain of the chimeric receptor can comprise a CD3zeta chain and a costimulatory signaling region.

[0123] The costimulatory signaling region refers to a portion of the chimeric receptor comprising the intracellular domain of a costimulatory molecule. A costimulatory molecule is a cell surface molecule other than an antigen receptor or their ligands that is required for a response of lymphocytes to an antigen. Examples of such molecules include CD27, CD28, 4-1BB (CD 137), OX40, CD30, CD40, lymphocyte function-associated antigen-1 (LFA-l), CD2, CD7, LIGHT, NKG2C, B7-H3, zeta chain associated protein kinase (ZAP70), and/or a ligand that specifically binds with CD83. In some alternatives, the costimulatory signaling domain can have at least 80%, 85%, 90%, or 95% amino acid sequence identity to the intracellular domain of CD28 as shown in **Table 5** or to 4-1BB having a sequence provided in **Table 2** or at least a percent sequence identity that is within a range defined by any two of the percent sequence identities listed. In an alternative, a variant of the CD28 intracellular domain comprises an amino acid substitution at positions 186-187, wherein LL is substituted with GG.

[0124] The intracellular signaling sequences of the chimeric receptor can be linked to each other in a random or specified order. In some alternatives, a short oligo- or polypeptide linker, preferably between 2 and 10 amino acids in length can form the linkage. In one alternative, the intracellular signaling domains comprises all or a portion of the signaling domain of CD3-zeta or variant thereof and all or a portion of the signaling domain of CD28 or a variant thereof. In another alternative, the intracellular signaling domain comprises all or a portion of the signaling domain of CD3-zeta or variant thereof and all or a portion of the signaling domain of 4-lBB or variant thereof. In yet another alternative, the intracellular signaling domain comprises all or a portion of the signaling domain of CD3-zeta or variant thereof, all or a portion of the signaling domain of CD28 or variant thereof, and all or a portion of the signaling domain of 4-lBB or variant thereof. In a specific alternative, the amino acid sequence of the intracellular signaling domain comprising a variant of CD3zeta and a portion of the 4-1BB intracellular signaling domain is provided in **Table 2.** A representative nucleic acid sequence is provided in **Table 1** (SEQ ID NO: 6; SEQ ID NO: 7). In some alternatives, the nucleic acid sequence comprises the sequence set forth in SEQ ID

NO: 6. In some alternatives, the nucleic acid sequence comprises the sequence set forth in SEQ ID NO: 7.

[0125] In an alternative, a polynucleotide coding for an intracellular signaling domain comprises a 4-1BB intracellular domain linked to a portion of a CD3zeta domain. In other alternatives, a 4-1BB intracellular domain and a CD28 intracellular domain are linked to a portion of a CD3 zeta domain.

[0126] A polynucleotide coding for an intracellular signaling domain can be readily prepared by synthetic or recombinant methods from the amino acid sequence. In some alternatives, the polynucleotide coding for an intracellular signaling domain can also have one or more restriction enzyme sites at the 5' and/or 3' ends of the coding sequence in order to provide for easy excision and replacement of the polynucleotide coding for an intracellular signaling domain with another polynucleotide coding for a different intracellular signaling domain. In some alternatives, the polynucleotide coding for an intracellular signaling domain is codon optimized for expression in mammalian cells. In some alternatives, the mammalian cells are human cells.

Marker sequences.

[0127] In some alternatives, the system further comprises one or more marker sequences under the control of an inducible promoter. A marker sequence can provide for selection of transduced cells, and/or identification of transduced cells. In some alternatives, the marker sequence is for a selection of transduced cells and/or identification of transduced cells. In some alternatives, the marker sequence is operably linked to a polynucleotide sequence coding for a linker sequence. In some alternatives, the linker sequence is a cleavable linker sequence. In some alternatives, the linker is a cleavable T2A linker.

[0128] A number of different marker sequences can be employed. Typically a marker sequence has a functional characteristic that allows for selection of transduced cells and/or detection of transduced cells. In some alternatives, the marker sequence is compatible with transduction of human lymphocytes. In some alternatives, the marker sequence allows for selection of transduced cells and/or detection of transduced cells.

[0129] The positive selectable marker can be a gene, which upon being introduced into the host cell, expresses a dominant phenotype permitting positive selection of cells carrying the gene. Genes of this type are known in the art, and include, inter alia, hygromycin-B phosphotransferase gene (hph) which confers resistance to hygromycin B, the amino

glycoside phosphotransferase gene (neo or aph) from Tn5, which codes for resistance to the antibiotic G418, the dihydrofolate reductase (DHFR) gene, which provides resistance to methotrexate, DHFR dm (exemplary polynucleotide and amino acid sequences in **Table 14**, SEQ ID NO: 46 and SEQ ID NO: 47, the pac gene that provides resistance to puromycin, Sh ble gene which inactivates zeocin, the adenosine deaminase gene (ADA), and the multi-drug resistance (MDR) gene. Transduced cells cultured in the presence of these agents will survive and be selected.

[0130] In an alternative, a first nucleic acid further comprises a polynucleotide coding for a marker sequence. In an alternative, the marker sequence is a truncated epidermal growth factor receptor as shown in **Table 2**. An exemplary polynucleotide for the truncated epidermal growth factor receptor is shown in Table 1. (SEQ ID NO: 9). In some alternatives, the marker sequence is a truncated Her2 sequence. An exemplary polynucleotide and amino acid for the truncated Her2 sequences is shown in **Table 13** and provided by SEQ ID NO: 44 and SEQ ID NO: 45, respectively.

[0131] In some alternatives, the polynucleotide coding for the marker sequence is operably linked to a polynucleotide coding for a linker sequence. In a specific alternative, the linker sequence is a cleavable linker sequence T2A, as shown in **Table 2.** An exemplary polynucleotide sequence coding for the T2A linker is provided in **Table 1.** (SEQ ID NO:8).

[0132] A polynucleotide coding for marker sequence can be readily prepared by synthetic or recombinant methods from the amino acid sequence. In some alternatives, a polynucleotide coding for a marker sequence is operably linked to a polynucleotide coding for an intracellular signaling domain. In some alternatives, the polynucleotide coding for a marker sequence can also have one or more restriction enzyme sites at the 5' and/or 3' ends of the coding sequence in order to provide for easy excision and replacement of the polynucleotide coding for a marker sequence with another polynucleotide coding for a different marker sequence. In some alternatives, the polynucleotide coding for a marker sequence is codon optimized for expression in mammalian cells, preferably humans.

[0133] In some alternatives, two or more marker sequences can be employed. In some alternatives, a first marker sequence is under control of a constitutive promoter and provides for an indication that the transduced cell is expressing the transgene. In other alternatives, a second marker sequence is under the control of the inducible promoter and provides an indication that the transgene expression has been induced. In some alternatives, the marker under the control of the inducible promoter can be used to select for cells in which

noninduced or basal expression is much lower than in other cells by selecting cells that have a lower expression of the marker sequence under the control of the inducible promoter and expand those cells for further applications.

Other Genetic Components under control of Inducible Promoter.

[0134] In some alternatives, the first nucleic acid comprises a polynucleotide sequence coding for genes that promote survival and proliferation, genes that prevent apoptosis, and/or genes that inhibit negative checkpoint signaling under the control of an inducible promoter. Such genes include genes encoding IL-2, IL-15, Chemokine receptors, Bcl2, CA-Akt, dn-TGFbetaRIII, dn-SHP1/2, and/or PD-1CD28 chimeras. These genes are also placed under the control of an inducible promoter as described herein. In some alternatives, the genes encode IL-2, IL-15, Chemokine receptors, Bcl2, CA-Akt, dn-TGFbetaRIII, dn-SHP1/2, and/or PD-1CD28 chimeras. In some alternatives, the gene that modulates checkpoint signaling encodes a polypeptide that inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises VISTA, LAG-3 and/or TIM3.

[0135] In some alternatives, a first nucleic acid comprises a first inducible promoter linked to a polynucleotide coding for a cytokine or chemokine receptor. Chemokines, also referred to as chemotactic cytokines, are a group of structurally related proteins that regulate cell trafficking of lymphocytes. In some alternatives, the chemokines are homeostatic or inflammatory. Chemokine receptors include CCR2, CCR7, or CCR15. Cytokines include interleukins such as IL2, IL-12, IL-7, and/or Il-15, interferons, such as interferon δ, tumor necrosis factor, and a TLR4 agonist. In some alternatives, the chemokine receptors comprise CCR2, CCR7, and/or CCR15. In some alternatives, the chemokine receptors include CCR2, CCR7, or CCR15. In some alternatives, the cytokines include interleukins, wherein the interleukins are IL2, IL-12, IL-7 and/or Il-15 or interferons, wherein the interferons comprise interferon δ, tumor necrosis factor, or a TLR4 agonist.

[0136] In some alternatives, a first nucleic acid comprises a first inducible promoter linked to a polynucleotide coding for a polypeptide that regulates apoptosis. In some alternatives, genes that inhibit apoptosis include, for example, Bcl2, and/or CA-Akt. In some alternatives, the polypeptide is Bcl2 or CA-Akt.

[0137] In some alternatives, a first nucleic acid comprises a first inducible promoter linked to a polynucleotide coding for a polypeptide that modulates checkpoint

signaling. Such genes include dn-TGFbetaRIII, dn-SHP1/2, and/or PD-1CD28 chimeras. In some alternatives, the polypeptide is dn-TGFbetaRIII, dn-SHP1/2, and/or PD-1CD28 chimeras. In some alternatives, the polypeptide that modulates checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises VISTA, LAG-3 and/or TIM3.

[0138] Exemplary sequences for polynucleotides encoding these genes are found at:

Gene	A.A.	A.A.	N.A.	N.A.
	Accession	**gI**	**Accession**	**gI**
CCR2	P41597	1168965	NM_001123041	183979979
CCR7	P32248	1352335	NM_001838	299473754
IL-2	AAH70338	47682793	BC070338	47682792
IL-12	AAD16432	4323579	AF101062	4323578
IL-7	AAC63047	386824	NM_000880	315467865
IL-15	CAG46804	49456967	CR542007	49456966
IFN-g	EAW97180	119617586	EAW97180	119617586
TNF	NP_000585	25952111	NM_000594	395132451
Bcl2	AAH27258	20072668	BC027258	20072667
CA-Akt	NP_001014432	62241015	NM_001014432	62241014
TGFbeta Receptor III	Q03167	311033535	NM_003243	307574689
SHP1	NP_002822	18104989	NM_002831	166064064
SHP2	Q06124	84028248	NM_002834	33356176
PD-1	NP_001129245	209413749	NM_001135773	209413748
CD28	AAA51945	180092	NM_006139	340545506

[0139] Any number of nucleic acids can be placed under the control of an inducible promoter including those coding for chimeric antigen receptor, a marker sequences, a cytokine, a chemokine, an inhibitor of apoptosis, and/ or an inhibitor of negative checkpoint signaling. In some alternatives, one or more inducible promoters can be utilized to provide for an adequate expression level of each of the nucleic acids. In some alternatives, constructs can

be prepared with a gene such as a cytokine under the control of an inducible promoter and a construct comprising a chimeric antigen receptor under the control of a constitutive promoter. Such constructs are useful to provide for cell survival and proliferation of transduced cells, for example lymphocytes expressing a chimeric antigen receptor.

Constitutive Promoter Systems.

[0140] In other alternatives, a system comprises a second nucleic acid that comprises a constitutive promoter or a second inducible promoter linked to a transcriptional activator. In other alternatives, a system comprises a second nucleic acid that comprises a promoter linked to a transcriptional activator. In some alternatives, the promoter is a constitutive promoter or an inducible promoter. In some alternatives, a constitutive promoter includes EF1α promoter, actin promoter, the myosin promoter, the hemoglobin promoter, and the creatine kinase promoter. In some alternatives, viral promoters such as the CMV promoter are excluded. In some alternatives, the constitutive promoter can be linked to one or more of a polynucleotide coding for marker, or a chimeric antigen receptor as described herein.

Constitutive Promoters.

[0141] A constitutive promoter provides for continuous gene expression of the gene under the control of the promoter. In some alternatives, the constitutive promoter is a promoter that provides for gene expression in a lentiviral construct and/or in lymphocytes. In some alternatives, the promoter is not derived from a xenogenic source such as a plant or a virus.

[0142] In a specific alternative, the constitutive promoter comprises EF1α promoter, actin promoter, the myosin promoter, the hemoglobin promoter, and/or the creatine kinase promoter. In some alternatives, viral promoters such as the CMV promoter are excluded.

Transcriptional Activators.

[0143] In some alternatives, the constitutive promoter is operably linked to a transcriptional activator. In some alternatives, the transcriptional activator activates an inducible promoter in the presence of the inducer (e.g. drug).

[0144] In some alternatives, an inducible promoter is induced in the presence of a transcriptional activator. In some alternatives, the transcriptional activator preferentially binds

to the promoter in the presence of the drug. In some alternatives, the transcriptional activator is TamR-tf(HEA3). Modification of the transcriptional activator can be made in the amino acid sequence that can affect the ability of activator to bind to the drug, the promoter, or both. For example, binding in the ER ligand binding domain would affect the binding of the drug to the transcriptional activator.

[0145] In some alternatives, the system employs a synthetic transcriptional activator which, in the presence of the drug (e.g.tamoxifen), binds a synthetic promoter upstream of a transgene to induce expression. In some alternatives, the transcriptional activator is TamR-tf (HEA3). The tamoxifen regulated transcription factor ("TamR-tf", also designated "HEA3") is a chimeric transcription factor composed of human subunits including the N-terminal DNA binding domain of Hepatocyte Nuclear Factor 1-alpha (HNF-1α) (e.g. amino acids 1-281 of SEQ ID NO: 40) fused in frame to the mutant tamoxifen-specific ligand binding domain of the estrogen receptor ligand binding domain (ER-LBD), that is in turn fused to the p65 activation domain of NF-κB (p65). An exemplary amino acid sequence is provided in **Table 10** and is identified as SEQ ID NO: 40. The mutant tamoxifen-specific ligand binding domain of the estrogen receptor ligand binding domain (ER-LBD) is found at amino acids 282-595 of the TamR-tf and has a mutation at position 521. The p65 activation domain of NF-κB (p65 or TAD) is found at amino acids 596 to 862.

[0146] Additional changes can be made to the transcriptional activator to increase the properties of the transcription factor including, without limitation, altering one or more amino acids in the estrogen receptor ligand binding domain and/or altering one or more amino acids in the p65 transactivating domain. Altering amino acids in the estrogen receptor binding domain can provide for more specific binding of the drug to the transcriptional activator. An example of a transcriptional activator with altered sequence in the ER-LBD is shown in **Table 11** (SEQ ID NO:43). Mutations are made at amino acid position 400, 543, and 544 of SEQ ID NO: 40. The transcriptional activator with altered sequence has increased affinity for tamoxifen or 4-OHT. Altering amino acids in the p65 transactivating domain can provide for increased expression of the transgene in the absence of activation of the transduced cells.

Marker.

[0147] In some alternatives, the constitutive promoter is operably linked to a polynucleotide coding for a marker polypeptide. Such marker polypeptides are described herein, and include EGFRt, Her2t, and/or DHFRdm.

Chimeric Antigen Receptor.

[0148] In some alternatives, the constitutive promoter is operably linked to a polynucleotide coding for a chimeric antigen receptor. In some alternatives, the chimeric antigen receptor comprises a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the spacer is optimized to provide for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. Examples of chimeric antigen receptors are described herein.

Vectors.

[0149] A variety of vector combinations can be constructed to provide for efficiency of transduction and transgene expression. In some alternatives, the vector is a dual packaged or single (all in one) viral vector. In other alternatives, the vectors can include a combination of viral vectors and plasmid vectors. Other viral vectors include foamy virus, adenoviral vectors, retroviral vectors, and lentiviral vectors. In some alternatives, the vector is a lentiviral vector. In some alternatives, the vector is a foamy viral vector, adenoviral vectors, retroviral vectors or lentiviral vectors.

[0150] In some alternatives, a plasmid vector or a viral vector comprises a first nucleic acid comprising an inducible promoter linked to a polynucleotide coding for a chimeric antigen receptor. In some alternatives, a plasmid vector or viral vector comprises a first nucleic acid sequence comprising a polynucleotide coding for a gene that enhances cell survival or proliferation, a gene that regulates apoptosis, and/or a gene that modulates

checkpoint signaling. In some alternatives, the modulation of checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises VISTA, LAG-3 and/or TIM3. Such polynucleotides code for a cytokine, or a chemokine receptor. In some alternatives, a plasmid vector or a viral vector comprises a first nucleic acid comprising an inducible promoter linked to a polynucleotide coding for a marker sequence. Marker sequences are described herein. In some alternatives, the marker sequence is compatible with transduction of human lymphocytes. In some alternatives, the marker sequence allows for selection of transduced cells and/or detection of transduced cells. In some alternatives, the marker is a gene that can include inter alia, hygromycin-B phosphotransferase gene (hph), which confers resistance to hygromycin B, the amino glycoside phosphotransferase gene (neo or aph) from Tn5, which codes for resistance to the antibiotic G418, the dihydrofolate reductase (DHFR) gene, which provides resistance to methotrexate, DHFR dm (exemplary polynucleotide and amino acid sequences in **Table 14**, SEQ ID NO:46 and SEQ ID NO:47, the pac gene that provides resistance to puromycin, Sh ble gene, which inactivates zeocin, the adenosine deaminase gene (ADA), and/or the multi-drug resistance (MDR) gene. A first nucleic acid can include a number of different polynucleotide sequences all under the control of the inducible promoter. For example, a polynucleotide coding for a chimeric antigenic receptor can be linked to a polynucleotide coding for a marker polypeptide and/or a polynucleotide coding for cytokine or chemokine receptor.

[0151] In some alternatives, a lentiviral vector comprises a second nucleic acid comprising a constitutive promoter linked to a nucleic acid sequence coding for transcriptional activator that binds to drug and activates expression of an inducible promoter. In some alternatives, a lentiviral vector with a constitutive promoter can also include a nucleic acid sequence including a marker gene, piggyback transposase, and/or a polynucleotide coding for a chimeric antigen receptor. Each element of the nucleic acid can be separated from one another with a sequence such as a T2A self-cleaving sequence. In some alternatives, the elements of the nucleic acid are separated from one another with a sequence self-cleaving sequence. In some alternatives, the self-cleaving sequence is T2A.

[0152] In other alternatives, the heterogeneous (heterogeneous to the vector, e,g, lentiviral vector) nucleic acid sequence is limited by the amount of additional genetic components that can be packaged in the vector. In some alternatives, a construct contains at least two genes heterogenous to the viral vector. In some alternatives, the construct contains

no more than 4 genes heterogenous to the viral vector. The number of genes heterogenous to the viral vector that can be packaged in the vector can be determined by detecting the expression of one or more transgenes, and selecting vector constructs that provide for transduction of at least 10% of the cells and/or detectable expression levels of the transgene in at least 10% of the cells.

[0153] In some alternatives, a lentivirus is a dual packaged virus. A dual packaged virus contains at least one conditional construct comprising an inducible promoter operably linked to a polynucleotide coding for a chimeric antigen receptor. Optionally the conditional construct comprises a marker gene, a nucleic acid for a cytokine, a nucleic acid for a chemokine receptor. In some alternatives, a dual packaged lentivirus contains a constitutive construct comprising a constitutive promoter. In an alternative, the constitutive construct comprises a constitutive promoter linked to a transcriptional activator for the inducible promoter. In some alternatives, the constitutive construct also includes a marker gene and/or a polynucleotide encoding a cytokine or chemokine. In some alternatives of a system with two constructs, each construct can be packaged in a separate viral vector and the viral vectors can be mixed together for transduction in a cell population.

[0154] When the constitutive and conditional constructs both contain a marker gene, the marker gene on each construct is the same or different from one another. In some alternatives, when the constitutive and conditional constructs both contain a polynucleotide coding for a chimeric antigen receptor, the chimeric antigen receptor can be targeted to the same antigen but have different ligand binding domains, can be targeted to the same antigen but different epitopes, or can be targeted to different antigens.

[0155] In some alternatives, the vector is a minicircle. Minicircles are episomal DNA vectors that are produced as circular expression cassettes devoid of any bacterial plasmid DNA backbone. Their smaller molecular size enables more efficient transfections and offers sustained expression over a period of weeks as compared to standard plasmid vectors that only work for a few days. In some alternatives, a minicircle contains a drug inducible promoter linked to a polynucleotide coding for a chimeric antigen receptor. In some alternatives, the inducible promoter can be linked to chemokine receptor, a marker gene, and/or a cytokine. One or more minicircles can be employed. In some alternatives, a minicircle comprises an inducible promoter linked to a polynucleotide coding for a first chimeric antigen receptor, another minicircle comprises an inducible promoter linked to a polynucleotide coding for a second and different chimeric antigen receptor, and/ or a

minicircle comprises an inducible promoter linked to a polynucleotide coding for a chemokine receptor, a chimeric antigen receptor, and a marker gene. Each element of the constructs is separated by a nucleic acid, such as that coding for a self-cleaving T2A sequence. In some alternatives, each element of the constructs is separated by a nucleic acid, such as that coding for a self-cleaving T2A sequence. In some alternatives each minicircle differs from one another in the chimeric antigen receptor including but not limited to the spacer length and sequence, the intracellular signaling domain, and/or the marker sequence. The minicircle vector can be used with a constitutive lentivirus vector coding for a transcriptional activator for the inducible promoter. In some alternatives, the minicircle vector is used with a constitutive lentivirus vector coding for a transcriptional activator for the inducible promoter.

[0156] In some alternatives, the vector is a piggy bac transposon. The PiggyBac (PB) transposon is a mobile genetic element that efficiently transposes between vectors and chromosomes via a "cut and paste" mechanism. During transposition, the PB transposase recognizes transposon-specific inverted terminal repeat sequences (ITRs) located on both ends of the transposon vector and efficiently moves the contents from the original sites and efficiently integrates them into TTAA chromosomal sites. The powerful activity of the PiggyBac transposon system enables genes of interest between the two ITRs in the PB vector to be easily mobilized into target genomes.

[0157] In some alternatives, a PB contains a drug inducible promoter linked to a polynucleotide coding for a chimeric antigen receptor. In some alternatives, the inducible promoter can be linked to chemokine receptor, a marker gene, and/or a cytokine. One or more PB transposons can be employed. In some alternatives, a PB comprises an inducible promoter linked to a polynucleotide coding for a first chimeric antigen receptor, another PB comprises an inducible promoter linked to a polynucleotide coding for a second and different chimeric antigen receptor, and/ or a PB comprises an inducible promoter linked to a polynucleotide coding for a chemokine receptor, a chimeric antigen receptor, and a marker gene. Each element of the constructs is separated by a nucleic acid, such as that coding for a self-cleaving T2A sequence. In some alternatives each PB differs from one another in the chimeric antigen receptor including but not limited to the spacer length and sequence, the intracellular signaling domain, and/or the marker sequence. The PB vector can be used with a constitutive lentivirus vector coding for a transcriptional activator for the inducible promoter and constitutive vector comprising the piggyback transposase linked to a constitutive promoter.

[0158] In some alternatives, a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; and d) a polynucleotide coding for an intracellular signaling domain; and a second nucleic acid comprising a second constitutive or inducible promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the first and second nucleic acid are in a single lentivirus vector. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor.

[0159] In some alternatives, the first nucleic acid further comprises a marker gene. In some alternatives, the second nucleic acid further comprises polynucleotide coding for a second and different chimeric antigen receptor. The first and second chimeric antigen receptor can differ from one another in the ligand binding domain, the target antigen, an epitope of the target antigen, the spacer domain in length and sequence (short medium or long), and in the intracellular signaling domains.

[0160] In some alternatives, in a single lentivirus construct the first and second nucleic acids can be separated by a genomic insulator nucleic acid such as the sea urchin insulator chromatin domain. In other alternatives, the inducible promoter of the first nucleic acid and the constitutive promoter of the second nucleic acid are in opposite orientation.

[0161] One or more of these vectors can be used in conjunction with one another to transduce target cells and provide for inducible expression of a chimeric antigen receptor.

Host Cells and Compositions: T lymphocyte populations.

[0162] The compositions described herein provide for genetically modified host cells with the vectors and/or constructs as described herein. In some alternatives, the host cells are CD4+ and/or CD8+ T lymphocytes.

[0163] T lymphocytes can be collected in accordance with known techniques and enriched or depleted by known techniques such as affinity binding to antibodies such as flow cytometry and/or immunomagnetic selection. After enrichment and/or depletion steps, *in vitro*

expansion of the desired T lymphocytes can be carried out in accordance with known techniques or variations thereof that will be apparent to those skilled in the art. In some alternatives, the T cells are autologous T cells obtained from the patient.

[0164] For example, the desired T cell population or subpopulation can be expanded by adding an initial T lymphocyte population to a culture medium in vitro, and then adding to the culture medium feeder cells, such as non-dividing peripheral blood mononuclear cells (PBMC), (e.g., such that the resulting population of cells contains at least 5, 10, 20, or 40 or more PBMC feeder cells for each T lymphocyte in the initial population to be expanded); and incubating the culture (e.g. for a time sufficient to expand the numbers of T cells). The non-dividing feeder cells can comprise gamma-irradiated PBMC feeder cells. In some alternatives, the PBMC are irradiated with gamma rays in the range of 3000 to 3600 rads to prevent cell division. In some alternatives, the PBMC are irradiated with gamma rays of 3000, 3100, 3200, 3300, 3400, 3500 or 3600 rads or any value of rads between any two endpoints of any of the listed values to prevent cell division. The order of addition of the T cells and feeder cells to the culture media can be reversed if desired. The culture can typically be incubated under conditions of temperature and the like that are suitable for the growth of T lymphocytes. For the growth of human T lymphocytes, for example, the temperature will generally be at least 25 degrees Celsius, preferably at least 30 degrees, more preferably 37 degrees. In some alternatives, the temperature for the growth of human T lymphocytes is 22, 24, 26, 28, 30, 32, 34, 36, 37 degrees Celsius or any other temperature between any two endpoints of any of the listed values.

[0165] The T lymphocytes expanded include $CD8^+$ cytotoxic T lymphocytes (CTL) and $CD4^+$ helper T lymphocytes that can be specific for an antigen present on a human tumor or a pathogen. In some alternatives, the cells include precursor T cells. In some alternatives, the cells are hematopoietic stem cells.

[0166] In some alternatives, the expansion method can further comprise adding non-dividing EBV-transformed lymphoblastoid cells (LCL) as feeder cells. LCL can be irradiated with gamma rays in the range of 6000 to 10,000 rads. In some alternatives, the LCL are irradiated with gamma rays in of 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500 or 10,000 rads or any amount of rads between two endpoints of any of the listed values. The LCL feeder cells can be provided in any suitable amount, such as a ratio of LCL feeder cells to initial T lymphocytes of at least 10:1.

[0167] In some alternatives, the expansion method can further comprise adding anti-CD3 and/or anti CD28 antibody to the culture medium (*e.g.*, at a concentration of at least 0.5 ng/ml). In some alternatives, the expansion method can further comprise adding IL-2 and/or IL-15 to the culture medium (*e.g.*, wherein the concentration of IL-2 is at least 10 units/ml).

[0168] After isolation of T lymphocytes both cytotoxic and helper T lymphocytes can be sorted into naïve, memory, and effector T cell subpopulations either before or after expansion.

[0169] CD8+ cells can be obtained by using standard methods. In some alternatives, CD8+ cells are further sorted into naïve, central memory, and effector memory cells by identifying cell surface antigens that are associated with each of those types of CD8+ cells. In some alternatives, memory T cells are present in both CD62L+ and CD62L- subsets of CD8+ peripheral blood lymphocytes. PBMC are sorted into CD62L-CD8+ and CD62L+CD8+ fractions after staining with anti-CD8 and anti-CD62L antibodies. In some alternatives, the expression of phenotypic markers of central memory T_{CM} include CD45RO, CD62L, CCR7, CD28, CD3, and/or CD127 and are negative or low for granzyme B. In some alternatives, central memory T cells are CD45RO+, CD62L+, and/or CD8+ T cells. In some alternatives, effector T_E are negative for CD62L, CCR7, CD28, and/or CD127, and positive for granzyme B and/or perforin. In some alternatives, naïve CD8+ T lymphocytes are characterized by the expression of phenotypic markers of naïve T cells including CD62L, CCR7, CD28, CD3, CD127, and/or CD45RA.

[0170] CD4+ T helper cells are sorted into naïve, central memory, and effector cells by identifying cell populations that have cell surface antigens. CD4+ lymphocytes can be obtained by standard methods. In some alternatives, naïve CD4+ T lymphocytes are CD45RO-, CD45RA+, CD62L+, and/or CD4+ T cells. In some alternatives, central memory CD4+ cells are CD62L+ and/or CD45RO+. In some alternatives, effector CD4+ cells are CD62L- and/or CD45RO-.

[0171] Whether a cell or cell population is positive for a particular cell surface marker can be determined by flow cytometry using staining with a specific antibody for the surface marker and an isotype matched control antibody. A cell population negative for a marker refers to the absence of significant staining of the cell population with the specific antibody above the isotype control, positive refers to uniform staining of the cell population above the isotype control. In some alternatives, a decrease in expression of one or markers

refers to loss of 1 log10 in the mean fluorescence intensity and/or decrease of percentage of cells that exhibit the marker of at least 20% of the cells, 25% of-the cells, 30% of the cells, 35% of the cells, 40% of the cells, 45% of the cells, 50% of the cells, 55% of the cells, 60% of the cells, 65% of the cells, 70% of the cells, 75% of the cells, 80% of the cells, 85% of the cells, 90% of the cell, 95% of the cells, and 100% of the cells or any % between 20 and 100% when compared to a reference cell population. In some alternatives, a cell population positive for one or markers refers to a percentage of cells that exhibit the marker of at least 50% of the cells, 55% of the cells, 60% of the cells, 65% of the cells, 70% of the cells, 75% of the cells, 80% of the cells, 85% of the cells, 90% of the cell, 95% of the cells, or 100% of the cells or any % between 50 and 100% when compared to a reference cell population.

[0172] Whether a cell or cell population is positive for a particular cell surface marker can be determined by flow cytometry using staining with a specific antibody for the surface marker and an isotype matched control antibody. A cell population negative for a marker refers to the absence of significant staining of the cell population with the specific antibody above the isotype control, positive refers to uniform staining of the cell population above the isotype control. In some alternatives, a decrease in expression of one or markers refers to loss of 1 log10 in the mean fluorescence intensity and/or decrease of percentage of cells that exhibit the marker of at least 20% of the cells, 25% of-the cells, 30% of the cells, 35% of the cells, 40% of the cells, 45% of the cells, 50% of the cells, 55% of the cells, 60% of the cells, 65% of the cells, 70% of the cells, 75% of the cells, 80% of the cells, 85% of the cells, 90% of the cell, 95% of the cells, and 100% of the cells or any % between 20 and 100% when compared to a reference cell population. In some alternatives, an increase refers to an increase in mean fluorescence intensity and/or to an increase in the number of cells in a cell population that are positive for one or a given marker, such as a population in which s refers to a percentage of cells that exhibit the marker of at least 50% of the cells, 55% of the cells, 60% of the cells, 65% of the cells, 70% of the cells, 75% of the cells, 80% of the cells, 85% of the cells, 90% of the cell, 95% of the cells, or 100% of the cells or any % between 50 and 100% exhibit the marker, e.g., when compared to a reference cell population.

[0173] In some alternatives, populations of CD4+ and CD8+ that are antigen specific can be obtained by stimulating naïve or antigen specific T lymphocytes with antigen. For example, antigen-specific T cell lines or clones can be generated to Cytomegalovirus antigens by isolating T cells from infected subjects and stimulating the cells in vitro with the same antigen. Naïve T cells can also be used. Any number of antigens from tumor cells can

be utilized as targets to elicit T cell responses. In some alternatives, the adoptive cellular immunotherapy compositions are useful in the treatment of a disease or disorder including a solid tumor, hematologic malignancy, breast cancer or melanoma.

Modification of T lymphocyte populations.

[0174] In some alternatives it can be desired to introduce functional genes into the T cells to be used in immunotherapy in accordance with the present disclosure. For example, the introduced gene or genes can improve the efficacy of therapy by promoting the viability and/or function of transferred T cells; or they can provide a genetic marker to permit selection and/or evaluation of *in vivo* survival or migration; or they can incorporate functions that improve the safety of immunotherapy, for example, by making the cell susceptible to controlled expression of the transgene. This can be carried out in accordance with known techniques that will be apparent to those skilled in the art based upon the present disclosure.

[0175] In some alternatives, T cells are modified with a vector coding for drug inducible chimeric receptors as described herein. In some alternatives, cells are modified with a vector comprising a polynucleotide coding for a chimeric antigen receptor under control of an inducible promoter. In other alternatives, cells are modified with a vector comprising a polynucleotide coding for a cytokine, chemokine receptor, a gene that regulates apoptosis, or a gene that modulates checkpoint signaling under the control of an inducible promoter. In some alternatives, the T cells are obtained from the subject to be treated, in other alternatives, the lymphocytes are obtained from allogeneic human donors, preferably healthy human donors. In some alternatives, the modulation of checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises VISTA, LAG-3 and/or TIM3.

[0176] Chimeric receptors can be constructed with a specificity for any cell surface marker by utilizing antigen binding fragments or antibody variable domains of, for example, antibody molecules. The antigen binding molecules can be linked to one or more cell signaling modules. In some alternatives, cell signaling modules include CD3 transmembrane domains, CD3 intracellular signaling domains, and/or CD28 transmembrane domains. In some alternatives, the intracellular signaling domain comprises a CD28 transmembrane and signaling domain linked to a CD3 zeta intracellular domain. In some alternatives, a chimeric receptor can also include a transduction marker such as tEGFR.

[0177] In some alternatives, the same or a different chimeric receptor can be introduced into each of population of CD4+ and/or CD8+ T lymphocytes. In some alternatives, the chimeric receptor in each of these populations has a ligand binding domain that specifically binds to the same ligand on the tumor or infected cell or a different antigen or epitope. The cellular signaling modules can differ. In some alternatives, the intracellular signaling domain of the CD8+ cytotoxic T cells is the same as the intracellular signaling domain of the CD4+ helper T cells. In other alternatives, the intracellular signaling domain of the CD8+ cytotoxic T cells is different than the intracellular signaling domain of the CD4+ helper T cells.

[0178] In some alternatives each of the CD4 or CD8 T lymphocytes can be sorted into naïve, central memory, effector memory or effector cells prior to transduction, as described herein. In some alternatives, each of the CD4 or CD8 T lymphocytes can be sorted into naïve, central memory, effector memory, or effector cells after transduction.

[0179] As described herein, in some alternatives, naïve CD4+ cells are CD45RO-, CD45RA+, CD62L+, and/or CD4+ positive T cells. In some alternatives, central memory CD4+ cells are CD62L positive and/or CD45RO positive. In some alternatives, effector CD4+ cells are CD62L negative and/or CD45RO positive. Each of these populations can be independently modified with a chimeric receptor.

[0180] As described, in some alternatives, memory T cells are present in both CD62L+ and CD62L- subsets of CD8+ peripheral blood lymphocytes. PBMC are sorted into CD62L-CD8+ and CD62L+CD8+ fractions after staining with anti-CD8 and anti-CD62L antibodies. In some alternatives, the expression of phenotypic markers of central memory T cells (TCM) include CD62L, CCR7, CD28, CD3, and/or CD127 and are negative or low for granzyme B. In some alternatives, central memory T cells are CD45RO+, CD62L+, and/or CD8+ T cells. In some alternatives, effector T cells (T_E) are negative for CD62L, CCR7, CD28, and/or CD127, and positive for granzyme B and/or perforin. In some alternatives, naïve CD8+ T lymphocytes are characterized by CD8+, CD62L+, CD45RO+, CCR7+, CD28+ CD127+, and/or CD45RO+. Each of these populations can be independently modified with a chimeric receptor.

[0181] Various transduction techniques have been developed which utilize recombinant infectious virus particles for gene delivery. This represents a currently preferred approach to the transduction of T lymphocytes of the present invention. The viral vectors, which have been used in this way include virus vectors derived from simian virus 40,

adenoviruses, adeno-associated virus (AAV), lentiviral vectors, and/or retroviruses. Thus, gene transfer and expression methods are numerous but essentially function to introduce and express genetic material in mammalian cells. Several of the above techniques have been used to transduce hematopoietic or lymphoid cells, including calcium phosphate transfection, protoplast fusion, electroporation, and infection with recombinant adenovirus, adeno-associated virus and retrovirus vectors. Primary T lymphocytes have been successfully transduced by electroporation and by retroviral or lentiviral infection.

[0182] Retroviral and lentiviral vectors provide a highly efficient method for gene transfer into eukaryotic cells. Moreover, retroviral or lentiviral integration takes place in a controlled fashion and results in the stable integration of one or a few copies of the new genetic information per cell.

[0183] It is contemplated that overexpression of a stimulatory factor (for example, a lymphokine or a cytokine) can be toxic to the treated individual. Therefore, it is within the scope of the invention to include gene segments that cause the T cells of the invention to be susceptible to negative selection in vivo. By "negative selection" is meant that the infused cell can be eliminated as a result of a change in the in vivo condition of the individual. The negative selectable phenotype can result from the insertion of a gene that confers sensitivity to an administered agent, for example, a compound. Negative selectable genes are known in the art, and include, inter alia the following: the Herpes simplex virus type I thymidine kinase (HSV-I TK) gene, which confers ganciclovir sensitivity; the cellular hypoxanthine phosphribosyltransferase (HPRT) gene, the cellular adenine phosphoribosyltransferase (APRT) gene, and bacterial cytosine deaminase.

[0184] In some alternatives it can be useful to include in the T cells a positive marker that enables the selection of cells of the negative selectable phenotype *in vitro*. The positive selectable marker can be a gene that upon being introduced into the host cell expresses a dominant phenotype permitting positive selection of cells carrying the gene. Genes of this type are known in the art, and include, inter alia, hygromycin-B phosphotransferase gene (hph), which confers resistance to hygromycin B, the amino glycoside phosphotransferase gene (neo or aph) from Tn5, which codes for resistance to the antibiotic G418, the dihydrofolate reductase (DHFR) gene, the adenosine deaminase gene (ADA), and/or the multi-drug resistance (MDR) gene.

[0185] A variety of methods can be employed for transducing T lymphocytes, as is well known in the art. In some alternatives, transduction is carried out using lentiviral vectors.

[0186] In some alternatives, CD4+ and CD8+ cells each can separately be modified with an expression vector encoding a chimeric receptor to form defined populations. In some alternatives, cells can be separately modified with a vector comprising a polynucleotide under the control of a constitutive promoter and a vector comprising a polynucleotide coding for a cytokine or chemokine receptor under control of an inducible promoter.

[0187] In some alternatives, these cells are then further sorted into subpopulations of naïve, central memory and effector cells as described above, by sorting for cell surface antigens unique to each of those cell populations. In addition, CD4+ or CD8+ cell populations can be selected by their cytokine profile or proliferative activities. For example, CD4+ T lymphocytes that have enhanced production of cytokines such as IL-2, IL-4, IL-10, TNFα, and/or IFNγ, as compared to sham transduced cells or transduced CD8+ cells when stimulated with antigen can be selected. In other alternatives, naïve or central memory CD4+ T cells that have enhanced production of IL-2 and/or TNFα are selected. Likewise, CD8+ cells that have enhanced IFNγ production are selected, as compared to sham transduced CD8+ cells.

[0188] In some alternatives, CD4+ and CD8+ cells are selected that are cytotoxic for antigen bearing cells. In some alternatives, CD4+ are expected to be weakly cytotoxic as compared to CD8+ cells. In a preferred alternative, transduced lymphocytes, such as CD8+ central memory cells, are selected that provide for tumor cell killing in vivo using an animal model established for the particular type of cancer.

[0189] In yet other alternatives, transduced chimeric receptor expressing T cells are selected that can persist *in vivo* using an animal model established for the particular type of cancer. In some alternatives, transduced chimeric receptor CD8+ central memory cells with a short spacer region have been shown to persist *in vivo* after introduction into the animal for 3 days or more, 10 days or more, 20 days or more, 30 days or more, 40 days or more, or 50 days or more.

[0190] The disclosure contemplates that combinations of CD4+ and CD8+ T cells will be utilized in the compositions. In one alternative, combinations of chimeric receptor transduced CD4+ cells can be combined with chimeric receptor transduced CD8+ cells of the

same ligand specificity or combined with $CD8^+$ T cells that are specific for a distinct tumor ligand. In other alternatives, chimeric receptor transduced CD8+ cells are combined with chimeric receptor transduced CD4+ cells specific for a different ligand expressed on the tumor. In yet another alternative, chimeric receptor modified CD4+ and CD8+ cells are combined. In some alternatives CD8+ and CD4+ cells can be combined in different ratios for example, a 1:1 ratio of CD8+ and CD4+, a ratio of 10:1 of CD8+ to CD4+, or a ratio of 100:1 of CD8+ to CD4+, or any other ratio of CD8+ to CD4+ that is between any of the listed ratios. In some alternatives, the combined population is tested for cell proliferation in vitro and/or in vivo, and the ratio of cells that provides for proliferation of cells is selected.

[0191] After transduction and/or selection for chimeric receptor bearing cells, the cell populations are preferably expanded in vitro until a sufficient number of cells are obtained to provide for at least one infusion into a human subject, typically around 10^4 cells/kg to 10^9 cells/kg In some alternatives, the transduced cells are cultured in the presence of antigen bearing cells, anti CD3, anti CD28, and IL 2, IL-7, IL 15, or IL-21 or combinations thereof.

[0192] In some alternatives, CD4+ and CD8+cells that proliferate in response to cytokine stimulation, antigen or tumor targets in vitro or in vivo are selected. For example, CD4+ or CD8+ transduced cells that proliferate vigorously when stimulated with antiCD3 and/ or anti-CD28 are selected. In some alternatives, stimulation of transduced cells provides for enhanced transgene expression in the presence of an inducer (e.g. drug) of those trans genes under the control of an inducible promoter.

[0193] Each of the subpopulations of CD4+ and CD8+ cells can be combined with one another. In a specific alternative, modified naïve or central memory CD4+ cells are combined with modified central memory CD8+ T cells to provide a synergistic cytotoxic effect on antigen bearing cells, such as tumor cells.

Compositions.

[0194] The disclosure provides for an adoptive cellular immunotherapy composition comprising a genetically modified T lymphocyte cell preparation as described herein. In some alternatives, the T lymphocyte cell preparation comprises CD4 + T cells that have a chimeric receptor comprising an extracellular antibody variable domain specific for a ligand associated with the disease or disorder, a spacer region, a transmembrane domain, and an intracellular signaling domain of a T cell receptor or other receptors under the control of a

drug inducible promoter as described herein. In other alternatives, an adoptive cellular immunotherapy composition further comprises a chimeric receptor modified tumor-specific CD8+ cytotoxic T lymphocyte cell preparation that provides a cellular immune response, wherein the cytotoxic T lymphocyte cell preparation comprises CD8+ T cells that have a chimeric receptor comprising an extracellular single chain antibody specific for a ligand associated with the disease or disorder, a spacer region, a transmembrane domain, and an intracellular signaling domain of a T cell receptor under the control of a drug inducible promoter as described herein. In some alternatives, the chimeric receptor modified T cell population of the disclosure can persist *in vivo* for at least 3 days or longer. In an alternative, each of these populations can be combined with one another or other cell types to provide a composition.

[0195] In some alternatives, the CD4+ T helper lymphocyte cell is naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, or bulk CD4+ T cells. In some alternatives, CD4+ helper lymphocyte cell is a naïve CD4+ T cell, wherein the naïve CD4+ T cell comprises a CD45RO-, CD45RA+, and/or is a CD62L+ CD4+ T cell.

[0196] In some alternatives, the CD8+ T cytotoxic lymphocyte cell is a naïve CD8+ T cell, central memory CD8+ T cell, effector memory CD8+ T cell and/or bulk CD8+ T cell. In some alternatives, the CD8+ cytotoxic T lymphocyte cell is a central memory T cell, wherein the central memory T cell comprises a CD45RO+, CD62L+, and/or CD8+ T cell. In yet other alternatives, the CD8+ cytotoxic T lymphocyte cell is a central memory T cell and the CD4+ helper T lymphocyte cell is a naïve or central memory CD4+ T cell.

[0197] In some alternatives, the compositions comprise T cell precursors. In some alternatives, the compositions comprise hematopoietic stem cells. In some alternatives, the composition comprises a host cell wherein the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells or a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells and a second host cell, wherein the second host cell is a precursor T cell. In some alternatives, the precursor T cell is a hematopoietic stem cell.

Methods.

[0198] The disclosure provides methods of making adoptive immunotherapy compositions and uses or methods of using these compositions for performing cellular immunotherapy in a subject having a disease or disorder. In some alternatives, a method of manufacturing the compositions comprises obtaining a modified naïve or central memory CD4+ T helper cell, wherein the modified helper T lymphocyte cell preparation comprises CD4+ T cells that have a chimeric receptor comprising a ligand binding domain specific for a tumor cell surface molecule, a spacer domain, a transmembrane domain, and an intracellular signaling domain under control of an inducible promoter as described herein. In other alternatives, CD4+ cells have a cytokine or chemokine receptor under the control of an inducible promoter.

[0199] In another alternative, a method further comprises obtaining a modified CD8+ central memory T cell, wherein the modified central memory CD8 T lymphocyte cell preparation comprises CD8+ cells that have a chimeric receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte, a spacer domain, a transmembrane domain, and an intracellular signaling domain under control of the inducible promoter as described herein. In other alternatives, CD4+ cells have a cytokine or chemokine receptor under the control of an inducible promoter.

[0200] The drug inducible promoter in both modified CD4+ T cells and modified CD8+ cytotoxic T cells can be the same or different. In some alternatives, in one population of cells the promoter linked to the chimeric antigen receptor is a constitutive promoter and in the other population it is an inducible promoter. For example, modified CD4+ T cells that have a chimeric receptor comprising a ligand binding domain specific for a tumor cell surface molecule, a spacer domain, a transmembrane domain, and an intracellular signaling domain under control of an constitutive promoter, while the CD8+ cytotoxic T cell comprises CD8+ cells that have a chimeric receptor comprising a ligand binding domain specific for a tumor cell surface molecule, a spacer domain, a transmembrane domain, and an intracellular signaling domain under control of the inducible promoter.

[0201] In some alternatives, the polynucleotide can code for a chimeric antigen receptor that differs in the CD4+ versus the CD8+ cell population. The difference between the two constructs can include the specificity or affinity of the ligand binding domain for an

antigen or epitope, the length and sequence of the spacer region, and the intracellular signaling components.

[0202] The preparation of the CD4+ and CD8+ cells that are modified with a chimeric receptor is described throughout this disclosure. Antigen specific T lymphocytes can be obtained from a patient having the disease or disorder or can be prepared by in vitro stimulation of T lymphocytes in the presence of antigen. Subpopulations of CD4+ and/or CD8+ T lymphocytes that are not selected for antigen specificity can also be isolated as described herein and combined in the methods of manufacturing.

[0203] In some alternatives, the combination of cell populations can be evaluated for uniformity of cell surface makers, the ability to proliferate through at least two generations, to have a uniform cell differentiation status. Quality control can be performed by co-culturing a cell line expressing the target ligand with chimeric receptor modified T cells and the drug that induces expression of the chimeric antigen receptor to determine if the chimeric receptor modified T cells recognize the cell line using cytotoxicity, proliferation, or cytokine production assays in the presence of the inducer that are known in the field. Cell differentiation status and cell surface markers on the chimeric receptor modified T cells can be determined by flow cytometry. In some alternatives, the markers and cell differentiation status on the CD8+ cells include CD3, CD8, CD62L, CD28, CD27, CD69, CD25, PD-1, CTLA-4, CD45RO, and/or CD45RA. In some alternatives, the markers and the cell differentiation status on the CD4+ cells include CD3, CD4, CD62L, CD28, CD27, CD69, CD25, PD-1, CTLA-4 CD45RO, and/or CD45RA.

[0204] In some alternatives, the chimeric receptor modified T cells as described herein are able to persist *in vivo* for at least 3 days, or at least 10 days. In some alternatives, the chimeric receptor modified T cells as described herein, can proliferate *in vivo* through at least 2, or at least 3 generations as determined by CFSE dye dilution. Proliferation and persistence of the chimeric receptor modified T cells can be determined by using an animal model of the disease or disorder and administering the cells and determining persistence and/or proliferative capacity of the transferred cells. In other alternatives, proliferation and activation can be tested in vitro by going through multiple cycles of activation with antigen bearing cells.

[0205] The disclosure also provides methods of performing cellular immunotherapy in a subject having a disease or disorder comprising: administering a

composition of lymphocytes expressing a chimeric receptor under the control of a drug inducible promoter as described herein, and administering the drug.

[0206] In some alternatives, the drug is tamoxifen, variants, derivatives, pharmaceutical salts, solvates, and hydrates thereof as described herein. In some alternatives, the drug is delivered prior to, at the same time as the composition, or at later time points after the composition has been administered.

[0207] In some alternatives, the drug is administered with the composition, and if a toxic effect of the composition is observed the drug is withdrawn until the toxic effects diminish. After the symptoms of toxicity diminish, the drug is administered again. In some alternatives, a drug can be administered again once symptoms of toxicity diminish.

[0208] In some alternatives, the drug is administered with the composition but once the subject has a decrease in the tumor load or cancer cells, the drug is withdrawn for a period of time to allow the modified cells to rest and if there is no activity of the modified cells, the modified cells are not needed because of remission of the cancer.

[0209] In other alternatives, a method comprises administering to the subject a genetically modified cytotoxic T lymphocyte cell preparation that provides a cellular immune response, wherein the cytotoxic T lymphocyte cell preparation comprises CD8 + T cells that have a chimeric receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte, a spacer domain, a transmembrane domain, and an intracellular signaling domain under the control of a drug inducible promoter as described herein, and/or a genetically modified helper T lymphocyte cell preparation that elicits direct tumor recognition and augments the genetically modified cytotoxic T lymphocyte cell preparations ability to mediate a cellular immune response, wherein the helper T lymphocyte cell preparation comprises CD4+ T cells that have a chimeric receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte, a spacer domain, a transmembrane domain, and an intracellular signaling domain under control of a constitutive or drug inducible promoter as described herein and administering the drug that induces the inducible promoter. In some alternatives, the administering of the drug is performed after

administering of the composition or host cells, wherein administering is performed 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 2 weeks, 4 weeks or two months, or any time in between any two values of time listed.

[0210] In other alternatives, a method comprises administering to the subject a genetically modified cytotoxic T lymphocyte cell preparation that provides a cellular immune response, wherein the cytotoxic T lymphocyte cell preparation comprises CD8 + T cells that have a chimeric receptor comprising a ligand binding domain specific for a tumor cell surface molecule, a spacer domain, a transmembrane domain, and an intracellular signaling domain under the control of a constitutive promoter as described herein, and/or a genetically modified helper T lymphocyte cell preparation that elicits direct tumor recognition and augments the genetically modified cytotoxic T lymphocyte cell preparations ability to mediate a cellular immune response, wherein the helper T lymphocyte cell preparation comprises CD4+ T cells that have a chimeric receptor comprising a ligand binding domain specific for a tumor cell surface molecule, a spacer domain, a transmembrane domain, and an intracellular signaling domain under control of a constitutive or drug inducible promoter as described herein and administering the drug that induces the inducible promoter. In some alternatives, the tumor specific molecule is a tumor surface molecule.

[0211] In other alternatives, a method comprises administering to the subject a genetically modified cytotoxic T lymphocyte cell preparation that provides a cellular immune response, wherein the cytotoxic T lymphocyte cell preparation comprises CD8 + T cells that express a cytokine, chemokine receptor, a polypeptide that regulates apoptosis, and/or a polypeptide that modulates checkpoint signaling under the control of an inducible promoter as described herein, and/or a genetically modified helper T lymphocyte cell preparation that elicits direct tumor recognition and augments the genetically modified cytotoxic T lymphocyte cell preparations ability to mediate a cellular immune response, wherein the helper T lymphocyte cell preparation comprises CD4+ T cells that express a cytokine, chemokine receptor, a polypeptide that regulates apoptosis, and/or a polypeptide that modulates checkpoint signaling under control of a constitutive or drug inducible promoter as described herein and administering the drug that induces the inducible promoter. In some alternatives, one or more of the cell populations expresses a chimeric antigen receptor under the control of a constitutive promoter. In some alternatives, the modulation of checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises VISTA, LAG-3 and/or TIM3.

[0212] An effective amount of the drug for induction is an amount of the drug that provides for induction of the chimeric antigen receptor in at least 5 %, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100% or any number in between any of the listed percent values of the transduced cells.

[0213] Another alternative describes a method of performing cellular immunotherapy in a subject having a disease or disorder comprising: analyzing a biological sample of the subject for the presence of a target molecule associated with the disease or disorder and administering the adoptive immunotherapy compositions described herein and administering the drug that induces the inducible promoter, wherein the chimeric receptor specifically binds to the target molecule.

[0214] Subjects that can be treated by the present invention are, in general, human and other primate subjects, such as monkeys and apes for veterinary medicine purposes. The subjects can be male or female and can be any suitable age, including infant, juvenile, adolescent, adult, and geriatric subjects.

[0215] The methods are useful in the treatment or inhibition of, for example, hematologic malignancy, melanoma, breast cancer, brain cancer, and other epithelial malignancies or solid tumors. In some alternatives, the molecule associated with the disease or disorder is an orphan tyrosine kinase receptor ROR1, Her2, EGFR, CE7, hB7H3, CD19, CD20, CD22, mesothelin, CEA, or a hepatitis B surface antigen.

[0216] Subjects that can be addressed using the methods described herein include subjects identified or selected as having cancer, including but not limited to colon, lung, liver, breast, renal, prostate, ovarian, skin (including melanoma), bone, and brain cancer, etc. Such identification and/or selection can be made by clinical or diagnostic evaluation. In some alternatives the tumor associated antigens or molecules are known, such as melanoma, breast cancer, brain cancer, squamous cell carcinoma, colon cancer, leukemia, myeloma, and/or prostate cancer. In other alternatives the tumor associated molecules can be targeted with genetically modified T cells expressing an engineered chimeric receptor. Examples include but are not limited to B cell lymphoma, breast cancer, brain cancer, prostate cancer, and/or leukemia.

[0217] Cells prepared as described above can be utilized in methods and compositions for adoptive immunotherapy in accordance with known techniques, or

variations thereof that will be apparent to those skilled in the art based on the instant disclosure.

[0218] In some alternatives, the cells are formulated by first harvesting them from their culture medium, and then washing and concentrating the cells in a medium and container system suitable for administration (a "pharmaceutically acceptable" carrier) in a treatment-effective amount. Suitable infusion medium can be any isotonic medium formulation, typically normal saline, Normosol R (Abbott) or Plasma-Lyte A (Baxter), but also 5% dextrose in water or Ringer's lactate can be utilized. The infusion medium can be supplemented with human serum albumin, fetal bovine serum or other human serum components.

[0219] In some alternatives, a treatment or inhibitory effective amount of cells in the composition is a transduced CD4 or CD8 cell or at least 2 cell subsets (for example, 1 CD8+ central memory T cell subset and 1 CD4+ helper T cell subset) or is more typically greater than 10^2 cells, and up to 10^6, up to and including 10^8 or 10^9 cells and can be more than 10^{10} cells. The number of cells will depend upon the ultimate use for which the composition is intended as will the type of cells included therein. For example, if cells that are specific for a particular antigen are desired, then the population will contain greater than 70%, generally greater than 80%, 85% and 90-95% of such cells. For uses provided herein, the cells are generally in a volume of a liter or less, can be 500 mls or less, even 250 mls or 100 mls or less or a volume in between any two listed volume values. Hence the density of the desired cells is typically greater than 10^4 cells/m1 and generally is greater than 10^7 cells/ml, generally 10^8 cells/ml or greater. The clinically relevant number of immune cells can be apportioned into multiple infusions that cumulatively equal or exceed 10^6, 10^7, 10^8, 10^8, 10^9, 10^{10} or 10^{11} cells or any amount of cells defined between any two endpoints of any of the listed values.

[0220] In some alternatives, the lymphocytes of the invention can be used to confer immunity to individuals. By "immunity" is meant a lessening of one or more physical symptoms associated with a response to infection by a pathogen, or to a tumor, to which the lymphocyte response is directed. The amount of cells administered is usually in the range present in normal individuals with immunity to the pathogen. Thus, the cells are usually administered by infusion, with each infusion in a range of from 2 cells, up to at least 10^6 to $3x10^{10}$ cells, preferably in the range of at least 10^7 to 10^9 cells. The T cells can be administered by a single infusion, or by multiple infusions over a range of time. However, since different individuals are expected to vary in responsiveness, the type and amount of

cells infused, as well as the number of infusions and the time range over which multiple infusions are given are determined by the attending physician, and can be determined by routine examination. The generation of sufficient levels of T lymphocytes (including cytotoxic T lymphocytes and/or helper T lymphocytes) is readily achievable using the rapid expansion method of the present invention, as exemplified herein.

[0221] In some alternatives, a composition as described herein is administered to an identified or selected subject, such as a subject identified or selected as having melanoma, breast cancer, brain cancer, squamous cell carcinoma, colon cancer, leukemia, myeloma, and/or prostate cancer, intravenously, intraperitoneally, intratumorly, into the bone marrow, into the lymph node, and /or into cerebrospinal fluid. In some alternatives, the chimeric receptor engineered compositions are delivered to the site of the tumor. Alternatively, the compositions as described herein can be combined with a compound that targets the cells to the tumor or the immune system compartments and avoid sites such as the lung.

[0222] In some alternatives, the compositions as described herein are administered with chemotherapeutic agents and/or immunosuppressants. In an alternative, a patient is first administered a chemotherapeutic agent that inhibits or destroys other immune cells followed by the compositions described herein. In some cases, chemotherapy can be avoided entirely.

[0223] In some alternatives, a method comprising administering the modified T cells as described herein in combination with the inducer (e.g. inducible drug) until the tumor burden is diminished. Once the tumor burden is diminished, the inducer drug can be withdrawn in order to switch the expression of the chimeric antigen receptor off and decrease the number of T cell expressing the receptor. In other alternatives, the inducer drug can be administered at different time in order to switch the expression of the chimeric antigen receptor on in the event of a relapse or increase in tumor growth.

[0224] In other alternatives, the inducer drug can be given for a period of time of days, weeks, or months, and then withdrawn for days, weeks or months, followed by re-administration of the inducer drug for days, weeks or months to allow for cycling of the expression of the chimeric antigen receptor to avoid anergy or nonresponsiveness due to chronic stimulation of the cells.

Vector construction and preparation of dual packaged lentivirus.

[0225] An inducible lentiviral vector encoding 7xHBD/mE1b-CD19t-her2t-T2A-epHIV7) was constructed. CD19t specific chimeric receptors were constructed using: (1) the VL and VH chain segments of the CD19-specific mAb FMC63 (SEQ ID NO: 3), linked by a $(G_4S)_3$ linker (SEQ ID NO: 12) peptide (VL-linker-VH); (2) a spacer domain derived from IgG4-Fc Hinge only (12 AA encoded by (SEQ ID NO: 4)). Spacers contained a S → P substitution within the Hinge domain located at position 108 of the native IgG4-Fc protein; the 27 AA transmembrane domain of human CD28 (Uniprot Database: P10747, (SEQ ID NO: 14)); (4) a signaling module comprising either (i) the 41 AA cytoplasmic domain of human CD28 with an LL → GG substitution located at position 186-187 of the native CD28 protein (SEQ ID NO: 14) ; and/or (ii) the 42 AA cytoplasmic domain of human 4-1BB (Uniprot Database: Q07011, (SEQ ID NO: 15)); linked to (iii) the 112 AA cytoplasmic domain of isoform 3 of human CD3ζ (Uniprot Database: P20963, (SEQ ID NO: 16)).

[0226] The nucleic acid sequences coding for the CD19t were linked with sequences coding for Her2t (SEQ Id NO: 44); and the self-cleaving T2A sequence (SEQ ID NO: 8).

[0227] A conditional lentiviral vector encoding 7xHBD/mEF1ap-ZsGreen-epHIV7 was constructed. The synthetic promoter 7xHBD/mEF1ap was constructed by combining seven minimal hepatocyte nuclear family-1 (HNF-1) binding sites cloned from the human albumin promoter and the huEF1α promoter TATA box and has a sequence of (SEQ ID NO: 41). In this way, only in the presence of tamoxifen does binding of HEA-3 to 7xHBD/EF1mp promoter induce the "ON" state of transgene expression.

[0228] A conditional lentiviral vector encoding 7xHBD/mEF1ap-CD19t--T2A-DHFRdm_epHIV7 was constructed. CD19t specific chimeric receptors were constructed using: (1) the VL and VH chain segments of the CD19-specific mAb FMC63 (SEQ ID NO: 3), linked by a $(G_4S)_3$ linker (SEQ ID NO: 12) peptide (VL-linker-VH); (2) a spacer domain derived from IgG4-Fc Hinge only (12 AA encoded by (SEQ ID NO: 4)). Spacers contained a S → P substitution within the Hinge domain located at position 108 of the native IgG4-Fc protein; the 27 AA transmembrane domain of human CD28 (Uniprot Database: P10747, (SEQ ID NO: 14)); (4) a signaling module comprising either (i) the 41 AA cytoplasmic domain of human CD28 with an LL → GG substitution located at position 186-

187 of the native CD28 protein (SEQ ID NO: 14) ; and/or (ii) the 42 AA cytoplasmic domain of human 4-1BB (Uniprot Database: Q07011, (SEQ ID NO: 15)); linked to (iii) the 112 AA cytoplasmic domain of isoform 3 of human CD3ζ (Uniprot Database: P20963, (SEQ ID NO: 16)).

[0229] The nucleic acid sequences coding for the CD19t were linked with sequences coding for the self-cleaving T2A sequence (SEQ ID NO: 8); and DHFRdm (SEQ ID NO: 46)

[0230] The transcriptional regulator, HEA-3, is a chimeric transcription factor composed of human subunits including the N-terminal DNA binding domain of Hepatocyte Nuclear Factor 1-alpha (HNF-1α) fused in frame to the mutant tamoxifen-specific ligand binding domain of the estrogen receptor ligand binding domain (ER-LBD), that is in turn fused to the p65 activation domain of NF-κβ (p65). In the absence of tamoxifen, HEA-3 is excluded from the nucleus by binding of cytosolic heat-shock protein 90 (HSP90) to the tamoxifen binding active site and transgene expression is in the "OFF" state. Nanomolar concentrations of cytosolic tamoxifen actively outcompete HSP90 for ER-LBD binding, resulting in HEA-3 translocation to the nucleus. Upon nuclear translocation, HEA-3 is readily available to bind its restricted synthetic promoter. Transcriptional responsiveness to HEA-3 in the presence of tamoxifen is achieved when transgenes are placed behind an HEA-3 responsive synthetic promoter (7xHBD/EF1mp).

[0231] A constitutive construct was constructed with a constitutive promoter EF-1 α linked to a polynucleotide coding for a transcriptional activator HEA3 (SEQ ID NO: 39) and a marker sequence EGFRt (SEQ ID NO: 9).

[0232] Human codon-optimized nucleotide sequences encoding each transgene were synthesized (LifeTechnologies, Carlsbad, CA) and cloned into the epHIV7 lentiviral vector using NheI and Not1 restriction sites. The epHIV7 lentiviral vector had been derived from the pHIV7 vector by replacing the cytomegalovirus promoter of pHIV7 with an EF-1 promoter.

[0233] The inducible CD19 chimeric receptor-encoding and the constitutive lentivirus was produced in 293T cells co-transfected with the lentiviral vector and the packaging vectors pCHGP-2, pCMV-Rev2 and pCMV-G using Calphos transfection reagent (Clontech). Medium was changed 16 hours after transfection, and lentivirus collected after 24, 48 and 72 hours.

Generation of Jurkat T-cell lines expressing the CD19 chimeric receptors and ZsGreen when induced with tamoxifen.

[0234] Jurkat cells were transduced with lentiviral supernatant (MOI = 3) supplemented with 1 μg/mL polybrene (Millipore) on day 3 after activation by centrifugation at 2,100 rpm for 45 minutes at 32°C. T cells were expanded in RPMI, 10% human serum, 2 mM L-glutamine and 1 % penicillin-streptomycin (CTL medium), supplemented with recombinant human (rh) IL-2 to a final concentration of 50 U/mL every 48 hours.

Additional Alternatives.

[0235] In some alternatives, system for inducible expression of a chimeric antigen receptor is provided, wherein the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. . In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the first promoter comprises a nucleic acid sequence of SEQ ID NO: 41. In some alternatives, the second promoter is a constitutive promoter. In some alternatives, the second promoter is the EF1αp. In some alternatives, the transcriptional activator comprises a sequence of SEQ ID NO: 40. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives, both vectors are packaged in a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the first and second nucleic acid comprise a vector. In some alternatives, the first nucleic acid further comprises a nucleic acid sequence coding for a

selectable marker. In some alternatives, the second nucleic acid further comprises a nucleic acid coding for a selectable marker.

[0236] In some alternatives, a system for inducible expression of chimeric antigen receptor comprises: a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine, a polypeptide that regulates apoptosis and/or a polypeptide that modulates checkpoint signaling; and a second nucleic acid comprising a second promoter operably linked to a nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the second promoter is constitutive or inducible. In an exemplary alternative, the second nucleic acid further comprises a polynucleotide coding for a chimeric antigen receptor, under the control of a constitutive promoter.

[0237] In another aspect, the present disclosure provides compositions to confer and/or augment immune responses mediated by cellular immunotherapy, such as by adoptively transferring tumor-specific, subset specific genetically modified CD4+ T cells, wherein the CD4+ T cells confer and/or augment the ability of CD8+ T cells to sustain anti-tumor reactivity and increase and/or maximize tumor-specific proliferation. In some alternatives, the CD4+ cells are genetically modified to express a chimeric receptor nucleic acid and/or chimeric receptor polypeptide under the control of a regulated promoter as described herein.

[0238] In another aspect, the present disclosure provides compositions to confer and/or augment immune responses mediated by cellular immunotherapy, such as by adoptively transferring tumor-specific, subset specific genetically modified CD8+ T cells. In some alternatives, the CD8+ T cells express a chimeric receptor nucleic acid and/or chimeric receptor polypeptide under the control of a regulated promoter, as described herein.

[0239] In one alternative, the present invention provides a method of performing cellular immunotherapy in a subject having a disease or disorder by administering to the subject a genetically modified T lymphocyte cell preparation that provides a cellular immune response and administering a drug that induces a transgene in the genetically modified T lymphocyte cells.

[0240] In some alternatives, the genetically modified CD8+ and genetically modified CD4+ cell population are co-administered. In some alternatives, the T cells are autologous or allogeneic T cells. Various modifications of the above method are possible. For

example, the chimeric receptor that is expressed by the CD4+ T cell and the CD8+ T cell can be the same or different.

[0241] In some alternatives, a system for inducible expression of a chimeric antigen receptor is provided, wherein the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the first promoter comprises a nucleic acid sequence of SEQ ID NO: 41. In some alternatives, the second promoter is a constitutive promoter. In some alternatives, the second promoter is the EF1αp. In some alternatives, the transcriptional activator comprises a sequence of SEQ ID NO: 40. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives, both vectors are packaged in a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the first and second nucleic acid comprise a vector. In some alternatives, the first nucleic acid further comprises a nucleic acid sequence coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a nucleic acid coding for a selectable marker. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor.

[0242] In some alternatives, a system for inducible expression of chimeric antigen receptor is provided, wherein the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a polypeptide that modulates checkpoint signaling; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional

activator for the inducible promoter. In some alternatives, the polypeptide that modulates checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises VISTA, LAG-3 and/or TIM3. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for chimeric antigen receptor comprising a ligand binding domain, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor; a polynucleotide coding for a transmembrane domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment. In some alternatives, the ligand binding domain is single chain variable fragment. In some alternatives, the tumor specific molecule is CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, or MAGE A3 TCR or combinations thereof. In some alternatives, the second promoter is an inducible promoter or a constitutive promoter.

[0243] In some alternatives, a chimeric receptor polypeptide is provided, wherein the chimeric receptor polypetide is coded for by a system. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte, a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain, and d) a polynucleotide coding for an intracellular signaling domain, and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter.. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the first promoter comprises a nucleic acid sequence of SEQ ID NO: 41. In some alternatives, the second promoter is a constitutive promoter. In some alternatives, the second promoter is the EF1αp. In some alternatives, the

transcriptional activator comprises a sequence of SEQ ID NO: 40. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives, both vectors are packaged in a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the first and second nucleic acid comprise a vector. In some alternatives, the first nucleic acid further comprises a nucleic acid sequence coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a nucleic acid coding for a selectable marker. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a polypeptide that modulates checkpoint signaling and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the polypeptide that modulates checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises VISTA, LAG-3 and/or TIM3. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for chimeric antigen receptor comprising a ligand binding domain, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment. In some alternatives, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR or combinations thereof. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a polypeptide that modulates checkpoint signaling; and b) a second nucleic acid comprising a second constitutive or inducible promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In

some alternatives, the polypeptide that modulates checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises VISTA, LAG-3 and/or TIM3. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for chimeric antigen receptor comprising a ligand binding domain, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor; a polynucleotide coding for a transmembrane domain In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment. In some alternatives, the ligand binding domain is single chain variable fragment. In some alternatives, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR or combinations thereof. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the second promoter is an inducible promoter. In some alternatives, the second promoter is constitutive promoter.

[0244] In some alternatives, a host cell is provided, wherein the host cell comprises a system. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte, a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain, and d) a polynucleotide coding for an intracellular signaling domain, and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter.. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the first promoter comprises a nucleic acid sequence of

SEQ ID NO: 41. In some alternatives, the second promoter is a constitutive promoter. In some alternatives, the second promoter is the EF1αp. In some alternatives, the transcriptional activator comprises a sequence of SEQ ID NO: 40. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives, both vectors are packaged in a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the first and second nucleic acid comprise a vector. In some alternatives, the first nucleic acid further comprises a nucleic acid sequence coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a nucleic acid coding for a selectable marker. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a polypeptide that modulates checkpoint signaling and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the polypeptide that modulates checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises VISTA, LAG-3 and/or TIM3. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for chimeric antigen receptor comprising a ligand binding domain, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment. In some alternatives, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR or combinations thereof. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a polypeptide that modulates checkpoint signaling; and b) a second nucleic acid comprising a

second constitutive or inducible promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the polypeptide that modulates checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises VISTA, LAG-3 and/or TIM3. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for chimeric antigen receptor comprising a ligand binding domain, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor; a polynucleotide coding for a transmembrane domain In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment. In some alternatives, the ligand binding domain is single chain variable fragment. In some alternatives, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR or combinations thereof. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the second promoter is an inducible promoter. In some alternatives, the second promoter is constitutive promoter. In some alternatives, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR or combinations thereof. In some alternatives, the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells. In some alternatives, the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the host cell is a precursor T cell. In some alternatives, the precursor T cell is a hematopoietic stem cell.

[0245] In some alternatives, a composition is provided, wherein the composition comprises a host cell in a pharmaceutically acceptable excipient. In some alternatives, the host cell comprises a system. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte, a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain, and d) a polynucleotide coding for an intracellular signaling domain, and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter.. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the first promoter comprises a nucleic acid sequence of SEQ ID NO: 41. In some alternatives, the second promoter is a constitutive promoter. In some alternatives, the second promoter is the EF1αp. In some alternatives, the transcriptional activator comprises a sequence of SEQ ID NO: 40. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives, both vectors are packaged in a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the first and second nucleic acid comprise a vector. In some alternatives, the first nucleic acid further comprises a nucleic acid sequence coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a nucleic acid coding for a selectable marker. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a polypeptide that modulates checkpoint signaling and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the polypeptide that modulates checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises VISTA, LAG-3 and/or TIM3. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for chimeric antigen receptor comprising a ligand binding domain, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population,

wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment. In some alternatives, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR or combinations thereof. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a polypeptide that modulates checkpoint signaling; and b) a second nucleic acid comprising a second constitutive or inducible promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the polypeptide that modulates checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises VISTA, LAG-3 and/or TIM3. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for chimeric antigen receptor comprising a ligand binding domain, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor; a polynucleotide coding for a transmembrane domain In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment. In some alternatives, the ligand binding domain is single chain variable fragment. In some alternatives, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR or combinations thereof. In some alternatives, the spacer is optimized for increased T cell proliferation and/or

cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the second promoter is an inducible promoter. In some alternatives, the second promoter is constitutive promoter. In some alternatives, the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells. In some alternatives, the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells. In some alternatives, the composition comprises a host cell wherein the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells and further comprises another host cell wherein the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells. In some alternatives, the composition comprises a host cell wherein the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells or a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells and a second host cell, wherein the second host cell is a precursor T cell. In some alternatives, the precursor T cell is a hematopoietic stem cell.

[0246] In some alternatives, an in vitro method for preparing a host cell is provided wherein the method comprises a) providing a system and b) introducing the system into a separate isolated T lymphocyte population and expanding each T lymphocyte population in vitro. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte, a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain, and d) a polynucleotide coding for an intracellular signaling domain, and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional

activator for the inducible promoter.. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the first promoter comprises a nucleic acid sequence of SEQ ID NO: 41. In some alternatives, the second promoter is a constitutive promoter. In some alternatives, the second promoter is the EF1αp. In some alternatives, the transcriptional activator comprises a sequence of SEQ ID NO: 40. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives, both vectors are packaged in a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the first and second nucleic acid comprise a vector. In some alternatives, the first nucleic acid further comprises a nucleic acid sequence coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a nucleic acid coding for a selectable marker. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a polypeptide that modulates checkpoint signaling and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the polypeptide that modulates checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises VISTA, LAG-3 and/or TIM3. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for chimeric antigen receptor comprising a ligand binding domain, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment. In some alternatives, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR or combinations thereof. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide

coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a polypeptide that modulates checkpoint signaling; and b) a second nucleic acid comprising a second constitutive or inducible promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the polypeptide that modulates checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises VISTA, LAG-3 and/or TIM3. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for chimeric antigen receptor comprising a ligand binding domain, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor; a polynucleotide coding for a transmembrane domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment. In some alternatives, the ligand binding domain is single chain variable fragment. In some alternatives, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR or combinations thereof. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the second promoter is an inducible promoter. In some alternatives, the second promoter is constitutive promoter. In some alternatives, wherein the T lymphocytes are expanded, the method further comprises culturing the cells in the presence of anti-CD3 and/or anti CD28, and at least one homeostatic cytokine until the cells expand sufficiently for use as a cell infusion. In some alternatives, the lymphocyte is CD8+ or CD4+. In some alternatives, the cells are precursor T cells. In some alternatives, the cells are hematopoietic stem cells. In some alternatives, the composition comprises a host cell wherein the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells or a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells

and a second host cell, wherein the second host cell is a precursor T cell. In some alternatives, the precursor T cell is a hematopoietic stem cell.

[0247] In some alternatives, a use of a host cell or a composition in combination with a drug that induces expression of a transgene in the host cell or composition for the treatment of cancer or a viral infection is provided. In some alternatives, the host cell comprises a system. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte, a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain, and d) a polynucleotide coding for an intracellular signaling domain, and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter.. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the first promoter comprises a nucleic acid sequence of SEQ ID NO: 41. In some alternatives, the second promoter is a constitutive promoter. In some alternatives, the second promoter is the EF1αp. In some alternatives, the transcriptional activator comprises a sequence of SEQ ID NO: 40. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives, both vectors are packaged in a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the first and second nucleic acid comprise a vector. In some alternatives, the first nucleic acid further comprises a nucleic acid sequence coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a nucleic acid coding for a selectable marker. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a polypeptide that modulates checkpoint signaling and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the polypeptide that modulates checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises VISTA, LAG-3 and/or TIM3. In

some alternatives, the second nucleic acid further comprises a polynucleotide coding for chimeric antigen receptor comprising a ligand binding domain, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment. In some alternatives, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR or combinations thereof. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a polypeptide that modulates checkpoint signaling; and b) a second nucleic acid comprising a second constitutive or inducible promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the polypeptide that modulates checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises VISTA, LAG-3 and/or TIM3. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for chimeric antigen receptor comprising a ligand binding domain, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor; a polynucleotide coding for a transmembrane domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment. In some alternatives, the ligand binding domain is single chain variable fragment. In some alternatives, the tumor specific molecule is selected from

the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR or combinations thereof. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the second promoter is an inducible promoter. In some alternatives, the second promoter is constitutive promoter. In some alternatives, the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells. In some alternatives, the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells. In some alternatives, the composition comprises a host cell in a pharmaceutically acceptable excipient. In some alternatives, the host cell comprises a system. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the first promoter comprises a nucleic acid sequence of SEQ ID NO:41. In some alternatives, the second promoter is a constitutive promoter. In some alternatives, the second promoter is the EF1αp. In some alternatives, the transcriptional activator comprises a sequence of SEQ ID NO:40. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives, both vectors are packaged in a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the first and second nucleic acid comprise a vector. In some alternatives, the first nucleic acid further comprises a nucleic acid sequence coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a nucleic acid coding for a

selectable marker. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment. In some alternatives, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR or combinations thereof. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a polypeptide that modulates checkpoint signaling; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment. In some alternatives, the ligand binding domain is single chain variable fragment. In some alternatives, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR or combinations thereof. In some alternatives, the host cell is a precursor T cell. In some alternatives, the precursor T cell is a hematopoietic stem cell. In some alternatives, the isolated T lymphocyte population

comprises precursor T cells. In some alternatives, the precursor T cells are hematopoietic stem cells. In some alternatives, the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells. In some alternatives, the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells. In some alternatives, the composition comprises a host cell wherein the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells and another host cell wherein the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells. In some alternatives, the cancer is a solid tumor or hematologic malignancy. In some alternatives, the solid tumor is selected from the group consisting of a breast cancer, brain cancer, lung cancer, colon cancer, renal cancer, pancreatic cancer, prostate cancer, and ovarian cancer. In some alternatives, the composition comprises a host cell wherein the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells or a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells and a second host cell, wherein the second host cell is a precursor T cell. In some alternatives, the precursor T cell is a hematopoietic stem cell.

[0248] In some alternatives, a method of performing cellular immunotherapy in a subject having cancer or a viral infection is provided wherein the method comprises administering a composition or a host cell to the subject and administering a drug that induces expression of a transgene in the composition or the host cells. In some alternatives, the host cell comprises a system. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte, a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain, and d) a

polynucleotide coding for an intracellular signaling domain, and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter.. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the first promoter comprises a nucleic acid sequence of SEQ ID NO: 41. In some alternatives, the second promoter is a constitutive promoter. In some alternatives, the second promoter is the EF1αp. In some alternatives, the transcriptional activator comprises a sequence of SEQ ID NO: 40. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives, both vectors are packaged in a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the first and second nucleic acid comprise a vector. In some alternatives, the first nucleic acid further comprises a nucleic acid sequence coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a nucleic acid coding for a selectable marker. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a polypeptide that modulates checkpoint signaling and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the polypeptide that modulates checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises VISTA, LAG-3 and/or TIM3. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for chimeric antigen receptor comprising a ligand binding domain, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment. In some alternatives, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR or combinations thereof. In

some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a polypeptide that modulates checkpoint signaling; and b) a second nucleic acid comprising a second constitutive or inducible promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the polypeptide that modulates checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises VISTA, LAG-3 and/or TIM3. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for chimeric antigen receptor comprising a ligand binding domain, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor; a polynucleotide coding for a transmembrane domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment. In some alternatives, the ligand binding domain is single chain variable fragment. In some alternatives, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR or combinations thereof. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the second promoter is an inducible promoter. In some alternatives, the second promoter is constitutive promoter.. In some alternatives, the host cell is a precursor T cell. In some alternatives, the host cell is a hematopoietic stem cell. In some alternatives, the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells. In some alternatives, the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells. In some alternatives, the composition comprises a host cell in a pharmaceutically acceptable excipient. In some alternatives, the

host cell comprises a system. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the first promoter comprises a nucleic acid sequence of SEQ ID NO: 41. In some alternatives, the second promoter is a constitutive promoter. In some alternatives, the second promoter is the EF1αp. In some alternatives, the transcriptional activator comprises a sequence of SEQ ID NO: 40. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives, both vectors are packaged in a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the first and second nucleic acid comprise a vector. In some alternatives, the first nucleic acid further comprises a nucleic acid sequence coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a nucleic acid coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment. In some alternatives, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR or

combinations thereof. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide that regulates apoptosis, or a polypeptide that modulates checkpoint signaling; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the polypeptide that modulates checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises VISTA, LAG-3 and/or TIM3. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for chimeric antigen receptor comprising a ligand binding domain, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and d) a polynucleotide coding for an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment. In some alternatives, the ligand binding domain is single chain variable fragment. In some alternatives, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR or combinations thereof. In some alternatives, the host cell is a precursor T cell. In some alternatives, the host cell is a hematopoietic stem cell. In some alternatives, the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells. In some alternatives, the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells. In some alternatives, the composition comprises a host cell wherein the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells and another host cell wherein the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells. In some alternatives, the cancer is selected from a solid

tumor or hematologic malignancy. In some alternatives, the solid tumor is selected from the group consisting of a breast cancer, brain cancer, lung cancer, colon cancer, renal cancer, pancreatic cancer, prostate cancer, and ovarian cancer. In some alternatives, the host cell is a precursor T cell. In some alternatives, the precursor T cell is a hematopoietic stem cell. In some alternatives, the isolated T lymphocyte population comprises precursor T cells. In some alternatives, the precursor T cells are hematopoietic stem cells. In some alternatives, the composition comprises a host cell wherein the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells or a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells and a second host cell, wherein the second host cell is a precursor T cell. In some alternatives, the precursor T cell is a hematopoietic stem cell.

More Alternatives.

[0249] In some alternatives, a system for inducible expression of a chimeric antigen receptor, the system is provided, wherein the system comprises a) a first nucleic acid comprising a first promoter, which is an inducible promoter, operably linked to a polynucleotide coding for a chimeric antigen receptor and b) a second nucleic acid comprising a second promoter operably linked to a polynucleotide coding for a transcriptional activator, which is capable of activating transcription from the first promoter in the presence of a drug or metabolite thereof. In some alternatives, a system for inducible expression is provided, wherein the system comprises a) an inducible promoter, b) a polynucleotide coding for a chimeric antigen receptor and c) a polynucleotide coding for a transcriptional activator, which transcriptional activator is capable of activating transcription from the inducible promoter in the presence of a drug or metabolite thereof. In some alternatives, the polynucleotide coding for the chimeric receptor is operably linked to the inducible promoter. In some alternatives, the system further comprises further comprising a polynucleotide encoding a recombinant protein, which polynucleotide is operably linked to the inducible promoter. In some alternatives, the drug or metabolite thereof comprises: (i) a drug tolerated when administered to a human subject daily or weekly, or a metabolite thereof; (ii) a molecule that specifically binds to a human receptor, optionally the estrogen receptor, or a metabolite thereof; and/or(iii) tamoxifen and/or a metabolite or analog of tamoxifen. In

some alternatives, the transcriptional activator comprises: (a) a DNA-binding domain; (b) a ligand-binding domain that specifically binds to the drug or metabolite thereof; and (c) a transactivation domain, optionally linked and/or fused in that order. In some alternatives of the system, (a) the DNA-binding domain comprises DNA binding sites not present in a protein naturally expressed in a lymphocyte or not present in a protein naturally expressed in a T cell; and/or (b) the drug or metabolite is the molecule that specifically binds to the human receptor, optionally estrogen receptor, or metabolite thereof, and the binding between the drug or metabolite and the ligand-binding domain is selective for the ligand-binding domain over the human receptor, whereby binding by the ligand-binding domain to the drug or metabolite is greater, optionally at least 1.5, 2, 3, or 4 times as strong, as the binding by the human receptor; and/or (c) the transactivation domain comprises a p65 transactivation domain or functional variant thereof; and/or (d) the first promoter comprises one or more binding sites for the DNA binding domain. In some alternatives, the first promoter does not comprise another binding site for any human DNA binding domain other than a DNA-binding domain or domains present in the transcriptional activator; and/or wherein the first promoter is a synthetic chimeric promoter and/or the transcriptional activator is a synthetic chimeric transcriptional activator; and/or wherein the DNA binding domain comprises a DNA binding domain present in a hepatocyte nuclear factor, which is optionally HNF1-alpha or HNF1-beta. In some alternatives of the system, the first promoter comprises the nucleic acid sequence of SEQ ID NO: 41. In some alternatives, the second promoter is a constitutive promoter. In some alternatives, the second promoter is or comprises an EF1α promoter or functional portion thereof. In some alternatives, the transcriptional activator comprises a polypeptide having the sequence of SEQ ID NO: 40. In some alternatives, the first nucleic acid is comprised within a first vector, which is further comprised by the system and the second nucleic acid is comprised within a second vector, which is further comprised by the system. In some alternatives, the first nucleic acid and the second nucleic acid or the first promoter, polynucleotide encoding the chimeric antigen receptor, second promoter, and polynucleotide encoding the transactivator, are comprised within a vector, which is further comprised by the system. In some alternatives of the system, the system is comprised in a single viral packaging vector. In some alternatives, the viral vector is a lentiviral vector. In some alternatives, the first nucleic acid further comprises a nucleic acid sequence coding for a selectable marker or wherein the system further comprises a selectable marker operably linked to the first promoter. In some alternatives, the second nucleic acid further comprises a

nucleic acid coding for a selectable marker or wherein the system further comprises a selectable marker operably linked to the second promoter.

[0250] In some alternatives, a system for inducible expression is provided, wherein the system comprises a) a first nucleic acid comprising a first promoter, which is an inducible promoter and is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide than inhibits apoptosis, or a polypeptide that inhibits negative checkpoint signaling and b) a second nucleic acid comprising a second promoter, which is a constitutive or inducible promoter, operably linked to a polynucleotide coding for a transcriptional activator capable of inducing transcription from the first promoter in the presence of a drug or metabolite or analog thereof. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for a recombinant antigen receptor, which optionally is a chimeric antigen receptor. In some alternatives, the chimeric antigen receptor comprises a a) ligand binding domain, which binds to a ligand that is optionally a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population that is suitable to mediate recognition and elimination by a lymphocyte b) a polypeptide spacer, wherein the spacer optionally provides for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor, c) a transmembrane domain and d) an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment. In some alternatives, the ligand binding domain is single chain variable fragment. In some alternatives, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, MAGE A3 TCR and combinations thereof.

[0251] In some alternatives, a chimeric receptor polypeptide encoded by the system is provided. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter, which is an inducible promoter, operably linked to a polynucleotide coding for a chimeric antigen receptor and b) a second nucleic acid comprising a second promoter operably linked to a polynucleotide coding for a transcriptional activator, which is capable of activating transcription from the first promoter in the presence of a drug or metabolite thereof. In some alternatives, a system for inducible expression is provided, wherein the system comprises a) an inducible promoter, b) a polynucleotide coding for a chimeric antigen receptor and c) a polynucleotide coding for a transcriptional activator,

which transcriptional activator is capable of activating transcription from the inducible promoter in the presence of a drug or metabolite thereof. In some alternatives, the polynucleotide coding for the chimeric receptor is operably linked to the inducible promoter. In some alternatives, the system further comprises further comprising a polynucleotide encoding a recombinant protein, which polynucleotide is operably linked to the inducible promoter. In some alternatives, the drug or metabolite thereof comprises: (i) a drug tolerated when administered to a human subject daily or weekly, or a metabolite thereof; (ii) a molecule that specifically binds to a human receptor, optionally the estrogen receptor, or a metabolite thereof; and/or(iii) tamoxifen and/or a metabolite or analog of tamoxifen. In some alternatives, the transcriptional activator comprises: (a) a DNA-binding domain; (b) a ligand-binding domain that specifically binds to the drug or metabolite thereof; and (c) a transactivation domain, optionally linked and/or fused in that order. In some alternatives of the system, (a) the DNA-binding domain comprises DNA binding sites not present in a protein naturally expressed in a lymphocyte or not present in a protein naturally expressed in a T cell; and/or (b) the drug or metabolite is the molecule that specifically binds to the human receptor, optionally estrogen receptor, or metabolite thereof, and the binding between the drug or metabolite and the ligand-binding domain is selective for the ligand-binding domain over the human receptor, whereby binding by the ligand-binding domain to the drug or metabolite is greater, optionally at least 1.5, 2, 3, or 4 times as strong, as the binding by the human receptor; and/or (c) the transactivation domain comprises a p65 transactivation domain or functional variant thereof; and/or (d) the first promoter comprises one or more binding sites for the DNA binding domain. In some alternatives, the first promoter does not comprise another binding site for any human DNA binding domain other than a DNA-binding domain or domains present in the transcriptional activator; and/or wherein the first promoter is a synthetic chimeric promoter and/or the transcriptional activator is a synthetic chimeric transcriptional activator; and/or wherein the DNA binding domain comprises a DNA binding domain present in a hepatocyte nuclear factor, which is optionally HNF1-alpha or HNF1-beta.The system of any of claims 1-8, wherein the first promoter comprises the nucleic acid sequence of SEQ ID NO: 41. In some alternatives, the second promoter is a constitutive promoter. In some alternatives, the second promoter is or comprises an EF1α promoter or functional portion thereof. In some alternatives, the transcriptional activator comprises a polypeptide having the sequence of SEQ ID NO: 40. In some alternatives, the first nucleic acid is comprised within a first vector, which is further comprised by the system and the

second nucleic acid is comprised within a second vector, which is further comprised by the system. In some alternatives, the first nucleic acid and the second nucleic acid or the first promoter, polynucleotide encoding the chimeric antigen receptor, second promoter, and polynucleotide encoding the transactivator, are comprised within a vector, which is further comprised by the system. In some alternatives of the system, the system is comprised in a single viral packaging vector. In some alternatives, the viral vector is a lentiviral vector. In some alternatives, the first nucleic acid further comprises a nucleic acid sequence coding for a selectable marker or wherein the system further comprises a selectable marker operably linked to the first promoter. In some alternatives, the second nucleic acid further comprises a nucleic acid coding for a selectable marker or wherein the system further comprises a selectable marker operably linked to the second promoter. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter, which is an inducible promoter and is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide than inhibits apoptosis, or a polypeptide that inhibits negative checkpoint signaling and b) a second nucleic acid comprising a second promoter, which is a constitutive or inducible promoter, operably linked to a polynucleotide coding for a transcriptional activator capable of inducing transcription from the first promoter in the presence of a drug or metabolite or analog thereof. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for a recombinant antigen receptor, which optionally is a chimeric antigen receptor. In some alternatives, the chimeric antigen receptor comprises a a) ligand binding domain, which binds to a ligand that is optionally a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population that is suitable to mediate recognition and elimination by a lymphocyte b) a polypeptide spacer, wherein the spacer optionally provides for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor, c) a transmembrane domain and d) an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment. In some alternatives, the ligand binding domain is single chain variable fragment. In some alternatives, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR and combinations thereof.

[0252] In some alternatives, a host cell comprising a system or viral packaging vector is provided. In some alternatives, the system comprises a) a first nucleic acid

comprising a first promoter, which is an inducible promoter, operably linked to a polynucleotide coding for a chimeric antigen receptor and b) a second nucleic acid comprising a second promoter operably linked to a polynucleotide coding for a transcriptional activator, which is capable of activating transcription from the first promoter in the presence of a drug or metabolite thereof. In some alternatives, a system for inducible expression is provided, wherein the system comprises a) an inducible promoter, b) a polynucleotide coding for a chimeric antigen receptor and c) a polynucleotide coding for a transcriptional activator, which transcriptional activator is capable of activating transcription from the inducible promoter in the presence of a drug or metabolite thereof. In some alternatives, the polynucleotide coding for the chimeric receptor is operably linked to the inducible promoter. In some alternatives, the system further comprises further comprising a polynucleotide encoding a recombinant protein, which polynucleotide is operably linked to the inducible promoter. In some alternatives, the drug or metabolite thereof comprises: (i) a drug tolerated when administered to a human subject daily or weekly, or a metabolite thereof; (ii) a molecule that specifically binds to a human receptor, optionally the estrogen receptor, or a metabolite thereof; and/or (iii) tamoxifen and/or a metabolite or analog of tamoxifen. In some alternatives, the transcriptional activator comprises: (a) a DNA-binding domain; (b) a ligand-binding domain that specifically binds to the drug or metabolite thereof; and (c) a transactivation domain, optionally linked and/or fused in that order. In some alternatives of the system, (a) the DNA-binding domain comprises DNA binding sites not present in a protein naturally expressed in a lymphocyte or not present in a protein naturally expressed in a T cell; and/or (b) the drug or metabolite is the molecule that specifically binds to the human receptor, optionally estrogen receptor, or metabolite thereof, and the binding between the drug or metabolite and the ligand-binding domain is selective for the ligand-binding domain over the human receptor, whereby binding by the ligand-binding domain to the drug or metabolite is greater, optionally at least 1.5, 2, 3, or 4 times as strong, as the binding by the human receptor; and/or (c) the transactivation domain comprises a p65 transactivation domain or functional variant thereof; and/or (d) the first promoter comprises one or more binding sites for the DNA binding domain. In some alternatives, the first promoter does not comprise another binding site for any human DNA binding domain other than a DNA-binding domain or domains present in the transcriptional activator; and/or wherein the first promoter is a synthetic chimeric promoter and/or the transcriptional activator is a synthetic chimeric transcriptional activator; and/or wherein the DNA binding domain comprises a DNA binding

domain present in a hepatocyte nuclear factor, which is optionally HNF1-alpha or HNF1-beta. In some alternatives, the first promoter comprises the nucleic acid sequence of SEQ ID NO: 41. In some alternatives, the second promoter is a constitutive promoter. In some alternatives, the second promoter is or comprises an EF1α promoter or functional portion thereof. In some alternatives, the transcriptional activator comprises a polypeptide having the sequence of SEQ ID NO: 40. In some alternatives, the first nucleic acid is comprised within a first vector, which is further comprised by the system and the second nucleic acid is comprised within a second vector, which is further comprised by the system. In some alternatives, the first nucleic acid and the second nucleic acid or the first promoter, polynucleotide encoding the chimeric antigen receptor, second promoter, and polynucleotide encoding the transactivator, are comprised within a vector, which is further comprised by the system. In some alternatives, the system described above is comprised in a single viral packaging vector. In some such alternatives, viral vector is a lentiviral vector. In some alternatives, the first nucleic acid further comprises a nucleic acid sequence coding for a selectable marker or wherein the system further comprises a selectable marker operably linked to the first promoter. In some alternatives, the second nucleic acid further comprises a nucleic acid coding for a selectable marker or wherein the system further comprises a selectable marker operably linked to the second promoter. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter, which is an inducible promoter and is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide than inhibits apoptosis, or a polypeptide that inhibits negative checkpoint signaling and b) a second nucleic acid comprising a second promoter, which is a constitutive or inducible promoter, operably linked to a polynucleotide coding for a transcriptional activator capable of inducing transcription from the first promoter in the presence of a drug or metabolite or analog thereof. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for a recombinant antigen receptor, which optionally is a chimeric antigen receptor. In some alternatives, the chimeric antigen receptor comprises a a) ligand binding domain, which binds to a ligand that is optionally a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population that is suitable to mediate recognition and elimination by a lymphocyte b) a polypeptide spacer, wherein the spacer optionally provides for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor, c) a transmembrane domain and d) an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the

second promoter. In some alternatives, the ligand binding domain is an antibody fragment. In some alternatives, the ligand binding domain is single chain variable fragment. In some alternatives, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR and combinations thereof.

[0253] In some alternatives, a host cell is provided, wherein the host cell is a primary human lymphocyte, wherein the host cell comprises a) a nucleic acid comprising a first promoter, which is an inducible synthetic promoter containing a binding site for a DNA binding domain not naturally present in the primary human lymphocyte and b) a polynucleotide coding for a transcriptional activator, the transcriptional activator comprising i) the DNA binding domain, wherein the DNA binding domain does not specifically bind to a DNA sequence naturally present in the primary human lymphocyte; ii) a domain that specifically binds to a drug or metabolite thereof and does not bind or does not bind with as great a degree of affinity to any molecule naturally present in the primary human lymphocyte and iii) a transactivation domain, wherein the transcriptional activator is capable of inducing transcription from the first promoter in the presence of the drug or metabolite thereof and/or upon binding of the drug or metabolite thereof to the domain in (ii). In some alternatives, the cell further comprises a polynucleotide encoding a chimeric antigen receptor, which optionally is operably linked to the first promoter. In some alternatives, In some alternatives, the cell further comprises a second promoter, which is operably linked to the polynucleotide encoding the transcriptional activator, wherein the second promoter is optionally constitutive. In some alternatives, the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cell. In some alternatives, the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells.

[0254] In some alternatives, a composition is provided, wherein the composition comprises a host cell in a pharmaceutically acceptable excipient. In some alternatives, the host cell the host cell is a primary human lymphocyte, wherein the host cell comprises a) a nucleic acid comprising a first promoter, which is an inducible synthetic promoter containing a binding site for a DNA binding domain not naturally present in the primary human lymphocyte and b) a polynucleotide coding for a transcriptional activator, the transcriptional activator comprising i) the DNA binding domain, wherein the DNA binding domain does not

specifically bind to a DNA sequence naturally present in the primary human lymphocyte; ii) a domain that specifically binds to a drug or metabolite thereof and does not bind or does not bind with as great a degree of affinity to any molecule naturally present in the primary human lymphocyte and iii) a transactivation domain, wherein the transcriptional activator is capable of inducing transcription from the first promoter in the presence of the drug or metabolite thereof and/or upon binding of the drug or metabolite thereof to the domain in (ii). In some alternatives, the cell further comprises a polynucleotide encoding a chimeric antigen receptor, which optionally is operably linked to the first promoter. In some alternatives, In some alternatives, the cell further comprises a second promoter, which is operably linked to the polynucleotide encoding the transcriptional activator, wherein the second promoter is optionally constitutive. In some alternatives, the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cell. In some alternatives, the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells. In some alternatives, the composition comprises a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cell and a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells.

[0255] In some alternatives, an *in vitro* method for preparing a host cell is provided and comprises introducing a system into a separate isolated T lymphocyte population and expanding each T lymphocyte population in vitro. In some alternatives, the host cell the host cell is a primary human lymphocyte, wherein the host cell comprises a) a nucleic acid comprising a first promoter, which is an inducible synthetic promoter containing a binding site for a DNA binding domain not naturally present in the primary human lymphocyte and b) a polynucleotide coding for a transcriptional activator, the transcriptional activator comprising i) the DNA binding domain, wherein the DNA binding domain does not specifically bind to a DNA sequence naturally present in the primary human lymphocyte; ii) a domain that specifically binds to a drug or metabolite thereof and does not bind or does not bind with as great a degree of affinity to any molecule naturally present in the primary human lymphocyte and iii) a transactivation domain, wherein the transcriptional activator is capable of inducing transcription from the first promoter in the presence of the drug or metabolite

thereof and/or upon binding of the drug or metabolite thereof to the domain in (ii). In some alternatives, the cell further comprises a polynucleotide encoding a chimeric antigen receptor, which optionally is operably linked to the first promoter. In some alternatives, In some alternatives, the cell further comprises a second promoter, which is operably linked to the polynucleotide encoding the transcriptional activator, wherein the second promoter is optionally constitutive. In some alternatives, the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cell. In some alternatives, the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter, which is an inducible promoter, operably linked to a polynucleotide coding for a chimeric antigen receptor and b) a second nucleic acid comprising a second promoter operably linked to a polynucleotide coding for a transcriptional activator, which is capable of activating transcription from the first promoter in the presence of a drug or metabolite thereof. In some alternatives, a system for inducible expression is provided, wherein the system comprises a) an inducible promoter, b) a polynucleotide coding for a chimeric antigen receptor and c) a polynucleotide coding for a transcriptional activator, which transcriptional activator is capable of activating transcription from the inducible promoter in the presence of a drug or metabolite thereof. In some alternatives, the polynucleotide coding for the chimeric receptor is operably linked to the inducible promoter. In some alternatives, the system further comprises further comprising a polynucleotide encoding a recombinant protein, which polynucleotide is operably linked to the inducible promoter. In some alternatives, the drug or metabolite thereof comprises: (i) a drug tolerated when administered to a human subject daily or weekly, or a metabolite thereof; (ii) a molecule that specifically binds to a human receptor, optionally the estrogen receptor, or a metabolite thereof; and/or (iii) tamoxifen and/or a metabolite or analog of tamoxifen. In some alternatives, the transcriptional activator comprises: (a) a DNA-binding domain; (b) a ligand-binding domain that specifically binds to the drug or metabolite thereof; and (c) a transactivation domain, optionally linked and/or fused in that order. In some alternatives of the system, (a) the DNA-binding domain comprises DNA binding sites not present in a protein naturally expressed in a lymphocyte or not present in a protein naturally expressed in a T cell; and/or (b) the drug or metabolite is the molecule that specifically binds to the human receptor, optionally estrogen receptor, or

metabolite thereof, and the binding between the drug or metabolite and the ligand-binding domain is selective for the ligand-binding domain over the human receptor, whereby binding by the ligand-binding domain to the drug or metabolite is greater, optionally at least 1.5, 2, 3, or 4 times as strong, as the binding by the human receptor; and/or (c) the transactivation domain comprises a p65 transactivation domain or functional variant thereof; and/or (d) the first promoter comprises one or more binding sites for the DNA binding domain. In some alternatives, the first promoter does not comprise another binding site for any human DNA binding domain other than a DNA-binding domain or domains present in the transcriptional activator; and/or wherein the first promoter is a synthetic chimeric promoter and/or the transcriptional activator is a synthetic chimeric transcriptional activator; and/or wherein the DNA binding domain comprises a DNA binding domain present in a hepatocyte nuclear factor, which is optionally HNF1-alpha or HNF1-beta. In some alternatives, the first promoter comprises the nucleic acid sequence of SEQ ID NO: 41. In some alternatives, the second promoter is a constitutive promoter. In some alternatives, the second promoter is or comprises an EF1α promoter or functional portion thereof. In some alternatives, the transcriptional activator comprises a polypeptide having the sequence of SEQ ID NO: 40. In some alternatives, the first nucleic acid is comprised within a first vector, which is further comprised by the system and the second nucleic acid is comprised within a second vector, which is further comprised by the system. In some alternatives, the first nucleic acid and the second nucleic acid or the first promoter, polynucleotide encoding the chimeric antigen receptor, second promoter, and polynucleotide encoding the transactivator, are comprised within a vector, which is further comprised by the system. In some alternatives, the system is comprised in a single viral packaging vector. In some alternatives, the viral vector is a lentiviral vector. In some alternatives, the first nucleic acid further comprises a nucleic acid sequence coding for a selectable marker or wherein the system further comprises a selectable marker operably linked to the first promoter. In some alternatives, the second nucleic acid further comprises a nucleic acid coding for a selectable marker or wherein the system further comprises a selectable marker operably linked to the second promoter. In some alternatives, the system comprises a) a first nucleic acid comprising a first promoter, which is an inducible promoter and is operably linked to a polynucleotide coding for a cytokine, a chemokine receptor, a polypeptide than inhibits apoptosis, or a polypeptide that inhibits negative checkpoint signaling and b) a second nucleic acid comprising a second promoter, which is a constitutive or inducible promoter, operably linked to a polynucleotide coding for a

transcriptional activator capable of inducing transcription from the first promoter in the presence of a drug or metabolite or analog thereof. In some alternatives, the second nucleic acid further comprises a polynucleotide coding for a recombinant antigen receptor, which optionally is a chimeric antigen receptor. In some alternatives, the chimeric antigen receptor comprises a a) ligand binding domain, which binds to a ligand that is optionally a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population that is suitable to mediate recognition and elimination by a lymphocyte b) a polypeptide spacer, wherein the spacer optionally provides for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor, c) a transmembrane domain and d) an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment. In some alternatives, the ligand binding domain is single chain variable fragment. In some alternatives, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, MAGE A3 TCR and combinations thereof. In some alternatives, the T lymphocytes in the population are expanded and wherein the method further comprises culturing the cells in the presence of anti-CD3 and/or anti CD28, and at least one homeostatic cytokine until the cells expand sufficiently for use as a cell infusion. In some alternatives, culturing in the presence of anti-CD3 and/or anti CD28, and at least one homeostatic cytokine can be performed before or after the introduction of the system.

[0256] In some alternatives, a use of the host cell or a composition in combination with the drug or a metabolite thereof for the treatment of cancer or a viral infection is provided. In some alternatives, the host cell is a primary human lymphocyte, wherein the host cell comprises a) a nucleic acid comprising a first promoter, which is an inducible synthetic promoter containing a binding site for a DNA binding domain not naturally present in the primary human lymphocyte and b) a polynucleotide coding for a transcriptional activator, the transcriptional activator comprising i) the DNA binding domain, wherein the DNA binding domain does not specifically bind to a DNA sequence naturally present in the primary human lymphocyte; ii) a domain that specifically binds to a drug or metabolite thereof and does not bind or does not bind with as great a degree of affinity to any molecule naturally present in the primary human lymphocyte and iii) a transactivation domain, wherein the transcriptional activator is capable of inducing transcription from the first promoter in the presence of the

drug or metabolite thereof and/or upon binding of the drug or metabolite thereof to the domain in (ii). In some alternatives, the cell further comprises a polynucleotide encoding a chimeric antigen receptor, which optionally is operably linked to the first promoter. In some alternatives, In some alternatives, the cell further comprises a second promoter, which is operably linked to the polynucleotide encoding the transcriptional activator, wherein the second promoter is optionally constitutive. In some alternatives, the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cell. In some alternatives, the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells. In some alternatives, the composition comprises a host cell in a pharmaceutically acceptable excipient. In some alternatives, the host cell the host cell is a primary human lymphocyte, wherein the host cell comprises a) a nucleic acid comprising a first promoter, which is an inducible synthetic promoter containing a binding site for a DNA binding domain not naturally present in the primary human lymphocyte and b) a polynucleotide coding for a transcriptional activator, the transcriptional activator comprising i) the DNA binding domain, wherein the DNA binding domain does not specifically bind to a DNA sequence naturally present in the primary human lymphocyte; ii) a domain that specifically binds to a drug or metabolite thereof and does not bind or does not bind with as great a degree of affinity to any molecule naturally present in the primary human lymphocyte and iii) a transactivation domain, wherein the transcriptional activator is capable of inducing transcription from the first promoter in the presence of the drug or metabolite thereof and/or upon binding of the drug or metabolite thereof to the domain in (ii). In some alternatives, the cell further comprises a polynucleotide encoding a chimeric antigen receptor, which optionally is operably linked to the first promoter. In some alternatives, the cell further comprises a second promoter, which is operably linked to the polynucleotide encoding the transcriptional activator, wherein the second promoter is optionally constitutive. In some alternatives, the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cell. In some alternatives, the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells. In some alternatives, the composition comprises a CD8+ T cytotoxic lymphocyte cell selected from the group

consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cell and a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells.

[0257] In some alternatives, a cell and a drug for use in treatment or inhibition of cancer or a viral infection is provided, wherein the cell comprises: (a) a polynucleotide encoding a chimeric antigen receptor that specifically binds to an antigen associated with the cancer or the viral infection, (b) an inducible synthetic promoter, and (c) a transcriptional activator containing a DNA binding domain that specifically binds to the synthetic promoter and a domain that specifically binds to the drug or a metabolite thereof and is capable of inducing transcription from the synthetic promoter in the presence of the drug or a metabolite thereof. In some alternatives, the cancer is a solid tumor or hematologic malignancy. In some alternatives, the solid tumor is selected from the group consisting of a breast cancer, lung cancer, colon cancer, renal cancer, pancreatic cancer, prostate cancer, and ovarian cancer.

[0258] In some alternatives, a method of performing cellular immunotherapy in a subject having cancer or a viral infection is provided wherein the method comprises administering a composition or a host cell to the subject and administering the drug or metabolite thereof, thereby inducing expression from the promoter. In some alternatives, the host cell is a primary human lymphocyte, wherein the host cell comprises a) a nucleic acid comprising a first promoter, which is an inducible synthetic promoter containing a binding site for a DNA binding domain not naturally present in the primary human lymphocyte and b) a polynucleotide coding for a transcriptional activator, the transcriptional activator comprising i) the DNA binding domain, wherein the DNA binding domain does not specifically bind to a DNA sequence naturally present in the primary human lymphocyte; ii) a domain that specifically binds to a drug or metabolite thereof and does not bind or does not bind with as great a degree of affinity to any molecule naturally present in the primary human lymphocyte and iii) a transactivation domain, wherein the transcriptional activator is capable of inducing transcription from the first promoter in the presence of the drug or metabolite thereof and/or upon binding of the drug or metabolite thereof to the domain in (ii). In some alternatives, the cell further comprises a polynucleotide encoding a chimeric antigen receptor, which optionally is operably linked to the first promoter. In some alternatives, In some alternatives, the cell further comprises a second promoter, which is operably linked to the polynucleotide encoding the transcriptional activator, wherein the second promoter is

optionally constitutive. In some alternatives, the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cell. In some alternatives, the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells. In some alternatives, the composition comprises a host cell in a pharmaceutically acceptable excipient. In some alternatives, the host cell the host cell is a primary human lymphocyte, wherein the host cell comprises a) a nucleic acid comprising a first promoter, which is an inducible synthetic promoter containing a binding site for a DNA binding domain not naturally present in the primary human lymphocyte and b) a polynucleotide coding for a transcriptional activator, the transcriptional activator comprising i) the DNA binding domain, wherein the DNA binding domain does not specifically bind to a DNA sequence naturally present in the primary human lymphocyte; ii) a domain that specifically binds to a drug or metabolite thereof and does not bind or does not bind with as great a degree of affinity to any molecule naturally present in the primary human lymphocyte and iii) a transactivation domain, wherein the transcriptional activator is capable of inducing transcription from the first promoter in the presence of the drug or metabolite thereof and/or upon binding of the drug or metabolite thereof to the domain in (ii). In some alternatives, the cell further comprises a polynucleotide encoding a chimeric antigen receptor, which optionally is operably linked to the first promoter. In some alternatives, the cell further comprises a second promoter, which is operably linked to the polynucleotide encoding the transcriptional activator, wherein the second promoter is optionally constitutive. In some alternatives, the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cell. In some alternatives, the host cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells. In some alternatives, the composition comprises a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cell and a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells. In some alternatives, the cancer is selected from a solid tumor or hematologic malignancy. In some alternatives, the solid tumor is selected from the group consisting of a

breast cancer, lung cancer, colon cancer, renal cancer, pancreatic cancer, prostate cancer, and ovarian cancer.

[0259] Another aspect of the disclosure includes a genetic system to deliver drug-regulated transgene expression in cells, such as drug-regulated expression of a recombinant protein such as a recombinant antigen receptor and/or a molecule expressed by a cell expressing a recombinant antigen receptor. In an alternative, regulated transgene expression is engineered into and/or contained within cells, such as lymphocytes, for example, for use in adoptive cell therapy, such as adoptive immunotherapy. Such systems provide rigorous safety attributes to cell therapies, such as chimeric antigen receptor (CAR) adoptive therapeutic strategies, generally without sacrificing curative intent. In some aspects, such features permit real-time clinician control of recombinant protein expression, e.g., CAR expression, *in vivo*. By engineering vectors that enable drug responsive transcriptional control of recombinant gene, e.g., CAR, expression, the activity of the recombinant gene, e.g., CARs, and/or other cell mediators can be turned "ON" and "OFF" *in vivo,* for example, based on a clinician prescribed pharmaceutical drug input that exhibits clinically permissive pharmacokinetics, tissue distribution, and partitioning between the extracellular space and cytosol of lymphocytes. The genetic system provides for drug regulated transgene expression to enforce a functional "OFF" state in the absence of the drug and a functional "ON" state transgene expression in the presence of the drug.

[0260] One alternative of such a drug is tamoxifen. Tamoxifen is an estrogen antagonist/partial agonist that is an FDA-approved and commercially available drug. It is taken orally and can be administered on a daily basis over an extended period of time. Tamoxifen has a proven safety record, favorable pharmacokinetic profile, excellent tissue distribution and a low partition coefficient between the extracellular space and cytosol. Functional analogs of tamoxifen also may be used. Other drugs can be selected, for example, based on safety record, favorable pharmacokinetic profile, excellent tissue distribution, a low partition coefficient between the extracellular space and cytosol, and/or low toxicities.

[0261] In some alternatives, the system employs a synthetic transcriptional regulator, e.g., synthetic transcriptional activator, which, in the presence of tamoxifen, can be induced to bind to a synthetic promoter operably linked to, e.g., upstream of, a transgene, to induce expression from the promoter, e.g., of the transgene. In some alternatives provided herein, the transcriptional activator is regulated by a drug or metabolite thereof, such as a tamoxifen-regulated transcriptional activator or transcription factor, which may be regulated,

e.g., induced, by tamoxifen or an analog or metabolite thereof. Exemplary tamoxifen-regulated transcription factors are chimeric transcription factors, such as those comprising a DNA binding domain specific for a synthetic promoter of the system, a domain that specifically binds tamoxifen and/or metabolite(s) thereof, for example, with affinity higher than the affinity of the domain for a natural molecule such as estrogen, and a transactivating domain, such as a strong transactivating domain. One tamoxifen regulated transcription factor ("TamR-tf", also designated "HEA3") is a chimeric transcription factor composed of human subunits including the N-terminal DNA binding domain of Hepatocyte Nuclear Factor 1-alpha (HNF-1α) fused in frame to a mutant (G521R) tamoxifen-specific form of an estrogen receptor ligand binding domain (ER-LBD), which binds to tamoxifen metabolites with high affinity as compared to estrogen, which is in turn fused to the p65 activation domain of NF-κB (p65). An exemplary amino acid sequence of a TamR-tf is provided in Table 10 and is identified as SEQ ID NO: 40. In this sequence, the mutant tamoxifen-specific form of the ligand binding domain of the estrogen receptor ligand binding domain (ER-LBD) is found at amino acids 282-595 of the TamR-tf and has a mutation at position 521, as compared to wild-type estrogen receptor ligand binding domain. The p65 activation domain of NF-κB (p65) is found at amino acids 596-862 of SEQ ID NO: 40. Changes can be made to the transcriptional activator, for example, to increase the properties of the transcription factor including, without limitation, altering one or more amino acids in the estrogen receptor ligand binding domain to increase the affinity of the factor for estrogen analogs and altering one or more amino acids in the p65 transactivating domain. In some alternatives, changes are made to the transcriptional activator that result in an altered transcription factor which retains or substantially retains one or more functions of tamR-tf, such as tamoxifen-specific binding and/or the same or substantially the same or at least the same specificity for tamoxifen or metabolite as compared to any natural molecule, at least the same or about the same specific DNA binding function, and/or the same or at least the same degree of transactivation activity.

[0262] In the absence of tamoxifen, the transcriptional activator, e.g., TamR-tf, is generally excluded from the nucleus by binding of cytosolic heat-shock protein 90 (HSP90) to the tamoxifen-binding active site, resulting in the expression of a transgene operably linked to the tamR-tf-inducible promoter being in the "OFF" state. Nanomolar concentrations of cytosolic tamoxifen actively out-compete HSP90 for ER-LBD binding, resulting in TamR-tf translocation to the nucleus. Upon nuclear translocation, TamR-tf is readily available to bind to a restricted synthetic promoter (e.g. 7xHBD/EF1αp). In the presence of tamoxifen, binding

of TamR-tf to the synthetic promoter, e.g., the 7xHBD/EF1αp promoter, induces the "ON" state of expression for a transgene operably linked to the synthetic promoter. In some alternatives, this transcriptional regulator can be modified to provide for a varying level of control of transgene expression. Amino acid substitutions in the LBD of TamR-tf permit selective responsiveness to tamoxifen and its metabolites, where 4-hydroxy tamoxifen (4-OHT) is the most pharmacologically active metabolite, in regards to TamR-tf activity, while lacking interaction with endogenous estrogen.In some alternatives, a system for inducible expression of chimeric antigen receptor is provided, wherein the system comprises: a first nucleic acid comprising a first promoter inducible by a drug operably linked to a polynucleotide, such as one coding for a chimeric antigen receptor, the chimeric antigen receptor optionally comprising a ligand binding domain, wherein the ligand binding domain binds to a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population that is suitable to mediate recognition and elimination by a lymphocyte, a polypeptide spacer, wherein the spacer optionally provides for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor, a transmembrane domain, and an intracellular signaling domain. In some alternatives, the system further includes a second nucleic acid encoding a transcriptional modulator for the inducible promoter, which is capable of modulating, e.g., activating, transcription from the first promoter, such as in the presence of the drug or metabolite thereof; typically the system comprises a second constitutive or inducible promoter operably linked to a nucleic acid coding for the transcriptional modulator.

[0263] In some alternatives of the inducible system, the first promoter is operably linked to a polynucleotide coding for a gene that promotes cell survival and proliferation, a gene that prevents apoptosis, and/or a gene that that inhibits negative checkpoint signaling. Such genes include genes encoding IL-2, IL-15, Chemokine receptors, Bcl2, CA-Akt, dn-TGFbetaRIII, dn-SHP1/2, and/or PD-1CD28 chimeras.

[0264] In some alternatives, the system employs a synthetic transcriptional activator which, in the presence of the drug (e.g.tamoxifen) or a metabolite thereof, such as following administration of the drug, is induced to bind a synthetic promoter upstream of a transgene to induce expression. In some alternatives, the transcriptional activator is TamR-tf (HEA3) or other tamoxifen-inducible transcriptional activator with analogous domains. The tamoxifen regulated transcription factor ("TamR-tf", also designated "HEA3") is a chimeric transcription factor composed of human subunits including the N-terminal DNA binding

domain of Hepatocyte Nuclear Factor 1-alpha (HNF-1α)(e.g. amino acids 1-281 of SEQ ID NO: 40) fused in frame to the mutant tamoxifen-specific ligand binding domain of the estrogen receptor ligand binding domain (ER-LBD), that is in turn fused to the p65 activation domain of NF-κB (p65).

[0265] In some alternatives of the compositions herein, the CD4+ T helper lymphocyte cell is naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, or bulk CD4+ T cells. In some alternatives, CD4+ helper lymphocyte cell is a naïve CD4+ T cell, wherein the naïve CD4+ T cell comprises a CD45RO-, CD45RA+, and/or is a CD62L+ CD4+ T cell. In some alternatives, at least 20, 30, 40, 50, 60, 70, 80, 90, 95, 99, or 100 % of the cells in the composition are CD4+; in some alternatives, at least 20, 30, 40, 50, 60, 70, 80, 90, 95, 99, or 100 % of the cells in the composition or the CD4+ cells in the composition are naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, or bulk CD4+ T cells.

[0266] In some alternatives of the compositions herein, the CD8+ T cytotoxic lymphocyte cell is a naïve CD8+ T cell, central memory CD8+ T cell, effector memory CD8+ T cell and/or bulk CD8+ T cell. In some alternatives, the CD8+ cytotoxic T lymphocyte cell is a central memory T cell, wherein the central memory T cell comprises a CD45RO+, CD62L+, and/or CD8+ T cell. In yet other alternatives, the CD8+ cytotoxic T lymphocyte cell is a central memory T cell and the CD4+ helper T lymphocyte cell is a naïve or central memory CD4+ T cell. In some alternatives, at least 20, 30, 40, 50, 60, 70, 80, 90, 95, 99, or 100 % of the cells in the composition are CD8+; in some alternatives, at least 20, 30, 40, 50, 60, 70, 80, 90, 95, 99, or 100 % of the cells in the composition or the CD8+ cells in the composition are naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells, or bulk CD8+ T cells. In some embodiments, at least 20, 30, 40, 50, 60, 70, 80, 90, 95, 99, or 100 % of the cells in the composition, and/or of the CD4+ and/or CD8+ cells in the composition, express the transgene or recombinant molecule, such as the CAR, and/or contain the expression system.

[0267] Additionally provided are methods of making compositions including adoptive immunotherapy compositions, such as those containing the systems, and uses or methods of using these compositions, such as for performing cellular immunotherapy in a subject having a disease or disorder.

[0268] In some alternatives, a method of manufacturing the compositions comprises obtaining generating a modified naïve or naïve-derived or central memory or

central memory-derived CD4+ T helper cell or population containing the same, wherein the modified helper T lymphocyte cell preparation comprises CD4+ T cells that have a chimeric receptor, such as one comprising a ligand binding domain specific for a tumor cell surface molecule, a spacer domain, a transmembrane domain, and an intracellular signaling domain under control of an inducible promoter as described herein. In other some alternatives, CD4+ cells have a cytokine or chemokine receptor under the control of an inducible promoter.

[0269] In some alternatives of the methods described herein, the T-cells are administered with other T-cells that are non-CAR expressing, and/or produce Tam-inducible proteins. In some alternatives, the transcriptional activator for the CAR comprises a DNA-binding domain, a tamofexin/metabolite binding domain, or a transactivation domain. In some alternatives, one or more, generally all, of the domains from the synthetic transcriptional activator is or are derived from human proteins, such as human or substantially human domains. Such features can reduce immunogenicity of the constructs upon administration to human subjects, for example, in cell therapy.

Expression in Jurkat Cells.

[0270] The "ON" and "OFF" state of Jurkat T cells expressing TamR ZsGreen was studied.

Constructs.

[0271] In some alternatives, this system involves two components: 1) constitutive expression of HEA-3 linked to a single transgene or set of transgenes by T2A skip-linker domains and 2) conditional expression of a transgene that is under control of the HEA-3 restricted synthetic promoter 7xHBD/mEF1ap whereby induction of the transgene occurs in response to tamoxifen. Depending on the desired use of TamRLV genetic control, a combination of delivery systems and vector compositions can be used. Construction of constructs is described above.

[0272] An alternative of the TamR-LV system allows for constitutive HEA3 expression and inducible expression of either ZsGreen or chimeric antigen receptors, permitting kinetic analysis and biological effect controlled in an "ON" and "OFF" manner by the presence or absence of tamoxifen. For this, two constructs were used: 1) HEA-3 is driven by the human EF1a promoter and linked to the constructed truncated EGFR (EGFRt)

transmembrane marker protein by a T2A skip-linker sequence and cloned into a third generation epHIV7 self-inactivating lentivirus packaging plasmid (construct A). (See **Table 10**; SEQ ID NO: 39; **Table 1:** SEQ ID NO: 8 and SEQ ID NO: 9) and 2) 7xHBD/mEF1ap controls tamoxifen-dependent expression of ZsGreen cloned into pcDNA3.1(-) which was engineered to lack the commercially used CMV promoter (construct B) (See **Table 12**; SEQ ID NO: 41). ZsGreen1 is a human codon-optimized ZsGreen variant that encodes the brightest commercially available green fluorescent protein. (Available from Clontech).

Methods.

[0273] Jurkat cells were transduced at a MOI of 5 with lentivirus packaging constructs described above in which a dual packaging approach in which each plasmid is co-transfected into 293T cells during lentiviral production. This construct utilized molar ratio of 1:2 of construct A to construct B. Construct A encodes the chimeric transcription factor HEA3 linked via a ribosomal cleavage sequence, T2A, to the tracking and selection marker EGFRt. Construct B encodes the synthetic, HEA3-responsive promoter, 7xHBD/mE1b which regulates expression of the transgene ZsGreen-DR1, a short half-life green fluorescent reporter gene.

[0274] After transduction, cells were expanded in culture, enriched for EGFRt expression utilizing Miltenyi magnetic bead selection, to a purity >99%. After further expansion in culture, cells were harvested and treated with Ethanol (Vehicle) as a negative control or 500 nM 4-hydroxytamoxifen (4OHT) for 24 hours, and then were harvested, stained with Erbitux-biotin followed by SA-APC, and analyzed for APC expression and ZsGreen expression via flow cytometry. The results are shown in **Figure 1**.

Dose response.

[0275] Cells were harvested and were then subjected to treatment with 4OHT with concentrations ranging from 0 nM-1000 nM, as indicated. Samples were harvested, washed, and stained with EGFRt-biotin, followed by strepavidin-APC, and then analyzed by flow cytometry for ZsGreen expression. The results are shown in **Figure 2**.

On and Off rate Kinetics.

[0276] The population was subjected to 500 nM 4OHT stimulation for 48 hours, and then sorted for ZsGreen + cells utilizing FACS, yielding a >99% ZsGreen + population on immediate post-sort analysis. Cells were subsequently expanded in culture for 3 weeks, and then prepared for kinetic studies. Cells were divided into 3 treatment groups: (1) no 4OHT (2) 200 nM 4OHT (added every 48 hours) (3) 24 hour 200 nM 4OHT, followed by washout 1x in PBS, then culture in 4OHT free media for 14 days, followed by a 24 hour re-stimulation with 200 nm 4OHT, and then washout in 1x PBS. At each time point, samples were harvested and analyzed via flow cytometry for ZsGreen expression. Results are shown in **Figure 3** and are presented as % ZsGreen+.

Results.

[0277] The results in **Figure 1** show that in the presence of 4-hydroxy tamoxifen (4-OHT), about 50% of the cells express ZsGreen, indicating these cells have both constructs A and B and that 4-OHT induced expression of construct B. The results of the dose response curve show that a concentration of 200 nM or greater of 4-OHT was effective to induce expression of the transgene in transduced cells. (**Figure 2**). The results in **Figure 3** show that as the 4-OHT is washed out of the culture, the Zs Green expression drops off to less than 10% of the max ZsGReen activity within 5 days. When 4-OHT is added back, ZsGreen activity returns to about 100% max activity within 2 days.

Discussion.

[0278] These experiments show that human T Jurkat cells can be transduced with a dual packaged lentivirus with a constitutive component and an inducible component. Expression of the ZsGreen gene is induced in the presence of 4-OHT in a dose responsive manner. In addition, washing out of 4-OHT from the cell culture resulted in a decrease in expression of ZsGreen that could be re-stimulated by addition of 4-OHT back to the cells.

Expression in Primary CD4 Central Memory Cells and CD8 central Memory cells.

[0279] "ON" and "OFF" state of CD4 and CD8 Central memory T cells expressing TamR ZsGreen was studied.

Constructs.

[0280] In some alternatives, this system involves two components: 1) constitutive expression of HEA-3 linked to a single transgene or set of transgenes by T2A skip-linker domains (construct A) (See **Table 10**; SEQ ID NO: 39; **Table 1** SEQ ID NO: 8 and SEQ ID NO: 9) and 2) conditional expression of a transgene that is under control of the HEA-3 restricted synthetic promoter 7xHBD/mEF1ap whereby induction of the transgene occurs in response to tamoxifen (construct B) (See **Table 12**; SEQ ID NO: 41). Depending on the desired use of TamRLV genetic control a combination of delivery systems and vector compositions can be used. Constructs were prepared as described above.

Methods.

[0281] CD4 central memory cells were obtained from peripheral blood by selecting cells through flow cytometry for markers CD4, and CD62L and negative for CD45RO. Cells were cultured with anti-CD3/anti-CD28 beads for 3 days.

[0282] CD8 central memory cells were obtained from peripheral blood by selecting cells through flow cytometry for markers CD8, and CD62L and negative for CD45RO. Cells were cultured with anti-CD3/anti-CD28 beads for 3 days.

[0283] After 3 days, CD4 or CD8 central memory cells were transduced at a MOI of 5 with lentivirus packaging constructs described above in which a dual packaging approach in which each plasmid is co-transfected into 293T cells during lentiviral production. This construct utilized molar ratio of 1:2 of construct A to construct B. Construct A encodes the chimeric transcription factor HEA3 linked via a ribosomal cleavage sequence, T2A, to the tracking and selection marker EGFRt. Construct B encodes the synthetic, HEA3-responsive promoter, 7xHBD/mE1b which regulates expression of the transgene ZsGreen-DR1, a short half-life green fluorescent reporter gene.

[0284] On day 21 after transduction, cells were enriched for EGFRt, and subsequently expanded with feeder cells, IL2, and IL15. Following expansion, cells were divided into 3 treatment groups, 4OHT alone (A), 4OHT combined with CD3/CD28 bead cotreatment (B) or 4OHT alone for 48 hours, followed by addition of CD3/CD28 beads (C). Corresponding samples without 4OHT were also obtained for comparison. All samples were harvested at indicated time points following 4OHT treatment, stained with EGFRt-biotin

antibody, followed by SA-APC, and analyzed for flow cytometry. The results are shown in **Figure 4** (CD4 central memory) and **Figure 5** (CD8 central memory).

Results.

[0285] The results show that the CD4 cells transduced with the dual plasmid vector required the presence of activation stimulus in order to express the ZsGreen even in the presence of tamoxifen. See **Figure 4B**. About 70% of the primary transduced CD4 cells in the presence of tamoxifen and antiCD3/anti-CD28 beads expressed ZsGreen. Gene expression was seen when activation occurred after a 48 hour treatment with tamoxifen. See **Figure 4C.**

[0286] The results show that the CD8 cells transduced with the dual plasmid vector also required the presence of activation stimulus in order to express the ZsGreen even in the presence of tamoxifen. See **Figure 5B**. About 37% of the primary transduced CD8 cells in the presence of tamoxifen and antiCD3/anti-CD28 beads expressed ZsGreen. Gene expression was seen when activation occurred after a 48 hour treatment with tamoxifen. See **Figure 5C**.

[0287] These results show that primary central memory T cells transduced with an inducible construct can express the transgene in the presence of the inducer upon activation with antiCD3/CD28 cells. However, nonactivated cells expressing the transgene can be readily isolated using immunomagnetic or flow cytometry sorting.

Construction of TamR---CD19CAR LV.

[0288] Construction of the vector was accomplished by dual packaging of transfer plasmids housing constructs as described above at a plasmid molar ratio of 1:1. (See **Figure 6C and 7C**) Construct A under the constitutive EF-1alpha promoter encodes TamR-tf (HEA3) linked via a ribosomal cleavage sequence, T2A, to the tracking and selection marker EGFRt.(See **Table 10**; SEQ ID NO: 39, **Table 1**: SEQ ID NO: 8 and SEQ ID NO: 9) Construct B contains the 7xHBD/mE1b promoter with downstream transgene cDNA including CD19CAR linked via 2a cleavage sequence to Her2t, a tracking and selection marker.(See **Table 12**; SEQ ID NO: 41; **Table 2** SEQ ID NO: 10; **Table 13**; SEQ ID NO: 44). Another construct was prepared adding an additional selective marker DHFRdm (See **Table 14**; SEQ ID NO: 46).

[0289] Human Jurkat cells were transduced at a MOI of 2, then were expanded in culture, and selected for EGFRt+ cells via magnetic bead selection. After further expansion, cells were either treated with vehicle alone (EtOH) or 500 nM 4OHT, and harvested for flow

cytometry. Samples were stained with EGFRt-APC antibody and Herceptin-biotin, followed by SA PE. Samples were also prepared for western blot, the primary antibody used is an anti-CD247 mouse mAb which recognizes the intracellular CD3 zeta chain on the CD19CAR (~48 kDa). The endogenous CD3 zeta migrates at ~23 kDa. The results are shown in **Figure 6**.

Jurkat T cells transduced with TamR CD19CAR LV containing a selective marker.

[0290] Construction of TamR CD19CAR LV was done through dual packaging of constructs A and B. Construct A contains HEA3 linked via a ribosomal cleavage sequence, T2A, to the tracking and selection marker EGFRt. Construct B contains the 7xHBD/mE1b promoter with downstream transgene consisting of CD19CAR linked via 2a cleavage sequence to Her2t, a tracking and selection marker, linked via another 2a cleavage sequence (P2A) to DHFRdm, a methotrexate selection gene.

[0291] Cells were transduced with TamR CD19CAR LV at a MOI of 1, expanded in culture, selected for EGFRt via magnetic bead selection. Following further expansion cells were treated with vector alone (EtOH) or 500 nM 4OHT, and harvested for flow cytometry, (upper panel). **Figure 7.** Samples were stained with EGFRt-APC antibody and Herceptin-biotin, followed by SA- PE. Parental (untransduced Jurkat) is shown for comparison. Samples were also prepared for western blot, the primary antibody used is an anti-CD247 mouse mAb which recognizes the intracellular CD3 zeta chain on the CD19CAR (~48 kDa). The endogenous CD3 zeta migrates at ~23 kDa.

Results.

[0292] The results show that EGFRt is detected in 94.6% of cells transduced with a construct not including the additional selective marker DHFRdm. See **Figure 6B. Figure 6C** shows that in the presence of tamoxifen, about 42.6% of the cells transduced with a construct not including DHFRdm expressed both EGFRt and Her2t. The cells expressing both markers expressed the CD3zeta chain as part of the CAR construct (48 kDa) as detected in Western blot. See **Figure 6B**.

[0293] **Figure 7** shows that 85.9% Jurkat cells transduced with a construct including another 2a cleavage sequence (P2A) to DHFRdm in the absence of tamoxifen express EGFRt. In the presence of tamoxifen, about 7.5% of the cells expressed both EGFRt

and Her2t. The cells expressing both markers expressed the CD3zeta chain as part of the CAR construct (48 kDa) as detected in Western blot. See **Figure 7B**.

[0294] Inducible expression of CD19 CAR is shown in transduced Jurkat cells. Induction can be measured by detecting expression of the marker Her2t as well as detecting expression of CD19CAR using western blot. Adding an additional selectable marker, DHFRdm, to the inducible construct did not improve inducible gene expression.

TamR CD19CAR LV Transduced human CD4 TCM T cells.

[0295] CD4 central memory cells were obtained from peripheral blood by selecting cells through flow cytometry for markers CD4, and CD62L and negative for CD45RO. Cells were cultured anti-CD3/anti-CD28 beads for 3 days. After 3 days, CD4 Central memory cells were transduced at a MOI of 5 with a lentivirus which packages constructs A and B. (**Figure 8B**). Construct A encodes the chimeric transcription factor HEA3 linked via a ribosomal cleavage sequence, T2A, to the tracking and selection marker EGFRt. (See **Table 10;** SEQ ID NO: 39; **Table 1**; SEQ ID NO: 8 and SEQ ID NO: 9). Construct B encodes the synthetic, HEA3-responsive promoter, 7xHBD/mE1b which regulates expression of the transgene CD19CAR- linked via a T2A to Her2t for tracking and selection, and via a second 2a sequence, P2A, to the methotrexate selection gene DHFRdm. (See **Table 12;** SEQ ID NO: 41; **Table 2** SEQ ID NO: 10; **Table 13**; SEQ ID NO: 44; **Table 14**; SEQ ID NO: 46).

[0296] On day 21 after transduction, cells were enriched for EGFRt utilizing magnetic bead selection, and subsequently expanded with irradiated feeder cells, IL2, and IL15. Two weeks after expansion, cells were cryopreserved. In the experiment depicted above, CD4+ CEM cells which were mock transduced, or CD4 +TamR CD19CAR LV were thawed, placed in culture with CD3/CD28 beads, IL-2, IL-15. CD4+TamRCD19CAR LV were treated or not with 500 nM 4OHT for 24 hours. All samples were harvested washed, then stained with an EGFRt-APC antibody and Her2t-biotin, followed by SA-PE, and analyzed via flow cytometry. The results are shown in **Figure 8**.

Results.

[0297] The results in **Figure 8** show that in the absence of tamoxifen 75.6% of transduced primary CD4 central memory cells express EGFRt, indicating the constitutive

expression of construct A. In the presence of tamoxifen and an activating stimulus (e.g. antiCD3/ antiCD28 beads), about 14.7 % of the primary cells express both EGFRt and Her2t.

[0298] Primary CD4 cells transduced with an inducible vector in the presence of the inducer and an activating stimulus express the transgene under control of the inducible promoter as detected by the expression of the marker gene Her2t. However, nonactivated cells expressing the transgene can be readily isolated using immunomagnetic or flow cytometry sorting. Further characterization of these cells will involve detecting of expression of CD19CAR.

[0299] The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein. All references and documents referred to herein are hereby incorporated by reference.

Table 1

Sequence of anti-CD19 short spacer chimeric receptor

GMCSFRss-CD19scFv-IgG4hinge-CD28tm-41BB-Zeta-T2A-EGFRt

*Atg*ctgctgctggtgaccagcctgctgctgtgcgagctgccccaccccgcctttctgctgatcccc (GMCSFRss) (SEQ ID NO:2)

Gacatccagatgacccagaccacctccagcctgagcgccagcctgggcgaccgggtgaccatcagctgccgggccagccaggaca tcagcaagtacctgaactggtatcagcagaagcccgacggcaccgtcaagctgctgatctaccacaccagccggctgcacagcggcg tgcccagccggtttagcggcagcggctccggcaccgactacagcctgaccatctccaacctggaacaggaagatatcgccacctactt ttgccagcagggcaacacactgccctacacctttggcggcggaacaaagctggaaatcaccggcagcacctccggcagcggcaagc ctggcagcggcgagggcagcaccaagggcgaggtgaagctgcaggaaagcggccctggcctggtggcccccagccagagcctga gcgtgacctgcaccgtgagcggcgtgagcctgcccgactacggcgtgagctggatccggcagccccccaggaagggcctggaatg gctgggcgtgatctggggcagcgagaccacctactacaacagcgccctgaagagccggctgaccatcatcaaggacaacagcaaga gccaggtgttcctgaagatgaacagcctgcagaccgacgacaccgccatctactactgcgccaagcactactactacggcggcagcta cgccatggactactggggccagggcaccagcgtgaccgtgagcagc (CD19scFv) (SEQ ID NO:3)

Gaatctaagtacggaccgcccctgccccccttgccct (IgG4hinge) (SEQ ID NO:4)

Atgttctgggtgctggtggtggtcggaggcgtgctggcctgctacagcctgctggtcaccgtggccttcatcatcttttgggtg (CD28tm-)(SEQ ID NO:5)

Aaacggggcagaaagaaactcctgtatatattcaaacaaccatttatgagaccagtacaaactactcaagaggaagatggctgtagctg ccgatttccagaagaagaagaaggaggatgtgaactg (41BB) (SEQ ID NO:6)

Cgggtgaagttcagcagaagcgccgacgcccctgcctaccagcagggccagaatcagctgtacaacgagctgaacctgggcagaa gggaagagtacgacgtcctggataagcggagaggccgggaccctgagatgggcggcaagcctcggcggaagaacccccaggaag

gcctgtataacgaactgcagaaagacaagatggccgaggcctacagcgagatcggcatgaagggcgagcggaggcggggcaagg gccacgacggcctgtatcagggcctgtccaccgccaccaaggatacctacgacgccctgcacatgcaggccctgccccaagg (CD3Zeta)- (SEQ ID NO:7)

Ctcgagggcggcggagagggcagaggaagtcttctaacatgcggtgacgtggaggagaatcccggccctagg (T2A) (SEQ ID NO:8)

Atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatcccacgcaaagtgtgtaacggaataggta ttggtgaatttaaagactcactctccataaatgctacgaatattaaacacttcaaaaactgcacctccatcagtggcgatctccacatcctgc cggtggcatttaggggtgactccttcacacatactcctcctctggatccacaggaactggatattctgaaaaccgtaaaggaaatcacag ggtttttgctgattcaggcttggcctgaaaacaggacggacctccatgcctttgagaacctagaaatcatacgcggcaggaccaagcaa catggtcagttttctcttgcagtcgtcagcctgaacataacatccttgggattacgctccctcaaggagataagtgatggagatgtgataatt tcaggaaacaaaaatttgtgctatgcaaatacaataaactggaaaaaactgtttgggacctccggtcagaaaaccaaaattataagcaac agaggtgaaaacagctgcaaggccacaggccaggtctgccatgccttgtgctccccgagggctgctggggcccggagcccaggg actgcgtctcttgccggaatgtcagccgaggcagggaatgcgtggacaagtgcaaccttctggagggtgagccaagggagtttgtgga gaactctgagtgcatacagtgccacccagagtgcctgcctcaggccatgaacatcacctgcacaggacggggaccagacaactgtatc cagtgtgcccactacattgacggccccccactgcgtcaagacctgcccggcaggagtcatgggagaaaacaacaccctggtctggaag tacgcagacgccggccatgtgtgccacctgtgccatccaaactgcacctacggatgcactgggccaggtcttgaaggctgtccaacga atgggcctaagatcccgtccatcgccactgggatggtgggggccctcctcttgctgctggtggtggccctggggatcggcctcttcatg*tga* (EGFRt) (SEQ ID NO:9)

Table 2

GMCSFRss

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

CD19scFv

```
DNA: TTTCTGCTGATCCCC:GACATCCAGATGACCCAGACCACCTCCAGCCTGAGC
 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
```

IgG4hinge

```
DNA: TACTGGGGCCAGGGCACCAGCGTGACCGTGAGCAGC:GAGAGCAAGTACGGA
 AA: Y  W  G  Q  G  T  S  V  T  V  S  S   E  S  K  Y  G
```

CD28tm

```
DNA: CCGCCCTGCCCCCCTTGCCCT:ATGTTCTGGGTGCTGGTGGTGGTCGGAGGC
 AA: P  P  C  P  P  C  P   M  F  W  V  L  V  V  V  G  G


DNA: GTGCTGGCCTGCTACAGCCTGCTGGTCACCGTGGCCTTCATCATCTTTTGG
 AA: V  L  A  C  Y  S  L  L  V  T  V  A  F  I  I  F  W
```

41BB

```
DNA: GTG:AAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAACCATTTATG
 AA: V   K  R  G  R  K  K  L  L  Y  I  F  K  Q  P  F  M
```

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

CD3**Zeta**

DNA: GAAGAAGAAGAAGGAGGATGTGAACTGCGGGTGAAG:TTCAGCAGAAGCGCC
AA: E E E E G G C E L R V K F S R S A

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

DNA: CTGGGCAGAAGGGAAGAGTACGACGTCCTGGATAAGCGGAGAGGCCGGGAC
AA: L G R R E E Y D V L D K R R G R D

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

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

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

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

T2A

DNA: CCAAGG:CTCGAGGGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGT
AA: P R L E G G G E G R G S L L T C G

EGFRt

DNA: GACGTGGAGGAGAATCCCGGCCCTAGG:**ATGCTTCTCCTGGTGACAAGCCTT**
AA: D V E E N P G P R M L L L V T S L

DNA: **CTGCTCTGTGAGTTACCACACCCAGCATTCCTCCTGATCCCACGCAAAGTG**
AA: L L C E L P H P A F L L I P R K V

DNA: **TGTAACGGAATAGGTATTGGTGAATTTAAAGACTCACTCTCCATAAATGCT**
AA: C N G I G I G E F K D S L S I N A

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

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

DNA: **GATCCACAGGAACTGGATATTCTGAAAACCGTAAAGGAAATCACAGGGTTT**
AA: D P Q E L D I L K T V K E I T G F

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

DNA: **AACCTAGAAATCATACGCGGCAGGACCAAGCAACATGGTCAGTTTTCTCTT**
AA: N L E I I R G R T K Q H G Q F S L

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

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

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

DNA: ATTATAAGCAACAGAGGTGAAAACAGCTGCAAGGCCACAGGCCAGGTCTGC
AA: I I S N R G E N S C K A T G Q V C

DNA: CATGCCTTGTGCTCCCCCGAGGGCTGCTGGGGCCCGGAGCCCAGGGACTGC
AA: H A L C S P E G C W G P E P R D C

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

DNA: CTTCTGGAGGGTGAGCCAAGGGAGTTTGTGGAGAACTCTGAGTGCATACAG
AA: L L E G E P R E F V E N S E C I Q

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

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

DNA: GTCAAGACCTGCCCGGCAGGAGTCATGGGAGAAAACAACACCCTGGTCTGG
AA: V K T C P A G V M G E N N T L V W

DNA: AAGTACGCAGACGCCGGCCATGTGTGCCACCTGTGCCATCCAAACTGCACC
AA: K Y A D A G H V C H L C H P N C T

DNA: TACGGATGCACTGGGCCAGGTCTTGAAGGCTGTCCAACGAATGGGCCTAAG
AA: Y G C T G P G L E G C P T N G P K

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

DNA: GTGGCCCTGGGGATCGGCCTCTTCATG*TGA* (SEQ ID NO:10)
AA: V A L G I G L F M * (SEQ ID NO:11)

Table 3
ZXR-014 Nucleotide and amino acid sequences (map of sections)

GMCSFRss: nt2084-2149 (SEQ ID NO: 50)

CD19scFv: nt2150-2884(SEQ ID NO: 51)

Igg4Hinge: nt2885-2920 (SEQ ID NO: 52)

CD28tm: nt2921-3004 (SEQ ID NO: 53)

41BB: nt3005-3130 (SEQ ID NO: 54)

Zeta: nt3131-3466 (SEQ ID NO: 55)

T2A: nt3467-3538 (SEQ ID NO: 56)

EGFRt: nt3539-4612 (SEQ ID NO: 57)

Primers for sequencing:

Oligo name	Sequence	Region
oJ02649	ATCAAAAGAATAGACCGAGATAGGGT	pre-U5(SEQ ID NO:22)
oJ02648	CCGTACCTTTAAGACCAATGACTTAC	delU3(SEQ ID NO:23)
oJ02650	TTGAGAGTTTTCGCCCCG	mid-Ampr(SEQ ID NO:24)
oJ02651	AATAGACAGATCGCTGAGATAGGT	post-Ampr(SEQ ID NO:25)
oJ02652	CAGGTATCCGGTAAGCGG	CoE1 ori(SEQ ID NO:26)
oJ02653	CGACCAGCAACCATAGTCC	SV40(SEQ ID NO:27)
oJ02654	TAGCGGTTTGACTCACGG	CMV(SEQ ID NO:28)
oJ02655	GCAGGGAGCTAGAACGATTC	psi(SEQ ID NO:29)
oJ02656	ATTGTCTGGTATAGTGCAGCAG	RRE(SEQ ID NO:30)
oJ02657	TCGCAACGGGTTTGCC	EF1p(SEQ ID NO:31)
oJ02658	AGGAAGATATCGCCACCTACT	CD19Rop(SEQ ID NO:32)
oJ02601	CGGGTGAAGTTCAGCAGAAG	Zeta(SEQ ID NO:33)
oJ02735	ACTGTGTTTGCTGACGCAAC	WPRE(SEQ ID NO:34)
oJ02715	ATGCTTCTCCTGGTGACAAG	EGFRt(SEQ ID NO:35)

Table 4 Uniprot P0861 IgG4-Fc(SEQ ID NO:13)

```
    10         20         30         40         50         60
ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS

        70         80         90        100        110        120
GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRVES KYGPPCPSCP APEFLGGPSV

       130        140        150        160        170        180
FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK PREEQFNSTY

       190        200        210        220        230        240
RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT LPPSQEEMTK

       250        260        270        280        290        300
NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSRL TVDKSRWQEG

       310        320
NVFSCSVMHE ALHNHYTQKS LSLSLGK
```

1-98 CH1 (SEQ ID NO: 59)

99-110 Hinge (SEQ ID NO: 60)

111-220 CH2 (SEQ ID NO: 61)

221-327 CH3 (SEQ ID NO: 62)

Position 108 S→P (SEQ ID NO: 63)

Table 5 Uniprot P10747 CD28(SEQ ID NO:14)

```
10         20         30         40         50         60
MLRLLLALNL FPSIQVTGNK ILVKQSPMLV AYDNAVNLSC KYSYNLFSRE FRASLHKGLD

        70         80         90        100        110        120
SAVEVCVVYG NYSQQLQVYS KTGFNCDGKL GNESVTFYLQ NLYVNQTDIY FCKIEVMYPP

       130        140        150        160        170        180
PYLDNEKSNG TIIHVKGKHL CPSPLFPGPS KPFWVLVVVG GVLACYSLLV TVAFIIFWVR

       190        200        210        220
SKRSRLLHSD YMNMTPRRPG PTRKHYQPYA PPRDFAAYRS
```

1-18 signal peptide (SEQ ID NO: 64)

19-152 extracellular domain (SEQ ID NO: 65)

153-179 transmembrane domain (SEQ ID NO: 66)

180-220 intracellular domain (SEQ ID NO: 67)

Position 186-187 LL→GG (SEQ ID NO: 68)

Table 6 Uniprot Q07011 4-1BB(SEQ ID NO:15)

```
    10         20         30         40         50         60
MGNSCYNIVA TLLLVLNFER TRSLQDPCSN CPAGTFCDNN RNQICSPCPP NSFSSAGGQR

        70         80         90        100        110        120
TCDICRQCKG VFRTRKECSS TSNAECDCTP GFHCLGAGCS MCEQDCKQGQ ELTKKGCKDC

       130        140        150        160        170        180
CFGTFNDQKR GICRPWTNCS LDGKSVLVNG TKERDVVCGP SPADLSPGAS SVTPPAPARE

       190        200        210        220        230        240
PGHSPQIISF FLALTSTALL FLLFFLTLRF SVVKRGRKKL LYIFKQPFMR PVQTTQEEDG

       250
CSCRFPEEEE GGCEL
```

1-23 signal peptide (SEQ ID NO: 69)

24-186 extracellular domain (SEQ ID NO: 70)

187-213 transmembrane domain (SEQ ID NO: 71)

214-255 intracellular domain (SEQ ID NO: 72)

Table 7 Uniprot P20963 human CD3ζ isoform 3 (SEQ ID NO:16)

```
10         20         30         40         50         60
MKWKALFTAA ILQAQLPITE AQSFGLLDPK LCYLLDGILF IYGVILTALF LRVKFSRSAD

        70         80         90        100        110        120
APAYQQGQNQ LYNELNLGRR EEYDVLDKRR GRDPEMGGKP QRRKNPQEGL YNELQKDKMA

       130        140        150        160
EAYSEIGMKG ERRRGKGHDG LYQGLSTATK DTYDALHMQA LPPR
```

1-21 signal peptide (SEQ ID NO: 73)

22-30 extracellular (SEQ ID NO: 74)

31-51 transmembrane (SEQ ID NO: 75)

52-164 intracellular domain (SEQ ID NO: 76)

61-89 ITAM1 (SEQ ID NO: 77)

100-128 ITAM2 (SEQ ID NO: 78)

131-159 ITAM3 (SEQ ID NO: 79)

Table 8 Exemplary Hinge region Sequences

Human IgG1 EPKSCDKTHTCPPCP (SEQ ID NO:17)

Human IgG2 ERKCCVECPPCP (SEQ ID NO:18)

Human IgG3 ELKTPLGDTHTCPRCP $(EPKSCDTPPPCPRCP)_3$ (SEQ ID NO:19)

Human IgG4 ESKYGPPCPSCP (SEQ ID NO:20)

Modified Human IgG4 ESKYGPPCPPCP (SEQ ID NO: 21)

Modified Human IgG4 YGPPCPPCP (SEQ ID NO:36)

Modified Human IgG4 KYGPPCPPCP (SEQ ID NO:37)

Modified Human IgG4 EVVKYGPPCPPCP (SEQ ID NO:38)

Table 9 Her 2 construct-short spacer (SEQ ID NO:1)

GMCSFss-Her2scFv-IgG4hinge-CD28tm-41BB-Zeta-T2A-EGFRt

Leader

Atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatccca (SEQ ID NO: 80)

Her2scFV

Gatatccagatgacccagtccccgagctccctgtccgcctctgtgggcgatagggtcaccatcacctgccgtgccagtcaggatgt
gaatactgctgtagcctggtatcaacagaaaccaggaaaagctccgaaactactgatttactcggcatccttcctctactctggagtcc
cttctcgcttctctggttccagatctgggacggatttcactctgaccatcagcagtctgcagccggaagacttcgcaacttattactgtca
gcaacattatactactcctcccacgttcggacagggtaccaaggtggagatcaaaggcagtactagcggcggtggctccgggggc
ggatccggtgggggcggcagcagcgaggttcagctggtggagtctggcggtggcctggtgcagccaggggctcactccgtttgt
cctgtgcagcttctggcttcaacattaaagacacctatatacactgggtgcgtcaggccccgggtaagggcctggaatgggttgcaa
ggatttatcctacgaatggttatactagatatgccgatagcgtcaagggccgtttcactataagcgcagacacatccaaaaacacagc
ctacctgcagatgaacagcctgcgtgctgaggacactgccgtctattattgttctagatggggaggggacggcttctatgctatggact
actggggtcaaggaaccctggtcaccgtctcgagt (SEQ ID NO: 81)

Hinge spacer

Gagagcaagtacggaccgccctgccccccttgccct (SEQ ID NO: 82)

CD28tm

Atgttctgggtgctggtggtggtcggaggcgtgctggcctgctacagcctgctggtcaccgtggccttcatcatcttttgggtg
(SEQ ID NO: 83)

4-1BB

Aaacggggcagaaagaaactcctgtatatattcaaacaaccatttatgagaccagtacaaactactcaagaggaagatggctgtag
ctgccgatttccagaagaagaagaaggaggatgtgaactg (SEQ ID NO:84)

CD3 zeta

Cgggtgaagttcagcagaagcgccgacgcccctgcctaccagcagggccagaatcagctgtacaacgagctgaacctgggcag
aagggaagagtacgacgtcctggataagcggagaggccgggaccctgagatgggcggcaagcctcggcggaagaacccccag
gaaggcctgtataacgaactgcagaaagacaagatggccgaggcctacagcgagatcggcatgaagggcgagcggaggcggg
gcaagggccacgacggcctgtatcagggcctgtccaccgccaccaaggatacctacgacgccctgcacatgcaggccctgcccc
caagg (SEQ ID NO: 85)

T2A

Ctcgagggcggcggagagggcagaggaagtcttctaacatgcggtgacgtggaggagaatcccggccctagg (SEQ ID NO: 86)

tEGFR

atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatcccacgcaaagtgtgtaacggaatagg
tattggtgaatttaaagactcactctccataaatgctacgaatattaaacacttcaaaaactgcacctccatcagtggcgatctccacatc
ctgccggtggcatttaggggtgactccttcacacatactcctcctctggatccacaggaactggatattctgaaaaccgtaaaggaaat
cacagggtttttgctgattcaggcttggcctgaaaacaggacggacctccatgcctttgagaacctagaaatcatacgcggcaggac
caagcaacatggtcagttttctcttgcagtcgtcagcctgaacataacatccttgggattacgctccctcaaggagataagtgatggag
atgtgataatttcaggaaacaaaaatttgtgctatgcaaatacaataaactggaaaaaactgtttgggacctccggtcagaaaaccaaa
attataagcaacagaggtgaaaacagctgcaaggccacaggccaggtctgccatgccttgtgctccccgagggctgctggggcc
cggagcccagggactgcgtctcttgccggaatgtcagccgaggcagggaatgcgtggacaagtgcaaccttctggagggtgagc
caagggagtttgtggagaactctgagtgcatacagtgccacccagagtgcctgcctcaggccatgaacatcacctgcacaggacgg
ggaccagacaactgtatccagtgtgcccactacattgacggccccccactgcgtcaagacctgcccggcaggagtcatgggagaaa
acaacaccctggtctggaagtacgcagacgccggccatgtgtgccacctgtgccatccaaactgcacctacggatgcactgggcc

aggtcttgaaggctgtccaacgaatgggcctaagatcccgtccatcgccactgggatggtgggggccctcctcttgctgctggtggt
ggccctggggatcggcctcttcatgtga (SEQ ID NO: 87)

Table 10 TamR-tf (HEA3) nucleic acid (SEQ ID NO: 39)

HEA3 nucleotide(SEQ ID NO:39) and amino acid sequence (SEQ ID NO:40)

```
                M  V  S  K  L  S  Q  L  Q  T  E  L  L  A  A  L  L  E  S  G  L  S  K  E  A  L  I  Q  A
               ATGGTG TCCAAGCTGT CCCAGCTGCA GACAGAACTG CTGGCAGCAC TGCTGGAAAG CGGCCTGAGC AAAGAGGCCC TGATTCAGGC
               TACCAC AGGTTCGACA GGGTCGACGT CTGTCTTGAC GACCGTCGTG ACGACCTTTC GCCGGACTCG TTTCTCCGGG ACTAAGTCCG

 L  G  E  P  G  P  Y  L  L  A  G  E  G  P  L  D  K  G  E  S  C  G  G  G  R  G  E  L  A  E  L  P  N
ACTCGGCGAA CCTGGACCTT ATCTGCTCGC TGGCGAAGGC CCTCTGGATA AGGGCGAGAG CTGTGGCGGA GGAAGAGGAG AGCTGGCGGA GCTGCCTAAC
TGAGCCGCTT GGACCTGGAA TAGACGAGCG ACCGCTTCCG GGAGACCTAT TCCCGCTCTC GACACCGCCT CCTTCTCCTC TCGACCGGCT CGACGGATTG

 G  L  G  E  T  R  G  S  E  D  E  T  D  D  D  G  E  D  F  T  P  P  I  L  K  E  L  E  N  L  S  P  E  E
GGCCTGGGCG AGACAAGAGG CAGCGAGGAC GAGACAGACG ACGACGGCGA GGACTTCACC CCCCCATCC TGAAAGAGCT GGAAAACCTG AGCCCCGAGG
CCGGACCCGC TCTGTTCTCC GTCGCTCCTG CTCTGTCTGC TGCTGCCGCT CCTGAAGTGG GGGGGTAGG ACTTTCTCGA CCTTTGGAC TCGGGGCTCC

 A  A  H  Q  K  A  V  V  E  T  L  L  Q  E  D  P  W  R  V  A  K  M  V  K  S  Y  L  Q  Q  H  N  I  P
AAGCCGCCCA CCAGAAAGCC GTGGTGGAGA CACTGCTGCA GGAAGATCCC TGGCGGGTCG CCAAGATGGT CAAGAGCTAC CTGCAGCAGC ACAACATCCC
TTCGGCGGGT GGTCTTTCGG CACCACCTCT GTGACGACGT CCTTCTAGGG ACCGCCCAGC GGTTCTACCA GTTCTCGATG GACGTCGTCG TGTTGTAGGG

 Q  R  E  V  V  D  T  T  G  L  N  Q  S  H  L  S  Q  H  L  N  K  G  T  P  M  K  T  Q  K  R  A  A  L
CCAGCGGGAG GTGGTGGACA CCACCGGCCT GAACCAGAGC CACCTGAGCC AGCACCTGAA CAAGGGCACC CCCATGAAAA CCCAGAAGAG AGCCGCCCTG
GGTCGCCCTC CACCACCTGT GGTGGCCGGA CTTGGTCTCG GTGGACTCGG TCGTGGACTT GTTCCCGTGG GGGTACTTTT GGGTCTTCTC TCGGCGGGAC

 Y  T  W  Y  V  R  K  Q  R  E  V  A  Q  Q  F  T  H  A  G  Q  G  G  L  I  E  E  P  T  G  D  E  L  P  T
TACACTTGGT ACGTGCGGAA GCAGAGAGAG GTGGCCCAGC AGTTTACACA CGCCGGCCAG GGCGGCCTGA TCGAGGAACC TACCGGCGAC GAGCTGCCCA
ATGTGAACCA TGCACGCCTT CGTCTCTCTC CACCGGGTCG TCAAATGTGT GCGGCCGGTC CGCCGGACT AGCTCCTTGG ATGGCCGCTG CTCGACGGGT

 K  K  G  R  R  N  R  F  K  W  G  P  A  S  Q  Q  I  L  F  Q  A  Y  E  R  Q  K  N  P  S  K  E  E  R
CCAAGAAGGG CAGACGGAAC CGGTTTAAGT GGGGCCCTGC ATCTCAGCAG ATCCTGTTCC AGGCCTACGA GCGGCAGAAG AACCCCAGCA AAGAGGAACG
GGTTCTTCCC GTCTGCCTTG GCCAAATTCA CCCCGGGACG TAGAGTCGTC TAGGACAAGG TCCGGATGCT CGCCGTCTTC TTGGGGTCGT TTCTCCTTGC

 E  T  L  V  E  E  C  N  R  A  E  C  I  Q  R  G  V  S  P  S  Q  A  Q  G  L  G  S  N  L  V  T  E  V
GGAGACACTG GTGGAAGAGT GCAACCGGGC CGAGTGCATC CAGAGAGGCG TGAGCCCTTC TCAGGCTCAG GGCCTCGGCA GCAATCTGGT CACCGAAGTG
CCTCTGTGAC CACCTTCTCA CGTTGGCCCG GCTCACGTAG GTCTCTCCGC ACTCGGGAAG AGTCCGAGTC CCGGAGCCGT CGTTAGACCA GTGGCTTCAC

 R  V  Y  N  W  F  A  N  R  R  K  E  E  A  F  R  H  K  L  S  A  G  D  M  R  A  A  N  L  W  P  S  P  L
CGGGTGTACA ATTGGTTCGC CAACCGGCGG AAAGAGGAAG CCTTCCGGCA CAAGCTGTCT GCTGGCGATA TGAGAGCCGC CAACCTGTGG CCCAGCCCCC
GCCCACATGT TAACCAAGCG GTTGGCCGCC TTTCTCCTTC GGAAGGCCGT GTTCGACAGA CGACCGCTAT ACTCTCGGCG GTTGGACACC GGGTCGGGGG

 M  I  K  R  S  K  K  N  S  L  A  L  S  L  T  A  D  Q  M  V  S  A  L  L  D  A  E  P  P  I  L  Y  S
TGATGATCAA GCGGAGCAAG AAGAACAGCC TGGCCCTGAG CCTGACCGCC GATCAGATGG TGTCCGCTCT GCTGGACGCC GAGCCCCCTA TCCTGTACAG
ACTACTAGTT CGCCTCGTTC TTCTTGTCGG ACCGGGACTC GGACTGGCGG CTAGTCTACC ACAGGCGAGA CGACCTGCGG CTCGGGGGAT AGGACATGTC
```

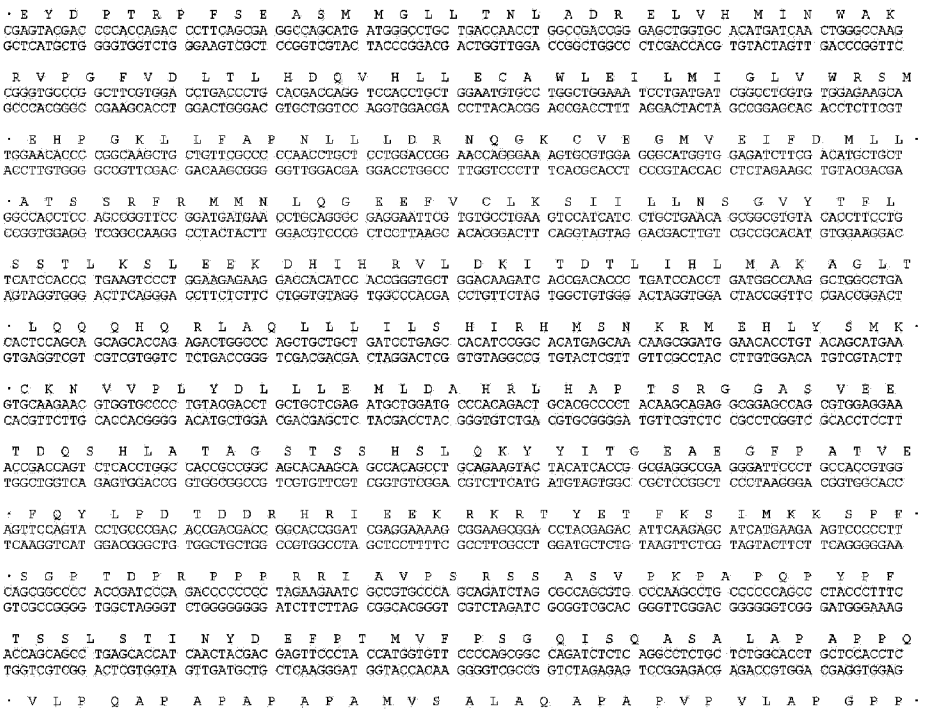


Table 11 TamR-tf (HEA4) nucleic acid (SEQ ID NO: 42) amino acid sequence (SEQ ID NO: 43)

HEA4 Nucleotide(SEQ ID NO: and Amino Acid Sequence



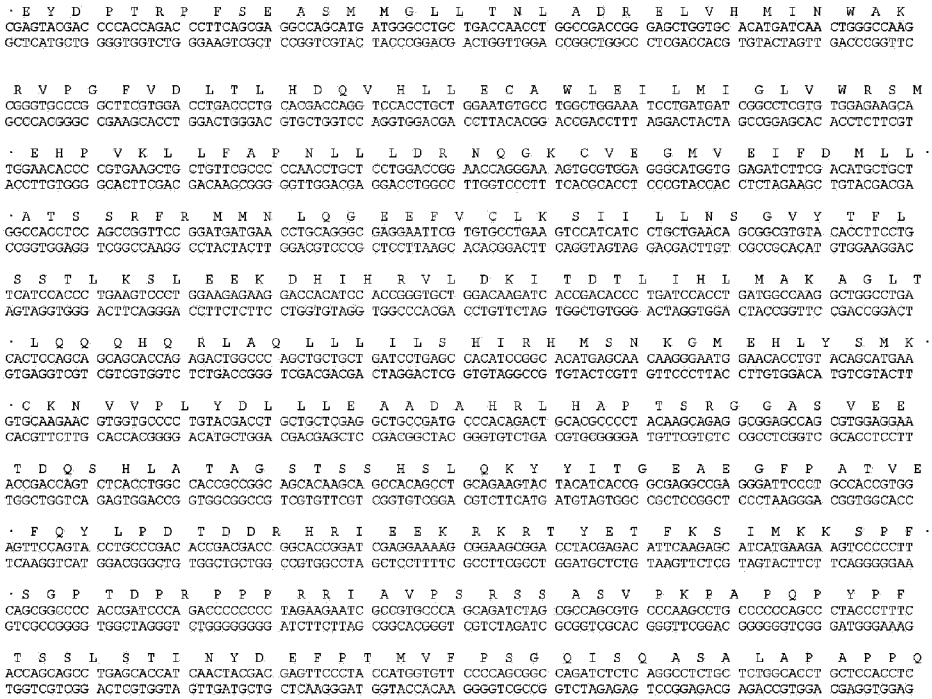

·E Y D P T R P F S E A S M M G L L T N L A D R E L V H M I N W A K
CGAGTACGAC CCCACCAGAC CCTTCAGCGA GGCCAGCATG ATGGGCCTGC TGACCAACCT GGCCGACCGG GAGCTGGTGC ACATGATCAA CTGGGCCAAG
GCTCATGCTG GGGTGGTCTG GGAAGTCGCT CCGGTCGTAC TACCCGGACG ACTGGTTGGA CCGGCTGGCC CTCGACCACG TGTACTAGTT GACCCGGTTC
R V P G F V D L T L H D Q V H L L E C A W L E I L M I G L V W R S M
CGGGTGCCCG GCTTCGTGGA CCTGACCCTG CACGACCAGG TCCACCTGCT GGAATGTGCC TGGCTGGAAA TCCTGATGAT CGGCCTCGTG TGGAGAAGCA
GCCCACGGGC CGAAGCACCT GGACTGGGAC GTGCTGGTCC AGGTGGACGA CCTTACACGG ACCGACCTTT AGGACTACTA GCCGGAGCAC ACCTCTTCGT
·E H P V K L L F A P N L L L D R N Q G K C V E G M V E I F D M L L·
TGGAACACCC CGTGAAGCTG CTGTTCGCCC CCAACCTGCT CCTGGACCGG AACCAGGGAA AGTGCGTGGA GGGCATGGTG GAGATCTTCG ACATGCTGCT
ACCTTGTGGG GCACTTCGAC GACAAGCGGG GGTTGGACGA GGACCTGGCC TTGGTCCCTT TCACGCACCT CCCGTACCAC CTCTAGAAGC TGTACGACGA
·A T S S R F R M M N L Q G E E F V C L K S I I L L N S G V Y T F L
GGCCACCTCC AGCCGGTTCC GGATGATGAA CCTGCAGGGC GAGGAATTCG TGTGCCTGAA GTCCATCATC CTGCTGAACA GCGGCGTGTA CACCTTCCTG
CCGGTGGAGG TCGGCCAAGG CCTACTACTT GGACGTCCCG CTCCTTAAGC ACACGGACTT CAGGTAGTAG GACGACTTGT CGCCGCACAT GTGGAAGGAC
S S T L K S L E E K D H I H R V L D K I T D T L I H L M A K A G L T
TCATCCACCC TGAAGTCCCT GGAAGAGAAG GACCACATCC ACCGGGTGCT GGACAAGATC ACCGACACCC TGATCCACCT GATGGCCAAG GCTGGCCTGA
AGTAGGTGGG ACTTCAGGGA CCTTCTCTTC CTGGTGTAGG TGGCCCACGA CCTGTTCTAG TGGCTGTGGG ACTAGGTGGA CTACCGGTTC CGACCGGACT
·L Q Q Q H Q R L A Q L L L I L S H I R H M S N K G M E H L Y S M K·
CACTCCAGCA GCAGCACCAG AGACTGGCCC AGCTGCTGCT GATCCTGAGC CACATCCGGC ACATGAGCAA CAAGGGAATG GAACACCTGT ACAGCATGAA
GTGAGGTCGT CGTCGTGGTC TCTGACCGGG TCGACGACGA CTAGGACTCG GTGTAGGCCG TGTACTCGTT GTTCCCTTAC CTTGTGGACA TGTCGTACTT
·C K N V V P L Y D L L L E A A D A H R L H A P T S R G G A S V E E
GTGCAAGAAC GTGGTGCCCC TGTACGACCT GCTGCTCGAG GCTGCCGATG CCCACAGACT GCACGCCCCT ACAAGCAGAG GCGGAGCCAG CGTGGAGGAA
CACGTTCTTG CACCACGGGG ACATGCTGGA CGACGAGCTC CGACGGCTAC GGGTGTCTGA CGTGCGGGGA TGTTCGTCTC CGCCTCGGTC GCACCTCCTT
T D Q S H L A T A G S T S S H S L Q K Y Y I T G E A E G F P A T V E
ACCGACCAGT CTCACCTGGC CACCGCCGGC AGCACAAGCA GCCACAGCCT GCAGAAGTAC TACATCACCG GCGAGGCCGA GGGATTCCCT GCCACCGTGG
TGGCTGGTCA GAGTGGACCG GTGGCGGCCG TCGTGTTCGT CGGTGTCGGA CGTCTTCATG ATGTAGTGGC CGCTCCGGCT CCCTAAGGGA CGGTGGCACC
·F Q Y L P D T D D R H R I E E K R K R T Y E T F K S I M K K S P F·
AGTTCCAGTA CCTGCCCGAC ACCGACGACC GGCACCGGAT CGAGGAAAAG CGGAAGCGGA CCTACGAGAC ATTCAAGAGC ATCATGAAGA AGTCCCCCTT
TCAAGGTCAT GGACGGGCTG TGGCTGCTGG CCGTGGCCTA GCTCCTTTTC GCCTTCGGCT GGATGCTCTG TAAGTTCTCG TAGTACTTCT TCAGGGGGAA
·S G P T D P R P P P R R I A V P S R S S A S V P K P A P Q P Y P F
CAGCGGCCCC ACCGATCCCA GACCCCCCCC TAGAAGAATC GCCGTGCCCA GCAGATCTAG CGCCAGCGTG CCCAAGCCTG CCCCCCAGCC CTACCCTTTC
GTCGCCGGGG TGGCTAGGGT CTGGGGGGGG ATCTTCTTAG CGGCACGGGT CGTCTAGATC GCGGTCGCAC GGGTTCGGAC GGGGGGTCGG GATGGGAAAG
T S S L S T I N Y D E F P T M V F P S G Q I S Q A S A L A P A P P Q
ACCAGCAGCC TGAGCACCAT CAACTACGAC GAGTTCCCTA CCATGGTGTT CCCCAGCGGC CAGATCTCTC AGGCCTCTGC TCTGGCACCT GCTCCACCTC
TGGTCGTCGG ACTCGTGGTA GTTGATGCTG CTCAAGGGAT GGTACCACAA GGGGTCGCCG GTCTAGAGAG TCCGGAGACG AGACCGTGGA CGAGGTGGAG



Table 12 7xHBD/mEF1α nucleic acid sequence (SEQ ID NO: 41)

Tagttaataatctacaatagttaataatctacaatagttaataatctacaatagttaataatctacaatagttaataatctacaatagttaataatctacaat agttaataatctacaa

Table 13 Her2t nucleic acid (SEQ ID NO: 44) and amino acid sequence (SEQ ID NO: 45)

HER2t(CHP) Nucleotide and Amino Acid Sequence

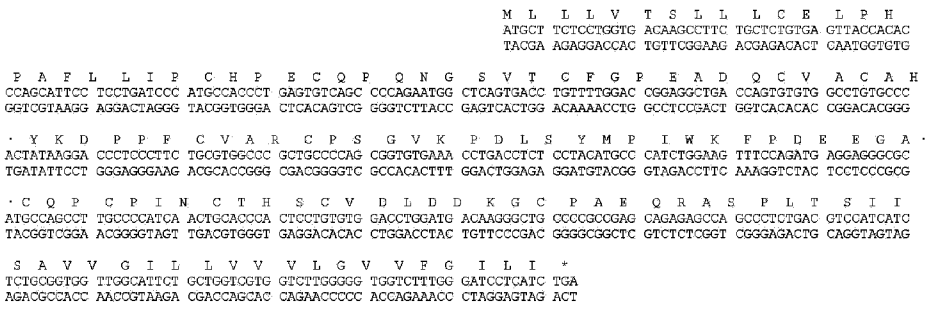


Table 14 DHFRdm nucleic acid (SEQ ID NO: 46) and amino acid (SEQ ID NO: 47)

DHFRdm Nucleotide and Amino Acid Sequence

```
M  V  G  S  L  N ·
ATGGTTG GTTCGCTAAA
TACCAAC CAAGCGATTT

·C  I  V  A  V  S  Q  N  M  G  I  G  K  N  G  D  F  P  W  P  P  L  R  N  E  S  R  Y  F  Q  R  M  T
CTGCATCGTC GCTGTGTCCC AGAACATGGG CATCGGCAAG AACGGGGACT TCCCCTGGCC ACCGCTCAGG AATGAATCCA GATATTTCCA GAGAATGACC
GACGTAGCAG CGACACAGGG TCTTGTACCC GTAGCCGTTC TTGCCCCTGA AGGGGACCGG TGGCGAGTCC TTACTTAGGT CTATAAAGGT CTCTTACTGG

 T  T  S  S  V  E  G  K  Q  N  L  V  I  M  G  K  K  T  W  F  S  I  P  E  K  N  R  P  L  K  G  R  I  N
ACAACCTCTT CAGTAGAAGG TAAACAGAAT CTGGTGATTA TGGGTAAGAA GACCTGGTTC TCCATTCCTG AGAAGAATCG ACCTTTAAAG GGTAGAATTA
TGTTGGAGAA GTCATCTTCC ATTTGTCTTA GACCACTAAT ACCCATTCTT CTGGACCAAG AGGTAAGGAC TCTTCTTAGC TGGAAATTTC CCATCTTAAT

· L  V  L  S  R  E  L  K  E  P  P  Q  G  A  H  F  L  S  R  S  L  D  D  A  L  K  L  T  E  Q  P  E  L ·
ATTTAGTTCT CAGCAGAGAA CTCAAGGAAC CTCCACAAGG AGCTCATTTT CTTTCCAGAA GTCTAGATGA TGCCTTAAAA CTTACTGAAC AACCAGAATT
TAAATCAAGA GTCGTCTCTT GAGTTCCTTG GAGGTGTTCC TCGAGTAAAA GAAAGGTCTT CAGATCTACT ACGGAATTTT GAATGACTTG TTGGTCTTAA

·A  N  K  V  D  M  V  W  I  V  G  G  S  S  V  Y  K  E  A  M  N  H  P  G  H  L  K  L  F  V  T  R  I
AGCAAATAAA GTAGACATGG TCTGGATAGT TGGTGGCAGT TCTGTTTATA AGGAAGCCAT GAATCACCCA GGCCATCTTA AACTATTTGT GACAAGGATC
TCGTTTATTT CATCTGTACC AGACCTATCA ACCACCGTCA AGACAAATAT TCCTTCGGTA CTTAGTGGGT CCGGTAGAAT TTGATAAACA CTGTTCCTAG

 M  Q  D  F  E  S  D  T  F  F  P  E  I  D  L  E  K  Y  K  L  L  P  E  Y  P  G  V  L  S  D  V  Q  E  E
ATGCAAGACT TTGAAAGTGA CACGTTTTTT CCAGAAATTG ATTTGGAGAA ATATAAACTT CTGCCAGAAT ACCCAGGTGT TCTCTCTGAT GTCCAGGAGG
TACGTTCTGA AACTTTCACT GTGCAAAAAA GGTCTTTAAC TAAACCTCTT TATATTTGAA GACGGTCTTA TGGGTCCACA AGAGAGACTA CAGGTCCTCC

· K  G  I  K  Y  K  F  E  V  Y  E  K  N  D
AGAAAGGCAT TAAGTACAAA TTTGAAGTAT ATGAGAAGAA TGAT
TCTTTCCGTA ATTCATGTTT AAACTTCATA TACTCTTCTT ACTA
```

2015243882 17 Jun 2020

CLAIMS:

1. A system for inducible expression of a polynucleotide comprising:

a) a first nucleic acid comprising a first promoter inducible by tamoxifen, a metabolite of tamoxifen, or an analog of tamoxifen, wherein the first promoter comprises the nucleotide sequence of SEQ ID NO: 41, and wherein the first promoter is operably linked to a polynucleotide encoding a chimeric antigen receptor (CAR), wherein the CAR comprises:

(i) a ligand binding domain specific for a ligand, wherein the ligand is a tumor specific molecule, a viral molecule, or a molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte when interacting with the ligand binding domain,

(ii) a polypeptide spacer,

(iii) a transmembrane domain, and

(iv) an intracellular signaling domain;

b) a second nucleic acid comprising a constitutive second promoter, wherein the second promoter is operably linked to a nucleic acid encoding a transcriptional activator for the first promoter and comprises the amino acid sequence of SEQ ID NO: 40;

c) tamoxifen, a metabolite of tamoxifen, or an analog of tamoxifen; and

d) an activating stimulator comprising an anti-CD3 and/or an anti-CD28.

2. The system of claim 1, wherein the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector.

3. The system of claim 2, wherein the first and second vectors are packaged in a viral vector, such as a lentivirus.

4. The system of any one of claims 1-3, wherein the first nucleic acid or the second nucleic acid further comprises a nucleotide sequence encoding a selectable marker.

5. The system of any one of claims 1-4, wherein the first promoter is in an opposite orientation to the second promoter.

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6. The system of any one of claims 1-5, wherein the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR.

7. The system of any one of claims 1-6, wherein the spacer comprises a spacer length optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor.

8. The system of any one of claims 1-7, further comprising a host cell comprising the first nucleic acid and the second nucleic acid.

9. The system of claim 8, wherein the host cell is selected from the group consisting of (i) a CD8+ cytotoxic T lymphocyte cell selected from the group consisting of a naïve CD8+ T cell, a central memory CD8+ T cell, an effector memory CD8+ T cell, and a bulk CD8+ T cell, (ii) a CD4+ helper T lymphocyte cell selected from the group consisting of a naïve CD4+ T cell, a central memory CD4+ T cell, an effector memory CD4+ T cell, and a bulk CD4+ T cell; (iii) a precursor T cell; and (iv) a hematopoietic stem cell.

10. An *in vitro* method for preparing the system of any one of claims 1-7, comprising:

a) providing the system of any one of claims 1-7;

b) introducing the first nucleic acid and the second nucleic acid into a separate isolated T lymphocyte population *in vitro*;

c) expanding the T lymphocyte population *in vitro*; and

d) culturing the T lymphocyte population in the presence of (i) the tamoxifen, the metabolite of tamoxifen, or the analog of tamoxifen, (ii) the activating stimulator comprising an anti-CD3 and/or an anti-CD28, and (iii) at least one homeostatic cytokine such that the T lymphocyte population expands sufficiently for use as a cell infusion.

11. The method of claim 10, wherein the T lymphocyte population comprises a cell selected from the group consisting of: (i) a CD8+ cytotoxic T lymphocyte cell selected from the group consisting of a naïve CD8+ T cell, a central memory CD8+ T cell, an effector memory CD8+ T cell, and a bulk CD8+ T cell; (ii) a CD4+ helper T lymphocyte cell selected from the group consisting of a naïve CD4+ T cell, a central memory CD4+ T cell, an effector memory CD4+ T cell, and a bulk CD4+ T cell; (iii) a precursor T cell; and (iv) a hematopoietic stem cell.

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12. An isolated host cell when used in the treatment of a cancer or a viral infection, wherein the host cell comprises the system of any one of claims 1-7.

13. The host cell according to claim 12, wherein the cancer is a solid tumor or a hematologic malignancy.

14. The host cell when used according to claim 13, wherein the solid tumor is selected from the group consisting of a breast cancer, a brain cancer, a lung cancer, a colon cancer, a renal cancer, a pancreatic cancer, a prostate cancer, and an ovarian cancer.

15. A method of performing cellular immunotherapy in a subject having a cancer or a viral infection, comprising administering the system of claim 8 or 9 to the subject.

16. The method of claim 15, wherein the cancer is a solid tumor or a hematologic malignancy.

17. The method of claim 16, wherein the solid tumor is selected from the group consisting of a breast cancer, a brain cancer, a lung cancer, a colon cancer, a renal cancer, a pancreatic cancer, a prostate cancer, and an ovarian cancer.

SEATTLE CHILDREN'S HOSPITAL
(DBA SEATTLE CHILDREN'S RESEARCH INSTITUTE)

WATERMARK INTELLECTUAL PROPERTY PTY LTD
P42276AU00

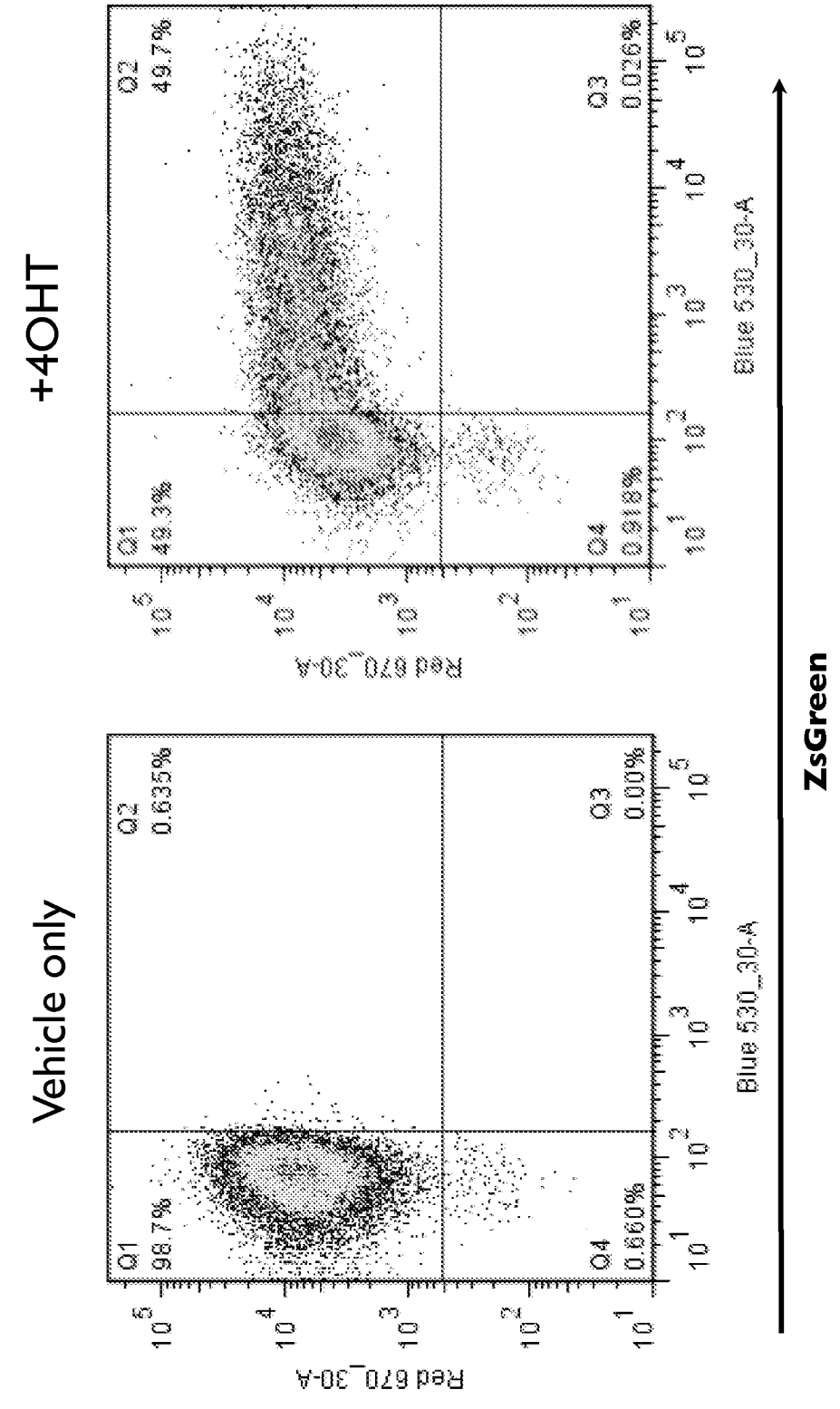

+4OHT
Vehicle only
Q1
49.3%
Q2
49.7%
Q3
0.0026%
Q4
0.918%
Q1
98.7%
Q2
0.635%
Q3
0.00%
Q4
0.660%
Red 670_30-A
Blue 530_30-A
EGFRt
ZsGreen

Fig 1A

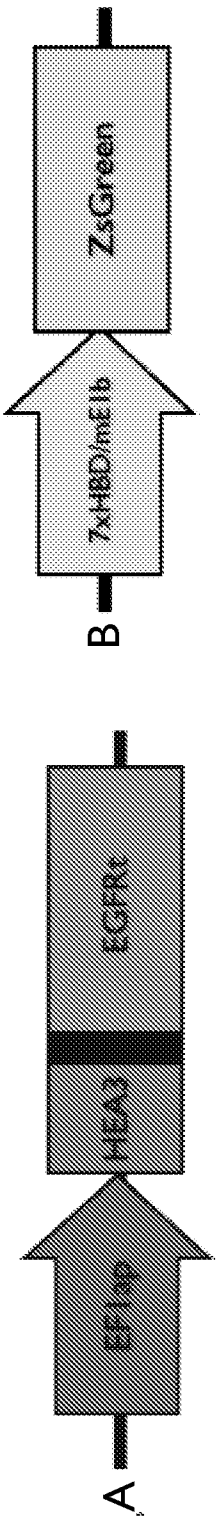

A
EF1αp
HEA3
EGFRt
B
7xHBD/mE1b
ZsGreen

Fig 1B

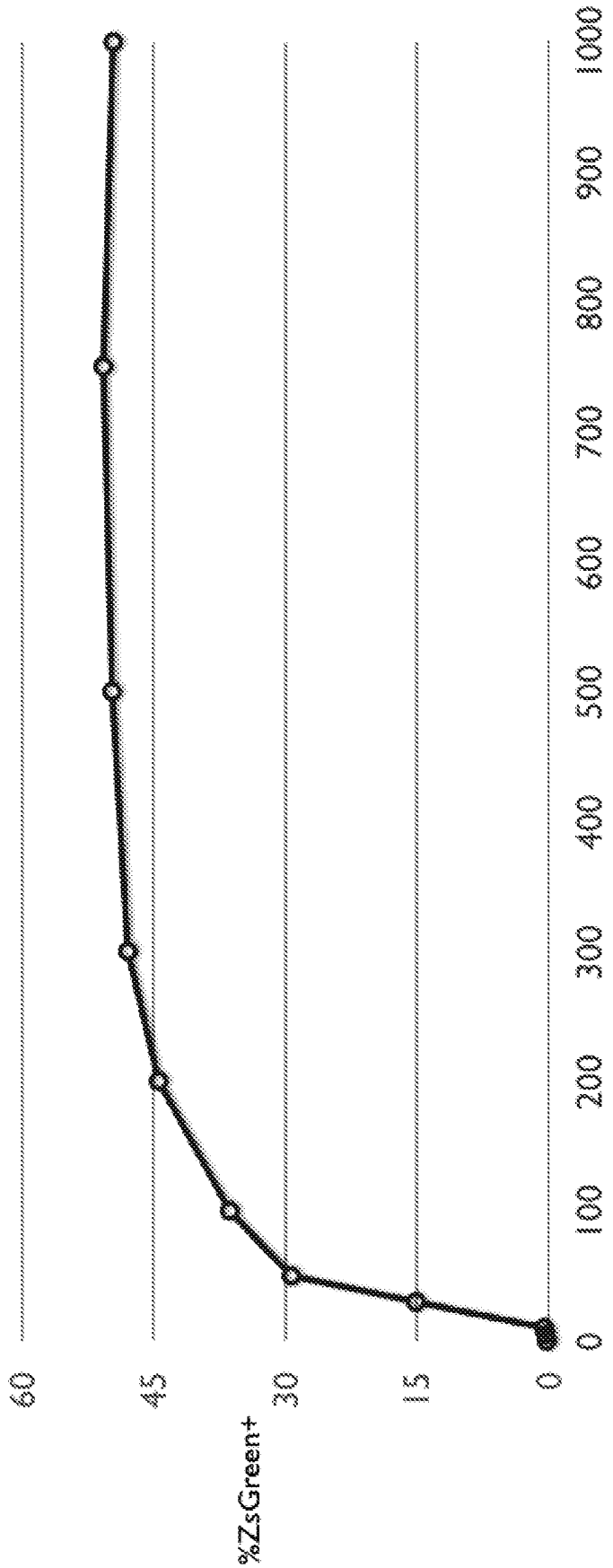

%ZsGreen+
60
45
30
15
0
0
100
200
300
400
500
600
700
800
900
1000
Concentration of 4OHT (nM)

Fig 2

Fig 3

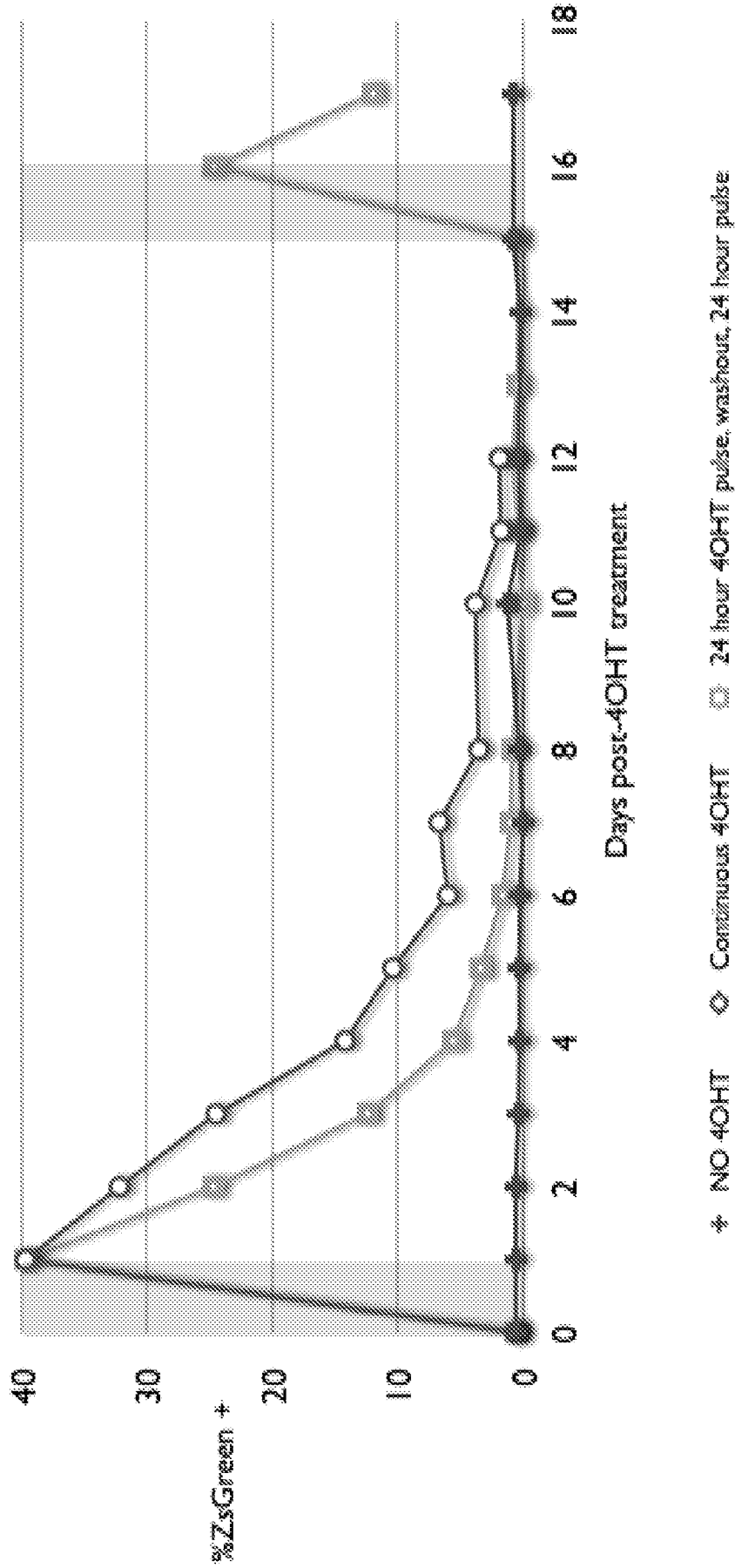
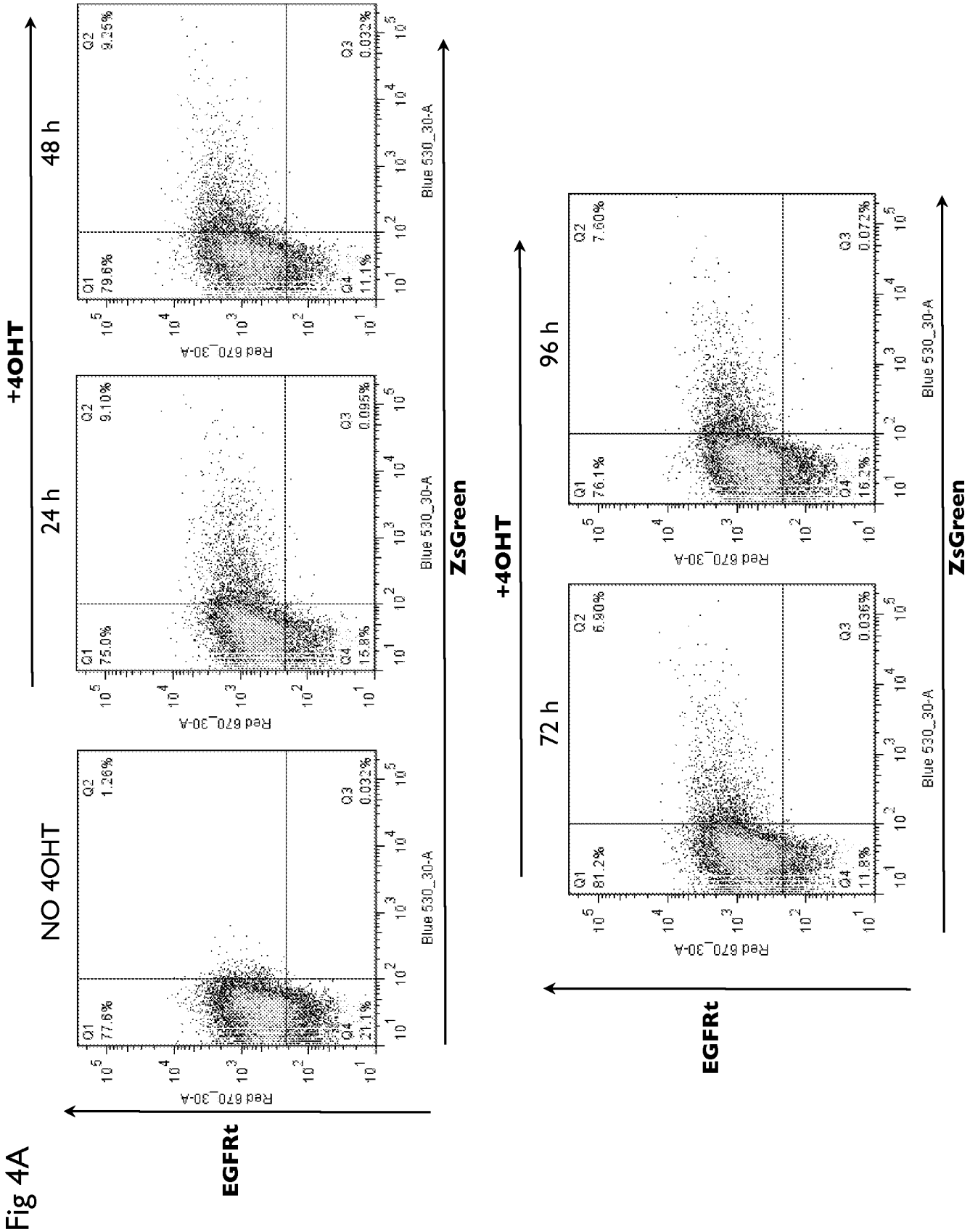

%ZsGreen +
0
10
20
30
40
0
2
4
6
8
10
12
14
16
18
Days post-4OHT treatment
NO 4OHT
Continuous 4OHT
24 hour 4OHT pulse, washout, 24 hour pulse

Fig 4A


NO 4OHT
+4OHT
24 h
48 h
72 h
96 h
EGFRt
ZsGreen
Red 670_30-A
Blue 530_30-A
Q1 77.6%
Q2 1.36%
Q3 0.032%
Q4 21.1%
Q1 75.0%
Q2 9.10%
Q3 0.095%
Q4 15.8%
Q1 79.6%
Q2 9.25%
Q3 0.032%
Q4 11.1%
Q1 81.2%
Q2 6.90%
Q3 0.036%
Q4 11.8%
Q1 76.1%
Q2 7.60%
Q3 0.072%
Q4 16.2%

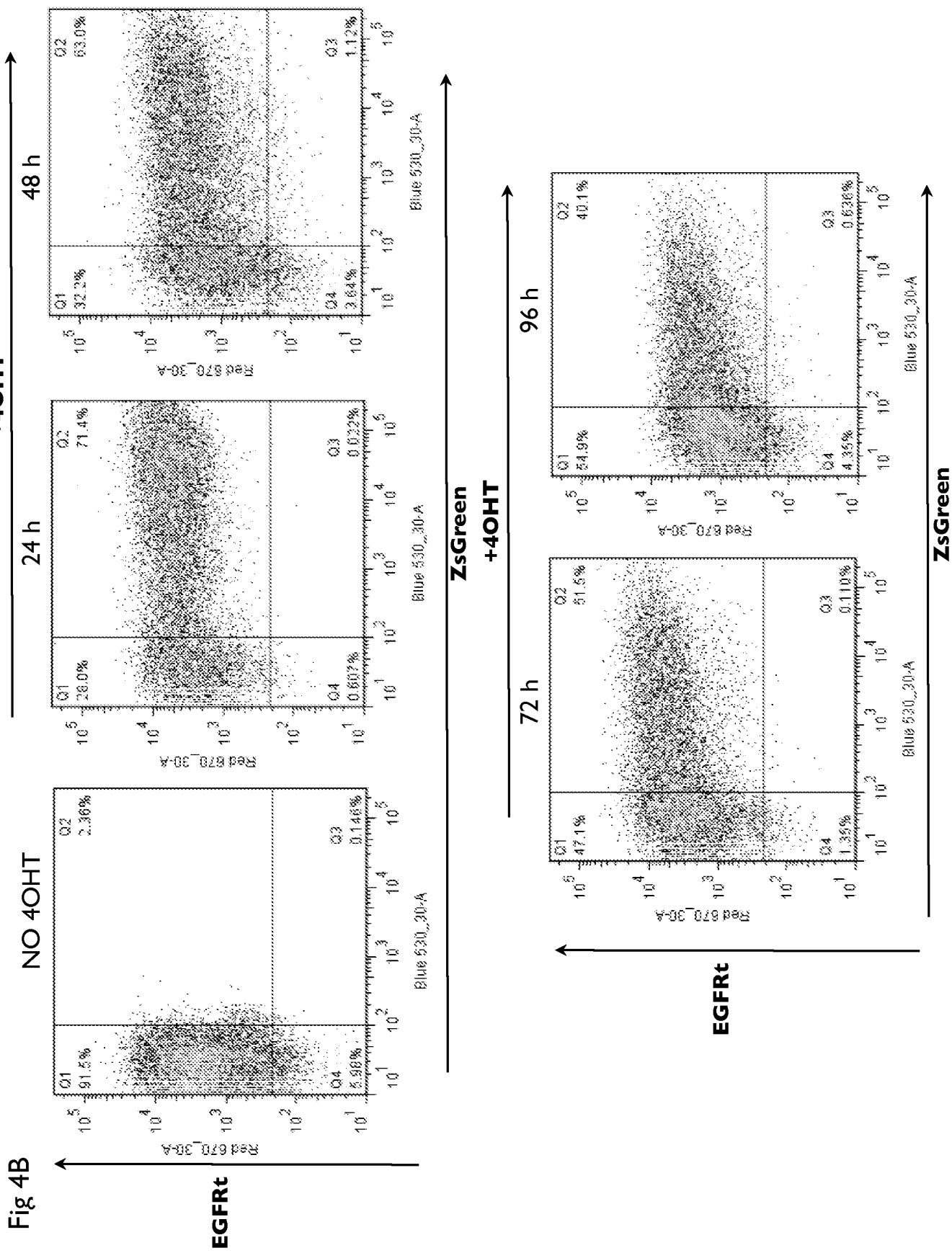
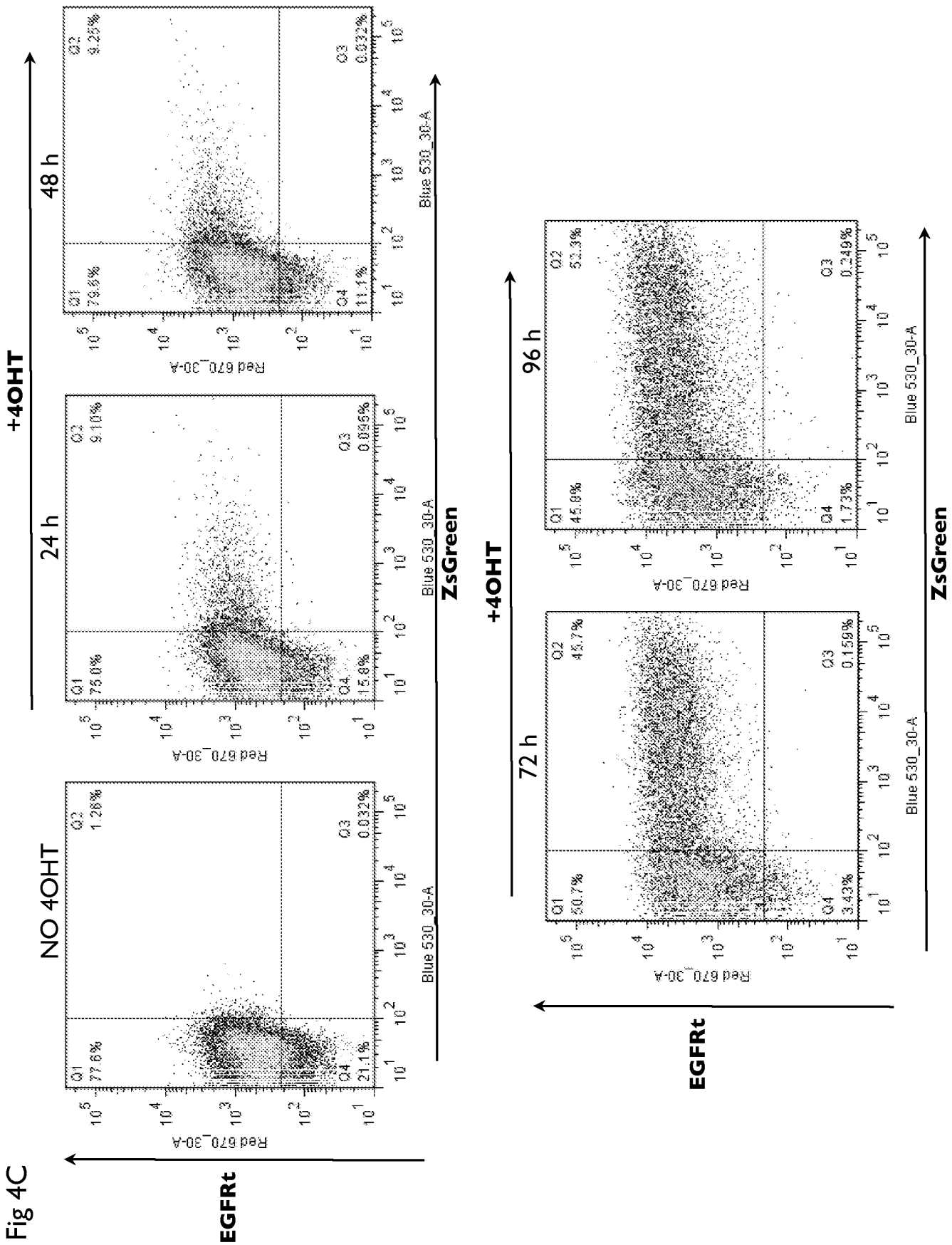

Fig 4B
NO 4OHT
+4OHT
24 h
48 h
72 h
96 h
EGFRt
ZsGreen
Red 670_30-A
Blue 530_30-A
Q1 91.5%
Q2 2.36%
Q3 0.146%
Q4 5.98%
Q1 28.0%
Q2 71.4%
Q3 0.0224%
Q4 0.607%
Q1 32.2%
Q2 63.0%
Q3 1.12%
Q4 3.64%
Q1 47.1%
Q2 51.5%
Q3 0.110%
Q4 1.35%
Q1 54.9%
Q2 40.1%
Q3 0.636%
Q4 4.35%

Fig 4C


NO 4OHT
+4OHT
24 h
48 h
72 h
96 h
EGFRt
ZsGreen
Red 670_30-A
Blue 530_30-A
Q1 77.6%
Q2 1.26%
Q3 0.0332%
Q4 21.1%
Q1 75.0%
Q2 9.10%
Q3 0.0959%
Q4 15.8%
Q1 79.6%
Q2 9.23%
Q3 0.0322%
Q4 11.1%
Q1 50.7%
Q2 45.7%
Q3 0.159%
Q4 3.43%
Q1 45.8%
Q2 52.3%
Q3 0.249%
Q4 1.73%

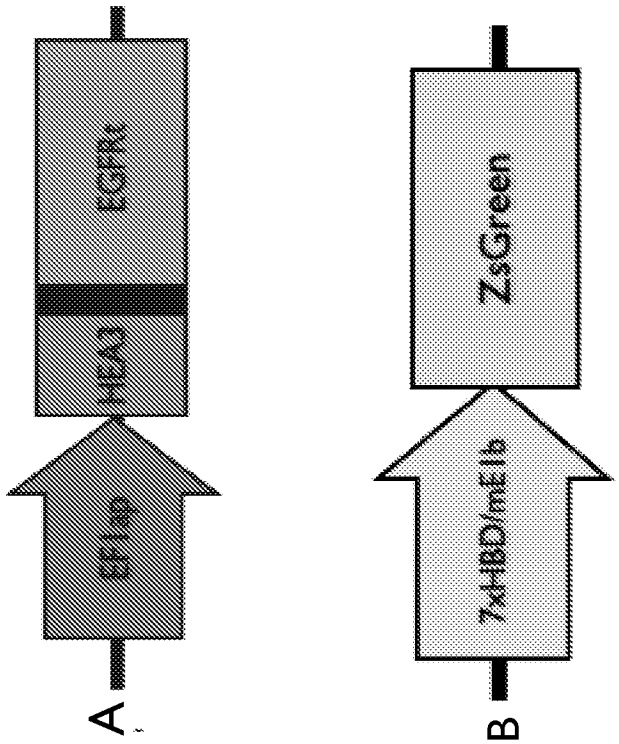


Fig 4D

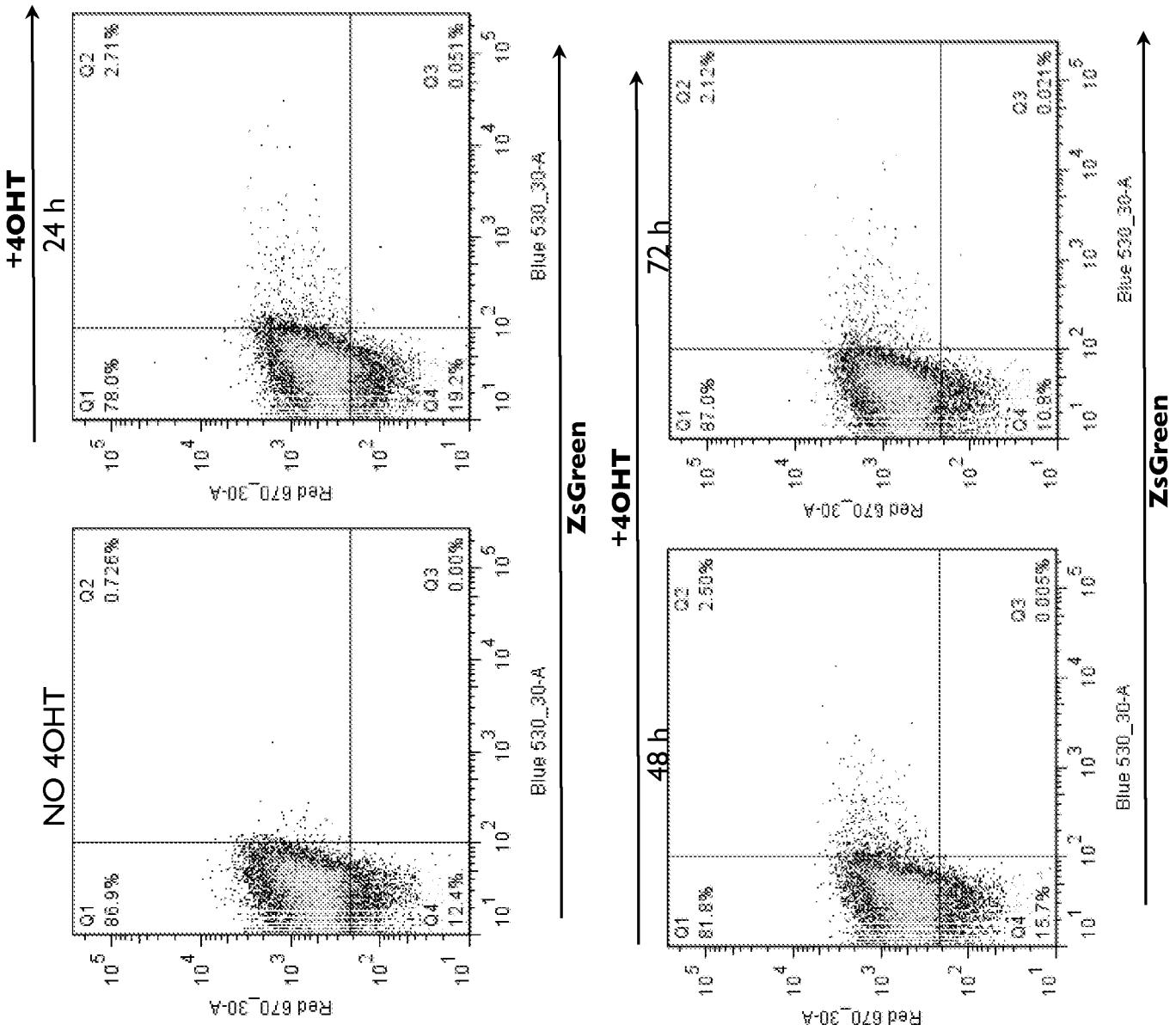

+4OHT
24 h
NO 4OHT
+4OHT
48 h
72 h
Red 670_30-A
Blue 530_30-A
ZsGreen
Q1
Q2
Q3
Q4

Fig 5A

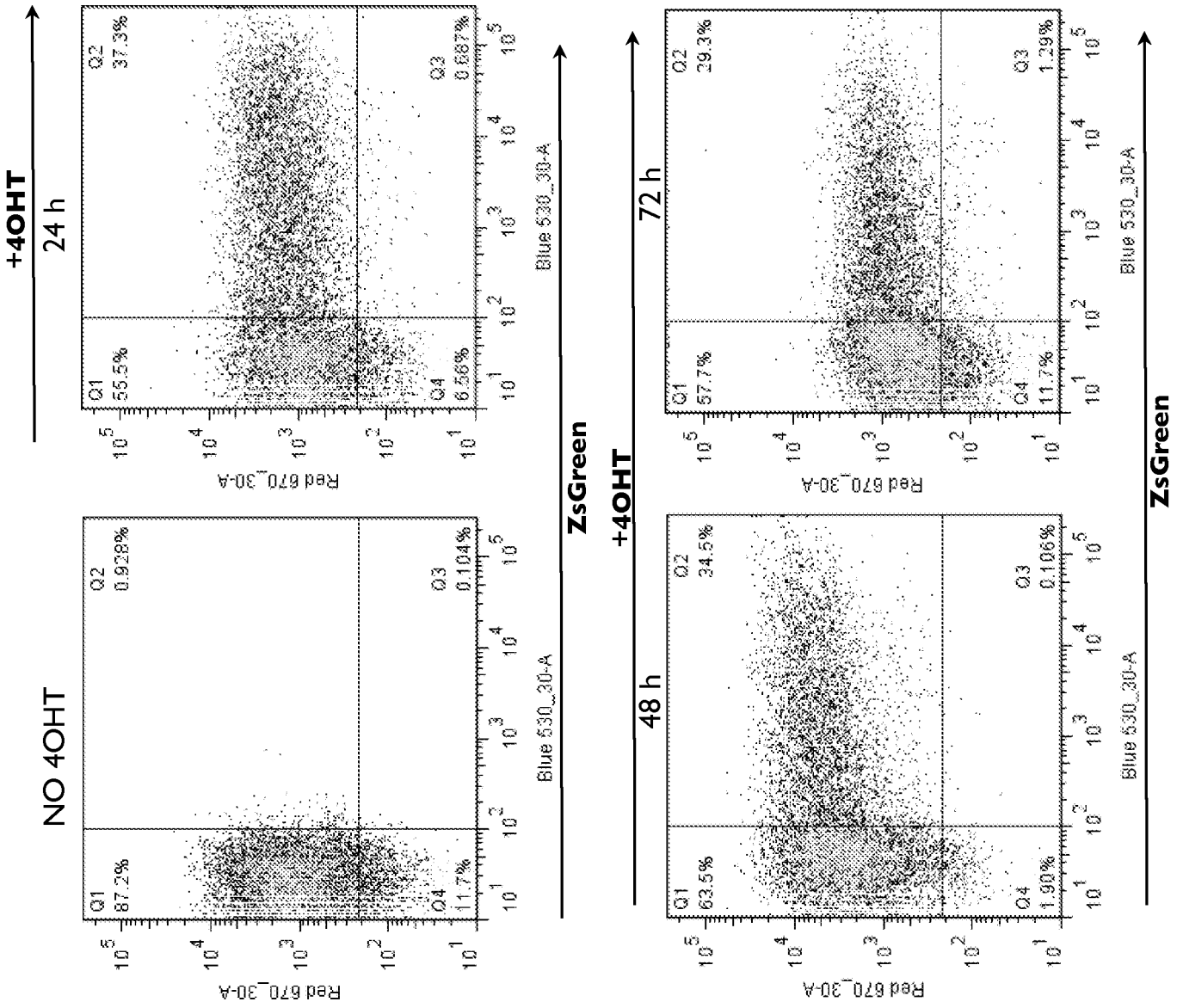

+4OHT
24 h
NO 4OHT
+4OHT
48 h
72 h
Red 670_30-A
Blue 530_30-A
ZsGreen
Q1 87.2%
Q2 0.928%
Q3 0.104%
Q4 11.7%
Q1 55.5%
Q2 37.3%
Q3 0.687%
Q4 6.56%
Q1 63.5%
Q2 34.6%
Q3 0.106%
Q4 1.90%
Q1 57.7%
Q2 29.3%
Q3 1.29%
Q4 11.7%

Fig 5B

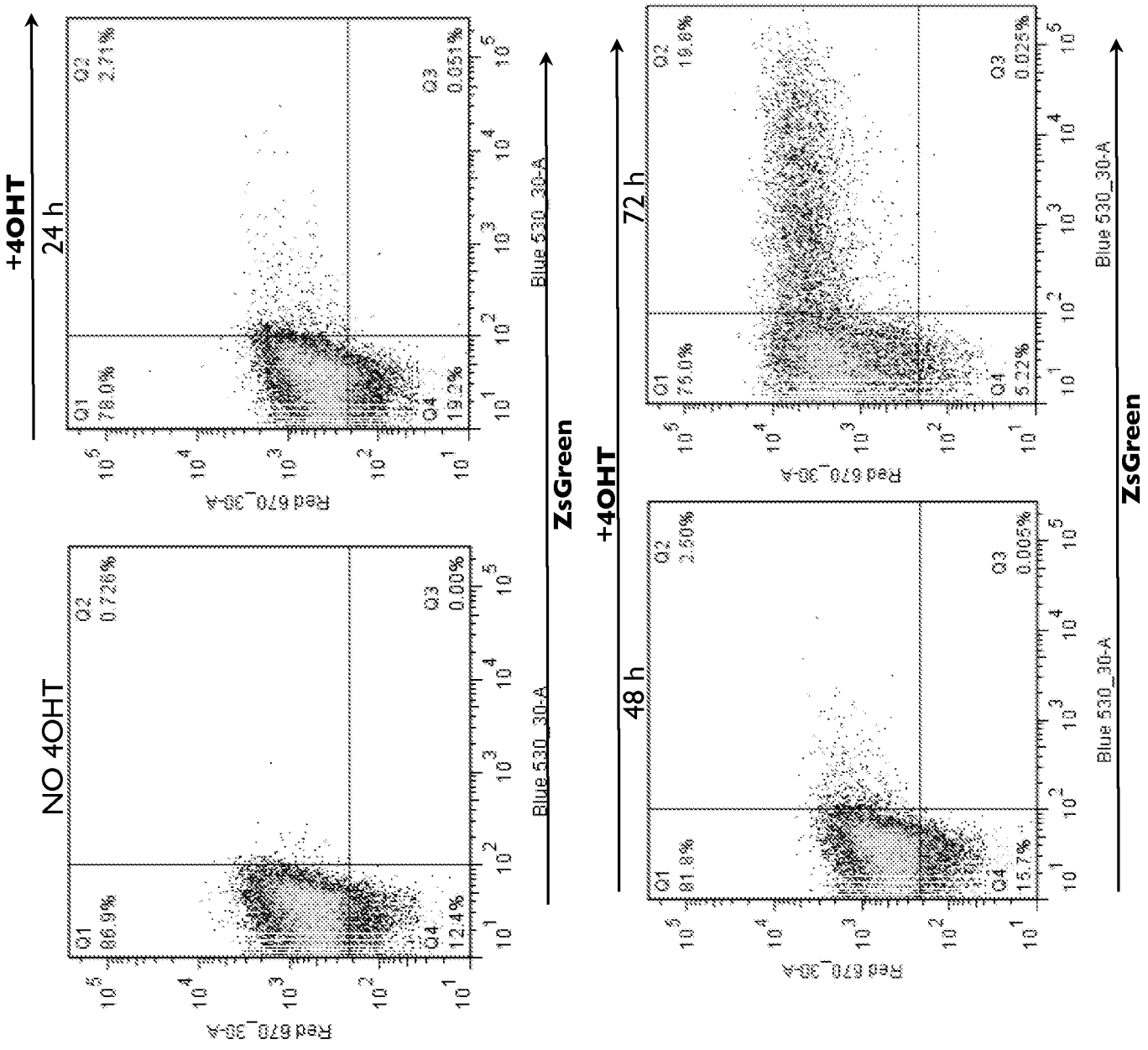

NO 4OHT
+4OHT
24 h
48 h
72 h
Q1 86.9%
Q2 0.726%
Q3 0.00%
Q4 12.4%
Q1 78.0%
Q2 2.71%
Q3 0.051%
Q4 19.2%
Q1 81.8%
Q2 2.50%
Q3 0.0065%
Q4 15.7%
Q1 75.0%
Q2 19.6%
Q3 0.025%
Q4 5.22%
Red 670_30-A
Blue 530_30-A
ZsGreen

Fig 5C

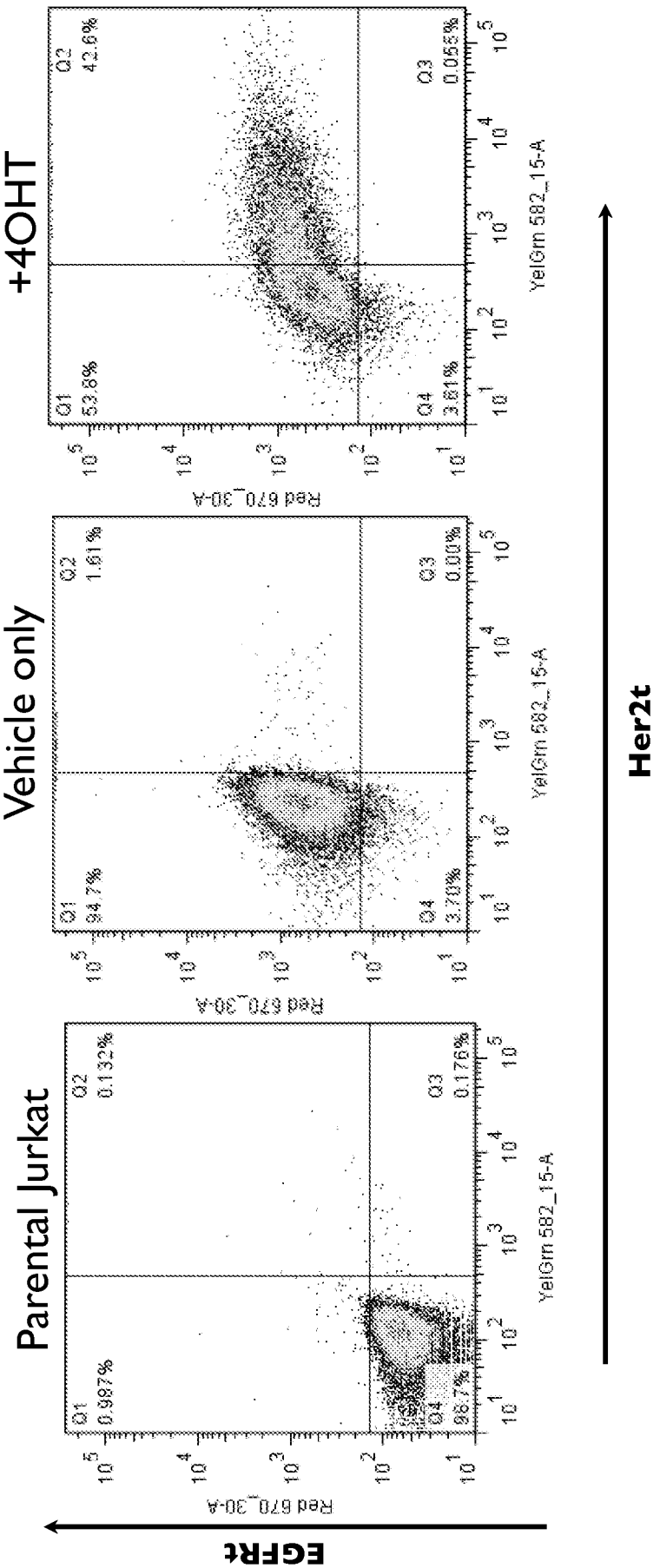

+4OHT
Vehicle only
Parental Jurkat
Q1 53.8%
Q2 42.6%
Q3 0.055%
Q4 3.61%
Q1 94.7%
Q2 1.61%
Q3 0.00%
Q4 3.70%
Q1 0.987%
Q2 0.132%
Q3 0.176%
Q4 98.7%
Red 670_30-A
YelGrn 582_15-A
EGFRt
Her2t

Fig 6A

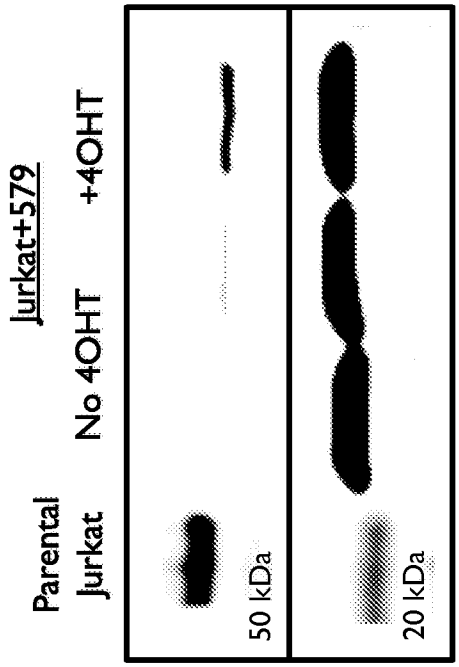

Jurkat+579
Parental Jurkat
No 4OHT
+4OHT
50 kDa
20 kDa

Fig 6B

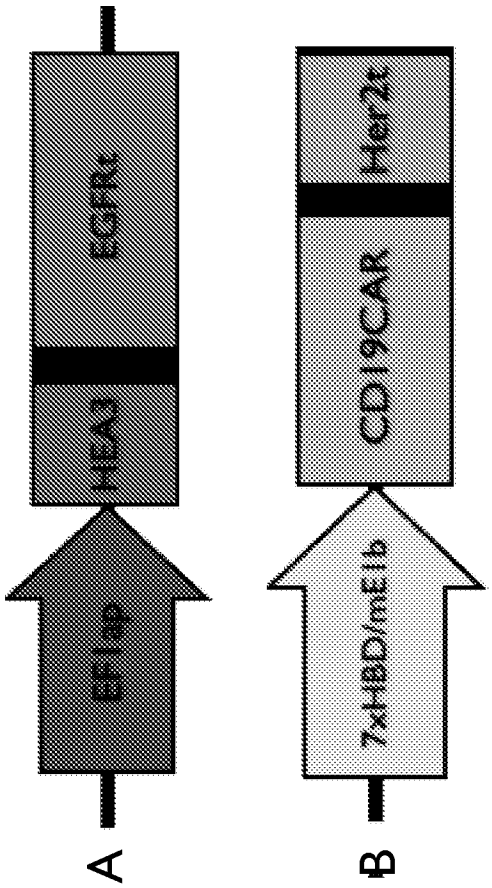

A
EF1ap
HEA3
EGFRt
B
7xHBD/mE1b
CD19CAR
Her2t

Fig 6C

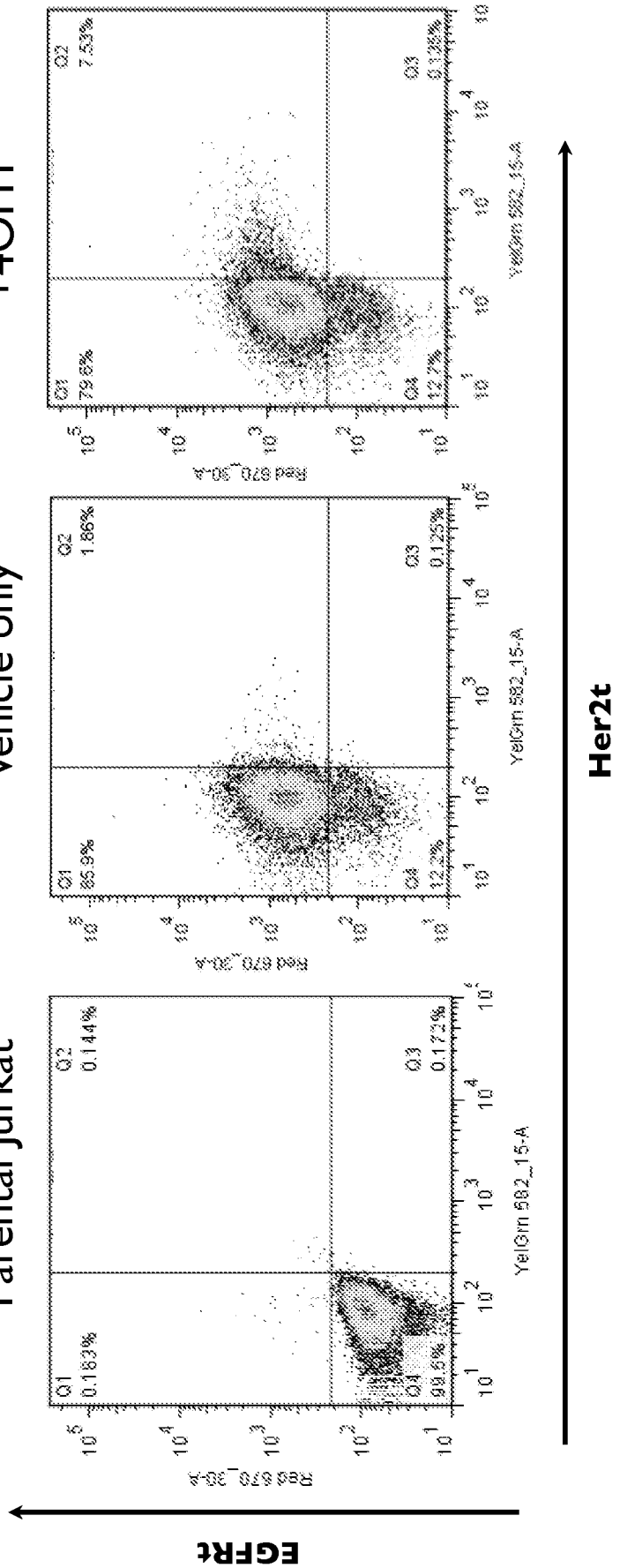

Parental Jurkat
Vehicle only
+4OHT
EGFRt
Her2t
Red 670_30-A
YelGrn 582_15-A
Q1
Q2
Q3
Q4
0.183%
0.144%
0.172%
99.6%
85.9%
1.86%
0.125%
12.2%
79.6%
7.53%
0.128%
12.7%

Fig 7A

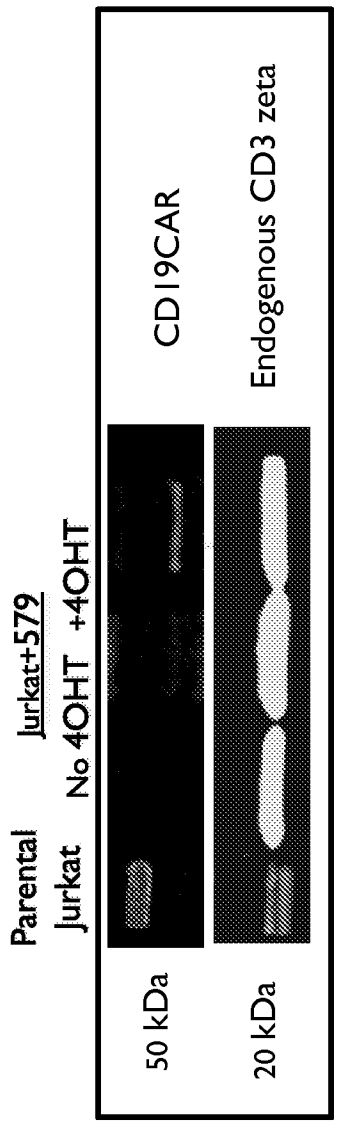

Parental Jurkat
Jurkat+579
No 4OHT +4OHT
50 kDa
20 kDa
CD19CAR
Endogenous CD3 zeta

Fig 7B

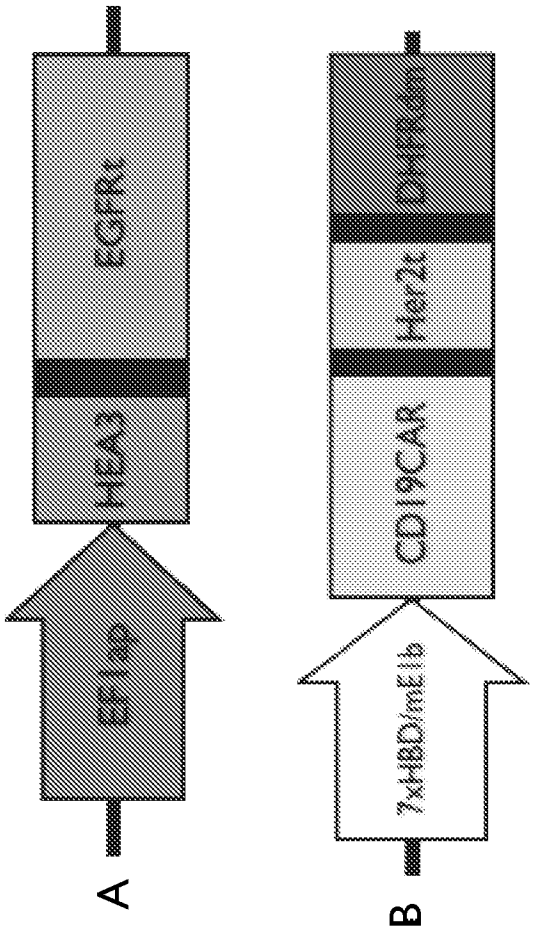

A
EF1αp
HEA3
EGFRt
B
7xHBD/mE1b
CD19CAR
Her2t

Fig 7C

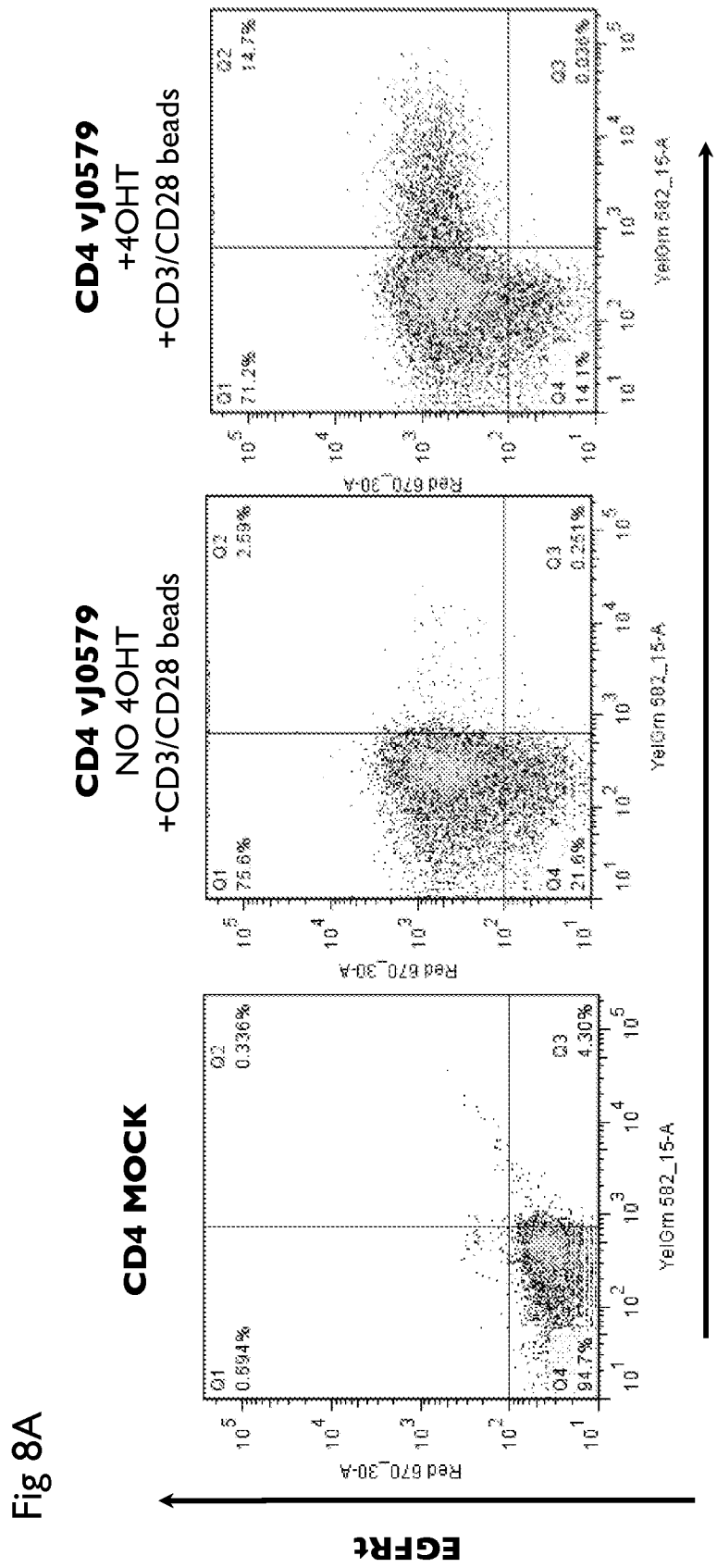

CD4 MOCK
CD4 vJ0579
NO 4OHT
+CD3/CD28 beads
CD4 vJ0579
+4OHT
+CD3/CD28 beads
EGFRt
Her2t
Red 670_30-A
YelOrn 582_15-A
Q1 0.694%
Q2 0.336%
Q3 4.30%
Q4 94.7%
Q1 75.6%
Q2 2.59%
Q3 0.251%
Q4 21.6%
Q1 71.2%
Q2 14.7%
Q3 0.836%
Q4 14.1%

Fig 8A

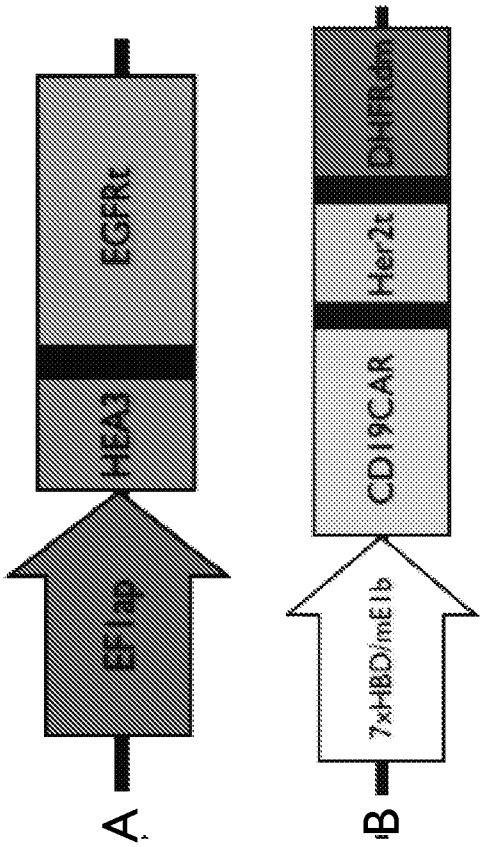


Fig 8B

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dba Seattle Children's Research Institute
Jensen, Michael C.

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cccgagggct gctggggccc ggagcccagg gactgcgtct cttgccggaa tgtcagccga 660
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gtcaagacct gcccggcagg agtcatggga gaaaacaaca ccctggtctg gaagtacgca 900
gacgccggcc atgtgtgcca cctgtgccat ccaaactgca cctacggatg cactgggcca 960
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<210> 10
<211> 2529
<212> DNA
<213> Artificial Sequence

<220>
<223> Protein that has domains linked as such GMCSFRss -
CD19scFv - IgG4hinge - CD28tm - 41BB - CD3Zeta -
T2A - EGFRt

<400> 10
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ggcgagggca gcaccaaggg cgaggtgaag ctgcaggaaa gcggccctgg cctggtggcc 480
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<210> 11
<211> 842

<212> PRT
<213> Artificial Sequence

<220>
<223> GMCSFRss - CD19scFv - IgG4hinge - CD28tm - 41BB
- CD3Zeta - T2A - EGFRt

<400> 11

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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
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 Glu Ser Lys Tyr Gly
            260                 265                 270
Pro Pro Cys Pro Pro Cys Pro Met Phe Trp Val Leu Val Val Val Gly
        275                 280                 285
Gly Val Leu Ala Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile Ile
    290                 295                 300
Phe Trp Val Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln
305                 310                 315                 320
Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser
                325                 330                 335
Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys
            340                 345                 350
Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln
        355                 360                 365
Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu
    370                 375                 380
Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg
385                 390                 395                 400
Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met
                405                 410                 415
Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly
            420                 425                 430
Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp
        435                 440                 445
Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg Leu Glu Gly
    450                 455                 460
Gly Gly Glu Gly Arg Gly Ser Leu Leu Thr Cys Gly Asp Val Glu Glu
465                 470                 475                 480
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Asn Pro Gly Pro Arg Met Leu Leu Leu Val Thr Ser Leu Leu Leu Cys
                485                 490                 495
Glu Leu Pro His Pro Ala Phe Leu Leu Ile Pro Arg Lys Val Cys Asn
            500                 505                 510
Gly Ile Gly Ile Gly Glu Phe Lys Asp Ser Leu Ser Ile Asn Ala Thr
        515                 520                 525
Asn Ile Lys His Phe Lys Asn Cys Thr Ser Ile Ser Gly Asp Leu His
    530                 535                 540
Ile Leu Pro Val Ala Phe Arg Gly Asp Ser Phe Thr His Thr Pro Pro
545                 550                 555                 560
Leu Asp Pro Gln Glu Leu Asp Ile Leu Lys Thr Val Lys Glu Ile Thr
                565                 570                 575
Gly Phe Leu Leu Ile Gln Ala Trp Pro Glu Asn Arg Thr Asp Leu His
            580                 585                 590
Ala Phe Glu Asn Leu Glu Ile Ile Arg Gly Arg Thr Lys Gln His Gly
        595                 600                 605
Gln Phe Ser Leu Ala Val Val Ser Leu Asn Ile Thr Ser Leu Gly Leu
    610                 615                 620
Arg Ser Leu Lys Glu Ile Ser Asp Gly Asp Val Ile Ile Ser Gly Asn
625                 630                 635                 640
Lys Asn Leu Cys Tyr Ala Asn Thr Ile Asn Trp Lys Lys Leu Phe Gly
                645                 650                 655
Thr Ser Gly Gln Lys Thr Lys Ile Ile Ser Asn Arg Gly Glu Asn Ser
            660                 665                 670
Cys Lys Ala Thr Gly Gln Val Cys His Ala Leu Cys Ser Pro Glu Gly
        675                 680                 685
Cys Trp Gly Pro Glu Pro Arg Asp Cys Val Ser Cys Arg Asn Val Ser
    690                 695                 700
Arg Gly Arg Glu Cys Val Asp Lys Cys Asn Leu Leu Glu Gly Glu Pro
705                 710                 715                 720
Arg Glu Phe Val Glu Asn Ser Glu Cys Ile Gln Cys His Pro Glu Cys
                725                 730                 735
Leu Pro Gln Ala Met Asn Ile Thr Cys Thr Gly Arg Gly Pro Asp Asn
            740                 745                 750
Cys Ile Gln Cys Ala His Tyr Ile Asp Gly Pro His Cys Val Lys Thr
        755                 760                 765
Cys Pro Ala Gly Val Met Gly Glu Asn Asn Thr Leu Val Trp Lys Tyr
    770                 775                 780
Ala Asp Ala Gly His Val Cys His Leu Cys His Pro Asn Cys Thr Tyr
785                 790                 795                 800
Gly Cys Thr Gly Pro Gly Leu Glu Gly Cys Pro Thr Asn Gly Pro Lys
                805                 810                 815
Ile Pro Ser Ile Ala Thr Gly Met Val Gly Ala Leu Leu Leu Leu Leu
            820                 825                 830
Val Val Ala Leu Gly Ile Gly Leu Phe Met
        835                 840
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<210> 12
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> (G4S)3 linker

<400> 12

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Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 1               5                  10                  15
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<210> 13
<211> 327
<212> PRT
<213> Artificial Sequence

<220>
<223> Uniprot P0861 IgG4-Fc

<400> 13
Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
 1   5   10   15
Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20   25   30
Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35   40   45
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50   55   60
Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr
65   70   75   80
Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85   90   95
Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro
            100   105   110
Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
        115   120   125
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
    130   135   140
Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp
145   150   155   160
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe
                165   170   175
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
            180   185   190
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu
        195   200   205
Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
    210   215   220
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys
225   230   235   240
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
                245   250   255
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
            260   265   270
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
        275   280   285
Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
    290   295   300
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
305   310   315   320
Leu Ser Leu Ser Leu Gly Lys
                325

<210> 14
<211> 220
<212> PRT
<213> Artificial Sequence

<220>
<223> CD28 transmembrane domain

<400> 14
Met Leu Arg Leu Leu Leu Ala Leu Asn Leu Phe Pro Ser Ile Gln Val
 1   5   10   15
Thr Gly Asn Lys Ile Leu Val Lys Gln Ser Pro Met Leu Val Ala Tyr
            20   25   30
Asp Asn Ala Val Asn Leu Ser Cys Lys Tyr Ser Tyr Asn Leu Phe Ser
        35   40   45
Arg Glu Phe Arg Ala Ser Leu His Lys Gly Leu Asp Ser Ala Val Glu
    50   55   60
Val Cys Val Val Tyr Gly Asn Tyr Ser Gln Gln Leu Gln Val Tyr Ser
65   70   75   80
Lys Thr Gly Phe Asn Cys Asp Gly Lys Leu Gly Asn Glu Ser Val Thr
                85   90   95
Phe Tyr Leu Gln Asn Leu Tyr Val Asn Gln Thr Asp Ile Tyr Phe Cys

```
            100                 105                 110
Lys Ile Glu Val Met Tyr Pro Pro Pro Tyr Leu Asp Asn Glu Lys Ser
        115                 120                 125
Asn Gly Thr Ile Ile His Val Lys Gly Lys His Leu Cys Pro Ser Pro
    130                 135                 140
Leu Phe Pro Gly Pro Ser Lys Pro Phe Trp Val Leu Val Val Val Gly
145                 150                 155                 160
Gly Val Leu Ala Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile Ile
                165                 170                 175
Phe Trp Val Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met
            180                 185                 190
Asn Met Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro
        195                 200                 205
Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser
    210                 215                 220


<210> 15
<211> 240
<212> PRT
<213> Artificial Sequence

<220>
<223> 4-1BB domain

<400> 15
Met Gly Asn Ser Cys Tyr Asn Ile Val Ala Thr Leu Leu Leu Val Leu
 1               5                  10                  15
Asn Phe Glu Arg Thr Arg Ser Leu Gln Asp Pro Cys Ser Asn Cys Pro
            20                  25                  30
Ala Gly Thr Phe Cys Asp Asn Asn Arg Asn Gln Ile Cys Ser Pro Cys
        35                  40                  45
Pro Pro Asn Ser Phe Ser Ser Ala Gly Gly Gln Arg Thr Cys Asp Ile
    50                  55                  60
Cys Arg Gln Cys Lys Gly Val Phe Arg Thr Arg Lys Glu Cys Ser Ser
65                  70                  75                  80
Thr Ser Asn Ala Glu Cys Asp Cys Thr Pro Gly Phe His Cys Leu Gly
                85                  90                  95
Ala Gly Cys Ser Met Cys Glu Gln Asp Cys Lys Gln Gly Gln Glu Leu
            100                 105                 110
Thr Lys Lys Gly Cys Lys Asp Cys Cys Phe Gly Thr Phe Asn Asp Gln
        115                 120                 125
Lys Arg Gly Ile Cys Arg Pro Trp Thr Asn Cys Ser Leu Asp Gly Lys
    130                 135                 140
Ser Val Leu Val Asn Gly Thr Lys Glu Arg Asp Val Val Cys Gly Pro
145                 150                 155                 160
Ser Pro Ala Asp Leu Ser Pro Gly Ala Ser Ser Val Thr Pro Pro Ala
                165                 170                 175
Pro Ala Arg Glu Pro Gly His Ser Pro Gln Ile Ile Ser Phe Phe Leu
            180                 185                 190
Ala Leu Thr Ser Thr Ala Leu Leu Phe Leu Leu Phe Phe Leu Thr Leu
        195                 200                 205
Arg Phe Ser Val Val Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe
    210                 215                 220
Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly
225                 230                 235                 240


<210> 16
<211> 164
<212> PRT
<213> Artificial Sequence

<220>
<223> Human CD3 Zeta isoform 3

<400> 16
Met Lys Trp Lys Ala Leu Phe Thr Ala Ala Ile Leu Gln Ala Gln Leu
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```
 1               5                  10                  15
Pro Ile Thr Glu Ala Gln Ser Phe Gly Leu Leu Asp Pro Lys Leu Cys
            20                  25                  30
Tyr Leu Leu Asp Gly Ile Leu Phe Ile Tyr Gly Val Ile Leu Thr Ala
        35                  40                  45
Leu Phe Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr
    50                  55                  60
Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg
65                  70                  75                  80
Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met
                85                  90                  95
Gly Gly Lys Pro Gln Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn
            100                 105                 110
Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met
        115                 120                 125
Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly
    130                 135                 140
Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala
145                 150                 155                 160
Leu Pro Pro Arg
```

<210> 17
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Human IgG1

<400> 17

```
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro
 1               5                  10                  15
```

<210> 18
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Human IgG2

<400> 18

```
Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro
 1               5                  10
```

<210> 19
<211> 61
<212> PRT
<213> Artificial Sequence

<220>
<223> Human IgG3

<400> 19

```
Glu Leu Lys Thr Pro Leu Gly Asp Thr His Thr Cys Pro Arg Cys Pro
 1               5                  10                  15
Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro Glu
            20                  25                  30
Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro Glu Pro
        35                  40                  45
Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro
    50                  55                  60
```

<210> 20
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Human IgG4

<400> 20
Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro
 1   5   10

<210> 21
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Modified human IgG4

<400> 21
Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro
 1   5   10

<210> 22
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> oJ02649

<400> 22
atcaaaagaa tagaccgaga tagggt 26

<210> 23
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> oJ02648

<400> 23
ccgtaccttt aagaccaatg acttac 26

<210> 24
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> oJ02650

<400> 24
ttgagagttt tcgccccg 18

<210> 25
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> oJ02651

<400> 25

aatagacaga tcgctgagat aggt 24

<210> 26
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> oJ02652

<400> 26
caggtatccg gtaagcgg 18

<210> 27
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> oJ02653

<400> 27
cgaccagcaa ccatagtcc 19

<210> 28
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> oJ02654

<400> 28
tagcggtttg actcacgg 18

<210> 29
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> oJ02655

<400> 29
gcagggagct agaacgattc 20

<210> 30
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> oJ02656

<400> 30
attgtctggt atagtgcagc ag 22

<210> 31
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> oJ02657

<400> 31
tcgcaacggg tttgcc 16

<210> 32
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> oJ02658

<400> 32
aggaagatat cgccacctac t 21

<210> 33
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> oJ02601

<400> 33
cgggtgaagt tcagcagaag 20

<210> 34
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> oJ02735

<400> 34
actgtgtttg ctgacgcaac 20

<210> 35
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> oJ02715

<400> 35
atgcttctcc tggtgacaag 20

<210> 36
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Modified Human IgG4 linker

<400> 36
Tyr Gly Pro Pro Cys Pro Pro Cys Pro
1 5

<210> 37
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Modified Human IgG4

<400> 37
Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro
1 5 10

<210> 38
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Modified Human IgG4

<400> 38
Glu Val Val Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro
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<210> 39
<211> 2586
<212> DNA
<213> Artificial Sequence

<220>
<223> TamR-tf(HEA3)

<400> 39
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gaaggccctc tggataaggg cgagagctgt ggcggaggaa gaggagagct ggccgagctg 180
cctaacggcc tgggcgagac aagaggcagc gaggacgaga cagacgacga cggcgaggac 240
ttcacccccc ccatcctgaa agagctggaa aacctgagcc ccgaggaagc cgcccaccag 300
aaagccgtgg tggagacact gctgcaggaa gatccctggc gggtcgccaa gatggtcaag 360
agctacctgc agcagcacaa catcccccag cgggaggtgg tggacaccac cggcctgaac 420
cagagccacc tgagccagca cctgaacaag ggcaccccca tgaaaaccca gaagagagcc 480
gccctgtaca cttggtacgt gcggaagcag agagaggtgg cccagcagtt tacacacgcc 540
ggccagggcg gcctgatcga ggaacctacc ggcgacgagc tgcccaccaa gaagggcaga 600
cggacccggt ttaagtgggg ccctgcatct cagcagatcc tgttccaggc ctacgagcgg 660
cagaagaacc ccagcaaaga ggaacgggag acactggtgg aagagtgcaa ccgggccgag 720
tgcatccaga gaggcgtgag cccttctcag gctcagggcc tcggcagcaa tctggtcacc 780
gaagtgcggg tgtacaattg gttcgccaac cggcggaaag aggaagcctt ccggcacaag 840
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agcaagaaga acagcctggc cctgagcctg accgccgatc agatggtgtc cgctctgctg 960
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aagctgctgt tcgcccccaa cctgctcctg gaccggaacc agggaaagtg cgtggagggc 1260
atggtggaga tcttcgacat gctgctggcc acctccagcc ggttccggat gatgaacctg 1320
cagggcgagg aattcgtgtg cctgaagtcc atcatcctgc tgaacagcgg cgtgtacacc 1380
ttcctgtcat ccaccctgaa gtccctggaa gagaaggacc acatccaccg ggtgctggac 1440
aagatcaccg acaccctgat ccacctgatg gccaaggctg gcctgacact ccagcagcag 1500
caccagagac tggcccagct gctgctgatc ctgagccaca tccggcacat gagcaacaag 1560
cggatggaac acctgtacag catgaagtgc aagaacgtgg tgcccctgta cgacctgctg 1620
ctcgagatgc tggatgccca cagactgcac gcccctacaa gcagaggcgg agccagcgtg 1680
gaggaaaccg accagtctca cctggccacc gccggcagca caagcagcca cagcctgcag 1740
aagtactaca tcaccggcga ggccgaggga ttccctgcca ccgtggagtt ccagtacctg 1800
cccgacaccg acgaccggca ccggatcgag gaaaagcgga agcggaccta cgagacattc 1860
aagagcatca tgaagaagtc ccccttcagc ggccccaccg atcccagacc ccccctaga 1920
agaatcgccg tgcccagcag atctagcgcc agcgtgccca agcctgcccc ccagccctac 1980
cctttcacca gcagcctgag caccatcaac tacgacgagt tccctaccat ggtgttcccc 2040
agcggccaga tctctcaggc ctctgctctg gcacctgctc cacctcaggt gctgcctcag 2100
gcccctgctc cagccccagc ccctgccatg gtgtctgcac tggcccaggc tccagctcct 2160
gtgcctgtgc tggcccctgg acctcctcag gctgtggccc ctcctgcccc taaacctacc 2220
caggccgggg agggaacact gtctgaggcc ctgctgcagc tccagttcga cgacgaggat 2280
ctgggagcac tgctgggcaa tagcaccgac cccgccgtgt ttaccgacct ggcctccgtg 2340
gacaacagcg agttccagca gctcctcaac cagggcatcc ctgtcgcccc acacaccacc 2400
gagcccatgc tgatggaata ccccgaggcc atcaccagac tggtcacagg cgcccagagg 2460
cctccagatc cagcaccagc tccactggga gcccctggcc tgcctaatgg gctgctgtct 2520
ggcgacgagg acttctccag cattgccgac atggacttca gcgccctgct gtcccagatc 2580
agcagc   2586

<210> 40
<211> 828
<212> PRT
<213> Artificial Sequence

<220>
<223> TamR-tf(HEA3)

<400> 40
Met Val Ser Lys Leu Ser Gln Leu Gln Thr Glu Leu Leu Ala Ala Leu
 1   5   10   15
Leu Glu Ser Gly Leu Ser Lys Glu Ala Leu Ile Gln Ala Leu Gly Glu
            20   25   30
Pro Gly Pro Tyr Leu Leu Ala Gly Glu Gly Pro Leu Asp Lys Gly Glu
        35   40   45
Ser Cys Gly Gly Gly Arg Gly Glu Leu Ala Glu Leu Pro Asn Gly Leu
    50   55   60
Gly Glu Thr Arg Gly Ser Glu Asp Glu Thr Asp Asp Asp Gly Glu Asp
65   70   75   80
Phe Thr Pro Pro Ile Leu Lys Glu Leu Glu Asn Leu Ser Pro Glu Glu
                85   90   95
Ala Ala His Gln Lys Ala Val Val Glu Thr Leu Leu Gln Glu Asp Pro
            100   105   110
Trp Arg Val Ala Lys Met Val Lys Ser Tyr Leu Gln Gln His Asn Ile
        115   120   125
Pro Gln Arg Glu Val Val Asp Thr Thr Gly Leu Asn Gln Ser His Leu
    130   135   140
Ser Gln His Leu Asn Lys Gly Thr Pro Met Lys Thr Gln Lys Arg Ala
145   150   155   160
Ala Leu Tyr Thr Trp Tyr Val Arg Lys Gln Arg Glu Val Ala Gln Gln
                165   170   175
Phe Thr His Ala Gly Gln Gly Gly Leu Ile Glu Glu Pro Thr Gly Asp
            180   185   190
Glu Leu Pro Thr Lys Lys Gly Arg Arg Asn Arg Phe Lys Trp Gly Pro
        195   200   205
Ala Ser Gln Gln Ile Leu Phe Gln Ala Tyr Glu Arg Gln Lys Asn Pro
    210   215   220
Ser Lys Glu Glu Arg Glu Thr Leu Val Glu Glu Cys Asn Arg Ala Glu
225   230   235   240
Cys Ile Gln Arg Gly Val Ser Pro Ser Gln Ala Gln Gly Leu Gly Ser
                245   250   255
Asn Leu Val Thr Glu Val Arg Val Tyr Asn Trp Phe Ala Asn Arg Arg
            260   265   270
Lys Glu Glu Ala Phe Arg His Lys Leu Ser Ala Gly Asp Met Arg Ala
        275   280   285
Ala Asn Leu Trp Pro Ser Pro Leu Met Ile Lys Arg Ser Lys Lys Asn
    290   295   300
Ser Leu Ala Leu Ser Leu Thr Ala Asp Gln Met Val Ser Ala Leu Leu
305   310   315   320
Asp Ala Glu Pro Pro Ile Leu Tyr Ser Glu Tyr Asp Pro Thr Arg Pro
                325   330   335
Phe Ser Glu Ala Ser Met Met Gly Leu Leu Thr Asn Leu Ala Asp Arg
            340   345   350
Glu Leu Val His Met Ile Asn Trp Ala Lys Arg Val Pro Gly Phe Val
        355   360   365
Asp Leu Thr Leu His Asp Gln Val His Leu Leu Glu Cys Ala Trp Leu
    370   375   380
Glu Ile Leu Met Ile Gly Leu Val Trp Arg Ser Met Glu His Pro Gly
385   390   395   400
Lys Leu Leu Phe Ala Pro Asn Leu Leu Leu Asp Arg Asn Gln Gly Lys
                405   410   415
Cys Val Glu Gly Met Val Glu Ile Phe Asp Met Leu Leu Ala Thr Ser
            420   425   430
Ser Arg Phe Arg Met Met Asn Leu Gln Gly Glu Glu Phe Val Cys Leu
        435   440   445
Lys Ser Ile Ile Leu Leu Asn Ser Gly Val Tyr Thr Phe Leu Ser Ser
    450   455   460

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Thr Leu Lys Ser Leu Glu Glu Lys Asp His Ile His Arg Val Leu Asp
465                 470                 475                 480
Lys Ile Thr Asp Thr Leu Ile His Leu Met Ala Lys Ala Gly Leu Thr
                485                 490                 495
Leu Gln Gln Gln His Gln Arg Leu Ala Gln Leu Leu Leu Ile Leu Ser
            500                 505                 510
His Ile Arg His Met Ser Asn Lys Arg Met Glu His Leu Tyr Ser Met
        515                 520                 525
Lys Cys Lys Asn Val Val Pro Leu Tyr Asp Leu Leu Leu Glu Met Leu
    530                 535                 540
Asp Ala His Arg Leu His Ala Pro Thr Ser Arg Gly Gly Ala Ser Val
545                 550                 555                 560
Glu Glu Thr Asp Gln Ser His Leu Ala Thr Ala Gly Ser Thr Ser Ser
                565                 570                 575
His Ser Leu Gln Lys Tyr Tyr Ile Thr Gly Glu Ala Glu Gly Phe Pro
            580                 585                 590
Ala Thr Val Glu Phe Gln Tyr Leu Pro Asp Thr Asp Asp Arg His Arg
        595                 600                 605
Ile Glu Glu Lys Arg Lys Arg Thr Tyr Glu Thr Phe Lys Ser Ile Met
    610                 615                 620
Lys Lys Ser Pro Phe Ser Gly Pro Thr Asp Pro Arg Pro Pro Pro Arg
625                 630                 635                 640
Arg Ile Ala Val Pro Ser Arg Ser Ser Ala Ser Val Pro Lys Pro Ala
                645                 650                 655
Pro Gln Pro Tyr Pro Phe Thr Ser Ser Leu Ser Thr Ile Asn Tyr Asp
            660                 665                 670
Glu Phe Pro Thr Met Val Phe Pro Ser Gly Gln Ile Ser Gln Ala Ser
        675                 680                 685
Ala Leu Ala Pro Ala Pro Pro Gln Val Leu Pro Gln Ala Pro Ala Pro
    690                 695                 700
Ala Pro Ala Pro Ala Met Val Ser Ala Leu Ala Gln Ala Pro Ala Pro
705                 710                 715                 720
Val Pro Val Leu Ala Pro Gly Pro Pro Gln Ala Val Ala Pro Pro Ala
                725                 730                 735
Pro Lys Pro Thr Gln Ala Gly Glu Gly Thr Leu Ser Glu Ala Leu Leu
            740                 745                 750
Gln Leu Gln Phe Asp Asp Glu Asp Leu Gly Ala Leu Leu Gly Asn Ser
        755                 760                 765
Thr Asp Pro Ala Val Phe Thr Asp Leu Ala Ser Val Asp Asn Ser Glu
    770                 775                 780
Phe Gln Gln Leu Leu Asn Gln Gly Ile Pro Val Ala Pro His Thr Thr
785                 790                 795                 800
Glu Pro Met Leu Met Glu Tyr Pro Glu Ala Ile Thr Arg Leu Val Thr
                805                 810                 815
Gly Ala Gln Arg Pro Pro Asp Pro Ala Pro Ala Pro Leu Gly Ala Pro
            820                 825                 830
Gly Leu Pro Asn Gly Leu Leu Ser Gly Asp Glu Asp Phe Ser Ser Ile
        835                 840                 845
Ala Asp Met Asp Phe Ser Ala Leu Leu Ser Gln Ile Ser Ser
    850                 855                 860


<210> 41
<211> 119
<212> DNA
<213> Artificial Sequence

<220>
<223> 7xHBD/mEF1-alpha

<400> 41
tagttaataa tctacaatag ttaataatct acaatagtta ataatctaca atagttaata 60
atctacaata gttaataatc tacaatagtt aataatctac aatagttaat aatctacaa  119

<210> 42
<211> 2586
<212> DNA
<213> Artificial Sequence

<220>
<223> TamR-tf (HEA4)
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<400> 42
atggtgtcca agctgtccca gctgcagaca gaactgctgg cagcactgct ggaaagcggc 60
ctgagcaaag aggccctgat tcaggcactc ggcgaacctg gaccttatct gctcgctggc 120
gaaggccctc tggataaggg cgagagctgt ggcggaggaa gaggagagct ggccgagctg 180
cctaacggcc tgggcgagac aagaggcagc gaggacgaga cagacgacga cggcgaggac 240
ttcacccccc ccatcctgaa agagctggaa aacctgagcc ccgaggaagc cgcccaccag 300
aaagccgtgg tggagacact gctgcaggaa gatccctggc gggtcgccaa gatggtcaag 360
agctacctgc agcagcacaa catcccccag cgggaggtgg tggacaccac cggcctgaac 420
cagagccacc tgagccagca cctgaacaag ggcaccccca tgaaaaccca gaagagagcc 480
gccctgtaca cttggtacgt gcggaagcag agagaggtgg cccagcagtt tacacacgcc 540
ggccagggcg gcctgatcga ggaacctacc ggcgacgagc tgcccaccaa gaagggcaga 600
cggaaccggt ttaagtgggg ccctgcatct cagcagatcc tgttccaggc ctacgagcgg 660
cagaagaacc ccagcaaaga ggaacgggag acactggtgg aagagtgcaa ccgggccgag 720
tgcatccaga gaggcgtgag cccttctcag gctcagggcc tcggcagcaa tctggtcacc 780
gaagtgcggg tgtacaattg gttcgccaac cggcggaaag aggaagcctt ccggcacaag 840
ctgtctgctg gcgatatgag agccgccaac ctgtggccca gccccctgat gatcaagcgg 900
agcaagaaga acagcctggc cctgagcctg accgccgatc agatggtgtc cgctctgctg 960
gacgccgagc cccctatcct gtacagcgag tacgacccca ccagaccctt cagcgaggcc 1020
agcatgatgg gcctgctgac caacctggcc gaccgggagc tggtgcacat gatcaactgg 1080
gccaagcggg tgcccggctt cgtggacctg accctgcacg accaggtcca cctgctggaa 1140
tgtgcctggc tggaaatcct gatgatcggc ctcgtgtgga gaagcatgga acaccccgtg 1200
aagctgctgt tcgcccccaa cctgctcctg gaccggaacc agggaaagtg cgtggagggc 1260
atggtggaga tcttcgacat gctgctggcc acctccagcc ggttccggat gatgaacctg 1320
cagggcgagg aattcgtgtg cctgaagtcc atcatcctgc tgaacagcgg cgtgtacacc 1380
ttcctgtcat ccaccctgaa gtccctggaa gagaaggacc acatccaccg ggtgctggac 1440
aagatcaccg acaccctgat ccacctgatg gccaaggctg gcctgacact ccagcagcag 1500
caccagagac tggcccagct gctgctgatc ctgagccaca tccggcacat gagcaacaag 1560
ggaatggaac acctgtacag catgaagtgc acgaacgtgg tgcccctgta cgacctgctg 1620
ctcgaggctg ccgatgccca cagactgcac gcccctacaa gcagaggcgg agccagcgtg 1680
gaggaaaccg accagtctca cctggccacc gccggcagca caagcagcca cagcctgcag 1740
aagtactaca tcaccggcga ggccgaggga ttccctgcca ccgtggagtt ccagtacctg 1800
cccgacaccg acgaccggca ccggatcgag gaaaagcgga agcggaccta cgagacattc 1860
aagagcatca tgaagaagtc ccccttcagc ggccccaccg atcccagacc ccccccctaga 1920
agaatcgccg tgcccagcag atctagcgcc agcgtgccca agcctgcccc ccagccctac 1980
cctttcacca gcagcctgag caccatcaac tacgacgagt tccctaccat ggtgttcccc 2040
agcggccaga tctctcaggc ctctgctctg gcacctgctc cacctcaggt gctgcctcag 2100
gcccctgctc cagccccagc ccctgccatg gtgtctgcac tggcccaggc tccagctcct 2160
gtgcctgtgc tggcccctgg acctcctcag gctgtggccc ctcctgcccc taaacctacc 2220
caggccgggg agggaacact gtctgaggcc ctgctgcagc tccagttcga cgacgaggat 2280
ctgggagcac tgctgggcaa tagcaccgac cccgccgtgt ttaccgacct ggcctccgtg 2340
gacaacagcg agttccagca gctcctcaac cagggcatcc ctgtcgcccc acacaccacc 2400
gagcccatgc tgatggaata ccccgaggcc atcaccagac tggtcacagg cgcccagagg 2460
cctccagatc cagcaccagc tccactggga gcccctggcc tgcctaatgg gctgctgtct 2520
ggcgacgagg acttctccag cattgccgac atggacttca gcgccctgct gtcccagatc 2580
agcagc 2586

<210> 43
<211> 862
<212> PRT
<213> Artificial Sequence

<220>
<223> TamR-tf (HEA4)

<400> 43
Met Val Ser Lys Leu Ser Gln Leu Gln Thr Glu Leu Leu Ala Ala Leu
 1   5   10   15
Leu Glu Ser Gly Leu Ser Lys Glu Ala Leu Ile Gln Ala Leu Gly Glu
            20   25   30
Pro Gly Pro Tyr Leu Leu Ala Gly Glu Gly Pro Leu Asp Lys Gly Glu
        35   40   45
Ser Cys Gly Gly Gly Arg Gly Glu Leu Ala Glu Leu Pro Asn Gly Leu
    50   55   60
Gly Glu Thr Arg Gly Ser Glu Asp Glu Thr Asp Asp Asp Gly Glu Asp
65   70   75   80
Phe Thr Pro Pro Ile Leu Lys Glu Leu Glu Asn Leu Ser Pro Glu Glu
                85   90   95
Ala Ala His Gln Lys Ala Val Val Glu Thr Leu Leu Gln Glu Asp Pro

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            100                 105                 110
Trp Arg Val Ala Lys Met Val Lys Ser Tyr Leu Gln Gln His Asn Ile
        115                 120                 125
Pro Gln Arg Glu Val Val Asp Thr Thr Gly Leu Asn Gln Ser His Leu
    130                 135                 140
Ser Gln His Leu Asn Lys Gly Thr Pro Met Lys Thr Gln Lys Arg Ala
145                 150                 155                 160
Ala Leu Tyr Thr Trp Tyr Val Arg Lys Gln Arg Glu Val Ala Gln Gln
                165                 170                 175
Phe Thr His Ala Gly Gln Gly Gly Leu Ile Glu Glu Pro Thr Gly Asp
            180                 185                 190
Glu Leu Pro Thr Lys Lys Gly Arg Arg Asn Arg Phe Lys Trp Gly Pro
        195                 200                 205
Ala Ser Gln Gln Ile Leu Phe Gln Ala Tyr Glu Arg Gln Lys Asn Pro
    210                 215                 220
Ser Lys Glu Glu Arg Glu Thr Leu Val Glu Glu Cys Asn Arg Ala Glu
225                 230                 235                 240
Cys Ile Gln Arg Gly Val Ser Pro Ser Gln Ala Gln Gly Leu Gly Ser
                245                 250                 255
Asn Leu Val Thr Glu Val Arg Val Tyr Asn Trp Phe Ala Asn Arg Arg
            260                 265                 270
Lys Glu Glu Ala Phe Arg His Lys Leu Ser Ala Gly Asp Met Arg Ala
        275                 280                 285
Ala Asn Leu Trp Pro Ser Pro Leu Met Ile Lys Arg Ser Lys Lys Asn
    290                 295                 300
Ser Leu Ala Leu Ser Leu Thr Ala Asp Gln Met Val Ser Ala Leu Leu
305                 310                 315                 320
Asp Ala Glu Pro Pro Ile Leu Tyr Ser Glu Tyr Asp Pro Thr Arg Pro
                325                 330                 335
Phe Ser Glu Ala Ser Met Met Gly Leu Leu Thr Asn Leu Ala Asp Arg
            340                 345                 350
Glu Leu Val His Met Ile Asn Trp Ala Lys Arg Val Pro Gly Phe Val
        355                 360                 365
Asp Leu Thr Leu His Asp Gln Val His Leu Leu Glu Cys Ala Trp Leu
    370                 375                 380
Glu Ile Leu Met Ile Gly Leu Val Trp Arg Ser Met Glu His Pro Val
385                 390                 395                 400
Lys Leu Leu Phe Ala Pro Asn Leu Leu Leu Asp Arg Asn Gln Gly Lys
                405                 410                 415
Cys Val Glu Gly Met Val Glu Ile Phe Asp Met Leu Leu Ala Thr Ser
            420                 425                 430
Ser Arg Phe Arg Met Met Asn Leu Gln Gly Glu Glu Phe Val Cys Leu
        435                 440                 445
Lys Ser Ile Ile Leu Leu Asn Ser Gly Val Tyr Thr Phe Leu Ser Ser
    450                 455                 460
Thr Leu Lys Ser Leu Glu Glu Lys Asp His Ile His Arg Val Leu Asp
465                 470                 475                 480
Lys Ile Thr Asp Thr Leu Ile His Leu Met Ala Lys Ala Gly Leu Thr
                485                 490                 495
Leu Gln Gln Gln His Gln Arg Leu Ala Gln Leu Leu Leu Ile Leu Ser
            500                 505                 510
His Ile Arg His Met Ser Asn Lys Gly Met Glu His Leu Tyr Ser Met
        515                 520                 525
Lys Cys Lys Asn Val Val Pro Leu Tyr Asp Leu Leu Leu Glu Ala Ala
    530                 535                 540
Asp Ala His Arg Leu His Ala Pro Thr Ser Arg Gly Gly Ala Ser Val
545                 550                 555                 560
Glu Glu Thr Asp Gln Ser His Leu Ala Thr Ala Gly Ser Thr Ser Ser
                565                 570                 575
His Ser Leu Gln Lys Tyr Tyr Ile Thr Gly Glu Ala Glu Gly Phe Pro
            580                 585                 590
Ala Thr Val Glu Phe Gln Tyr Leu Pro Asp Thr Asp Asp Arg His Arg
        595                 600                 605
Ile Glu Glu Lys Arg Lys Arg Thr Tyr Glu Thr Phe Lys Ser Ile Met
    610                 615                 620
Lys Lys Ser Pro Phe Ser Gly Pro Thr Asp Pro Arg Pro Pro Pro Arg
625                 630                 635                 640
Arg Ile Ala Val Pro Ser Arg Ser Ser Ala Ser Val Pro Lys Pro Ala
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                645                 650                 655
Pro Gln Pro Tyr Pro Phe Thr Ser Ser Leu Ser Thr Ile Asn Tyr Asp
            660                 665                 670
Glu Phe Pro Thr Met Val Phe Pro Ser Gly Gln Ile Ser Gln Ala Ser
        675                 680                 685
Ala Leu Ala Pro Ala Pro Pro Gln Val Leu Pro Gln Ala Pro Ala Pro
    690                 695                 700
Ala Pro Ala Pro Ala Met Val Ser Ala Leu Ala Gln Ala Pro Ala Pro
705                 710                 715                 720
Val Pro Val Leu Ala Pro Gly Pro Pro Gln Ala Val Ala Pro Pro Ala
                725                 730                 735
Pro Lys Pro Thr Gln Ala Gly Glu Gly Thr Leu Ser Glu Ala Leu Leu
            740                 745                 750
Gln Leu Gln Phe Asp Asp Glu Asp Leu Gly Ala Leu Leu Gly Asn Ser
        755                 760                 765
Thr Asp Pro Ala Val Phe Thr Asp Leu Ala Ser Val Asp Asn Ser Glu
    770                 775                 780
Phe Gln Gln Leu Leu Asn Gln Gly Ile Pro Val Ala Pro His Thr Thr
785                 790                 795                 800
Glu Pro Met Leu Met Glu Tyr Pro Glu Ala Ile Thr Arg Leu Val Thr
                805                 810                 815
Gly Ala Gln Arg Pro Pro Asp Pro Ala Pro Ala Pro Leu Gly Ala Pro
            820                 825                 830
Gly Leu Pro Asn Gly Leu Leu Ser Gly Asp Glu Asp Phe Ser Ser Ile
        835                 840                 845
Ala Asp Met Asp Phe Ser Ala Leu Leu Ser Gln Ile Ser Ser
    850                 855                 860


<210> 44
<211> 408
<212> DNA
<213> Artificial Sequence

<220>
<223> Her2t

<400> 44
atgcttctcc tggtgacaag ccttctgctc tgtgagttac cacacccagc attcctcctg 60
atcccatgcc accctgagtg tcagccccag aatggctcag tgacctgttt tggaccggag 120
gctgaccagt gtgtggcctg tgcccactat aaggaccctc ccttctgcgt ggcccgctgc 180
cccagcggtg tgaaacctga cctctcctac atgcccatct ggaagtttcc agatgaggag 240
ggcgcatgcc agccttgccc catcaactgc acccactcct gtgtggacct ggatgacaag 300
ggctgccccg ccgagcagag agccagccct ctgacgtcca tcatctctgc ggtggttggc 360
attctgctgg tcgtggtctt gggggtggtc tttgggatcc tcatctga              408

<210> 45
<211> 135
<212> PRT
<213> Artificial Sequence

<220>
<223> Her2t

<400> 45
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 Cys His Pro Glu Cys Gln Pro Gln Asn Gly
            20                  25                  30
Ser Val Thr Cys Phe Gly Pro Glu Ala Asp Gln Cys Val Ala Cys Ala
        35                  40                  45
His Tyr Lys Asp Pro Pro Phe Cys Val Ala Arg Cys Pro Ser Gly Val
    50                  55                  60
Lys Pro Asp Leu Ser Tyr Met Pro Ile Trp Lys Phe Pro Asp Glu Glu
65                  70                  75                  80
Gly Ala Cys Gln Pro Cys Pro Ile Asn Cys Thr His Ser Cys Val Asp
                85                  90                  95
Leu Asp Asp Lys Gly Cys Pro Ala Glu Gln Arg Ala Ser Pro Leu Thr
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100 105 110
Ser Ile Ile Ser Ala Val Val Gly Ile Leu Leu Val Val Val Leu Gly
115 120 125
Val Val Phe Gly Ile Leu Ile
130 135

<210> 46
<211> 561
<212> DNA
<213> Artificial Sequence

<220>
<223> DHFRdm

<400> 46
atggttggtt cgctaaactg catcgtcgct gtgtcccaga acatgggcat cggcaagaac 60
ggggacttcc cctggccacc gctcaggaat gaatccagat atttccagag aatgaccaca 120
acctcttcag tagaaggtaa acagaatctg gtgattatgg gtaagaagac ctggttctcc 180
attcctgaga agaatcgacc tttaaagggt agaattaatt tagttctcag cagagaactc 240
aaggaacctc cacaaggagc tcattttctt tccagaagtc tagatgatgc cttaaaactt 300
actgaacaac cagaattagc aaataaagta gacatggtct ggatagttgg tggcagttct 360
gtttataagg aagccatgaa tcacccaggc catcttaaac tatttgtgac aaggatcatg 420
caagactttg aaagtgacac gttttttcca gaaattgatt tggagaaata taaacttctg 480
ccagaatacc caggtgttct ctctgatgtc caggaggaga aaggcattaa gtacaaattt 540
gaagtatatg agaagaatga t 561

<210> 47
<211> 187
<212> PRT
<213> Artificial Sequence

<220>
<223> DHFRdm

<400> 47
Met Val Gly Ser Leu Asn Cys Ile Val Ala Val Ser Gln Asn Met Gly
1 5 10 15
Ile Gly Lys Asn Gly Asp Phe Pro Trp Pro Pro Leu Arg Asn Glu Ser
20 25 30
Arg Tyr Phe Gln Arg Met Thr Thr Thr Ser Ser Val Glu Gly Lys Gln
35 40 45
Asn Leu Val Ile Met Gly Lys Lys Thr Trp Phe Ser Ile Pro Glu Lys
50 55 60
Asn Arg Pro Leu Lys Gly Arg Ile Asn Leu Val Leu Ser Arg Glu Leu
65 70 75 80
Lys Glu Pro Pro Gln Gly Ala His Phe Leu Ser Arg Ser Leu Asp Asp
85 90 95
Ala Leu Lys Leu Thr Glu Gln Pro Glu Leu Ala Asn Lys Val Asp Met
100 105 110
Val Trp Ile Val Gly Gly Ser Ser Val Tyr Lys Glu Ala Met Asn His
115 120 125
Pro Gly His Leu Lys Leu Phe Val Thr Arg Ile Met Gln Asp Phe Glu
130 135 140
Ser Asp Thr Phe Phe Pro Glu Ile Asp Leu Glu Lys Tyr Lys Leu Leu
145 150 155 160
Pro Glu Tyr Pro Gly Val Leu Ser Asp Val Gln Glu Glu Lys Gly Ile
165 170 175
Lys Tyr Lys Phe Glu Val Tyr Glu Lys Asn Asp
180 185

<210> 48

<400> 48
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<210> 49

<400> 49
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<210> 50

<400> 50
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<210> 51

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<210> 58

<400> 58
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<210> 59
<211> 98
<212> PRT
<213> Artificial Sequence

<220>
<223> CH3 domain

<400> 59
Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
 1   5   10   15
Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20   25   30
Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35   40   45
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50   55   60
Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr
65   70   75   80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95
Arg Val

<210> 60
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Hinge domain

<400> 60
Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro
1 5 10

<210> 61
<211> 110
<212> PRT
<213> Artificial Sequence

<220>
<223> CH2 domain

<400> 61
Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
1 5 10 15
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
20 25 30
Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr
35 40 45
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
50 55 60
Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
65 70 75 80
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
85 90 95
Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys
100 105 110

<210> 62
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> CH3 protein domain

<400> 62
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu
1 5 10 15
Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
20 25 30
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
35 40 45
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
50 55 60
Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly
65 70 75 80
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
85 90 95
Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
100 105

<210> 63

<400> 63
 000

<210> 64
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Signal peptide

<400> 64
Met Leu Arg Leu Leu Leu Ala Leu Asn Leu Phe Pro
 1   5   10

<210> 65
<211> 134
<212> PRT
<213> Artificial Sequence

<220>
<223> Extracellular domain

<400> 65
Asn Lys Ile Leu Val Lys Gln Ser Pro Met Leu Val Ala Tyr Asp Asn
 1   5   10   15
Ala Val Asn Leu Ser Cys Lys Tyr Ser Tyr Asn Leu Phe Ser Arg Glu
            20   25   30
Phe Arg Ala Ser Leu His Lys Gly Leu Asp Ser Ala Val Glu Val Cys
        35   40   45
Val Val Tyr Gly Asn Tyr Ser Gln Gln Leu Gln Val Tyr Ser Lys Thr
    50   55   60
Gly Phe Asn Cys Asp Gly Lys Leu Gly Asn Glu Ser Val Thr Phe Tyr
65   70   75   80
Leu Gln Asn Leu Tyr Val Asn Gln Thr Asp Ile Tyr Phe Cys Lys Ile
                85   90   95
Glu Val Met Tyr Pro Pro Pro Tyr Leu Asp Asn Glu Lys Ser Asn Gly
            100   105   110
Thr Ile Ile His Val Lys Gly Lys His Leu Cys Pro Ser Pro Leu Phe
        115   120   125
Pro Gly Pro Ser Lys Pro
    130

<210> 66
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Transmembrane domain

<400> 66
Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu
 1   5   10   15
Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val
            20   25

<210> 67
<211> 50
<212> PRT
<213> Artificial Sequence

<220>
<223> Intracellular domain

<400> 67
Thr Val Ala Phe Ile Ile Phe Trp Val Arg Ser Lys Arg Ser Arg Leu
 1   5   10   15
Leu His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly Pro Thr
            20   25   30
Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr
        35   40   45
Arg Ser
    50

<210> 68

<220>
<223> Signal peptide

<400> 68
 000

<210> 69
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> Signal peptide

<400> 69
Met Gly Asn Ser Cys Tyr Asn Ile Val Ala Thr Leu Leu Leu Val Leu
 1   5   10   15
Asn Phe Glu Arg Thr Arg Ser
            20

<210> 70
<211> 166
<212> PRT
<213> Artificial Sequence

<220>
<223> Extracellular domain

<400> 70
Thr Arg Ser Leu Gln Asp Pro Cys Ser Asn Cys Pro Ala Gly Thr Phe
 1   5   10   15
Cys Asp Asn Asn Arg Asn Gln Ile Cys Ser Pro Cys Pro Pro Asn Ser
            20   25   30
Phe Ser Ser Ala Gly Gly Gln Arg Thr Cys Asp Ile Cys Arg Gln Cys
        35   40   45
Lys Gly Val Phe Arg Thr Arg Lys Glu Cys Ser Ser Thr Ser Asn Ala
    50   55   60
Glu Cys Asp Cys Thr Pro Gly Phe His Cys Leu Gly Ala Gly Cys Ser
65   70   75   80
Met Cys Glu Gln Asp Cys Lys Gln Gly Gln Glu Leu Thr Lys Lys Gly
                85   90   95
Cys Lys Asp Cys Cys Phe Gly Thr Phe Asn Asp Gln Lys Arg Gly Ile
            100   105   110
Cys Arg Pro Trp Thr Asn Cys Ser Leu Asp Gly Lys Ser Val Leu Val
        115   120   125
Asn Gly Thr Lys Glu Arg Asp Val Val Cys Gly Pro Ser Pro Ala Asp
    130   135   140
Leu Ser Pro Gly Ala Ser Ser Val Thr Pro Pro Ala Pro Ala Arg Glu
145   150   155   160
Pro Gly His Ser Pro Gln
                165

<210> 71
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Transmembrane domain

<400> 71
Ile Ile Ser Phe Phe Leu Ala Leu Thr Ser Thr Ala Leu Leu Phe Leu
 1   5   10   15
Leu Phe Phe Leu Thr Leu Arg Phe Ser Val Val
            20   25

<210> 72
<211> 42
<212> PRT
<213> Artificial Sequence

<220>
<223> Intracellular domain

<400> 72
Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met
 1   5   10   15
Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe
            20   25   30
Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu
        35   40

<210> 73
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> Signal peptide

<400> 73
Met Lys Trp Lys Ala Leu Phe Thr Ala Ala Ile Leu Gln Ala Gln Leu
 1   5   10   15
Pro Ile Thr Glu Ala
            20

<210> 74
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Extracellular domain

<400> 74
Gln Ser Phe Gly Leu Leu Asp Pro Lys
 1   5

<210> 75
<211> 21
<212> PRT
<213> Artificial Sequence

<220>

<223> Transmembrane domain

<400> 75
Leu Cys Tyr Leu Leu Asp Gly Ile Leu Phe Ile Tyr Gly Val Ile Leu
 1   5   10   15
Thr Ala Leu Phe Leu
            20

<210> 76
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Intracellular domain

<400> 76
Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly
 1   5   10   15
Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr
            20   25   30
Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys
        35   40   45
Pro Gln Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln
    50   55   60
Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu
65   70   75   80
Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr
                85   90   95
Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro
            100   105   110
Arg

<210> 77
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> ITAM I

<400> 77
Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn
 1   5   10   15
Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg
            20   25

<210> 78
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> ITAM 2

<400> 78
Pro Gln Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln
 1   5   10   15
Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met
            20   25

<210> 79
<211> 29

<212> PRT
<213> Artificial Sequence

<220>
<223> ITAM 3

<400> 79
Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser
 1   5   10   15
Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln
            20   25

<210> 80
<211> 66
<212> DNA
<213> Artificial Sequence

<220>
<223> Leader

<400> 80
atgcttctcc tggtgacaag ccttctgctc tgtgagttac cacacccagc attcctcctg 60
atccca 66

<210> 81
<211> 734
<212> DNA
<213> Artificial Sequence

<220>
<223> Her2scFV

<400> 81
gatatccaga tgacccagtc cccgagctcc ctgtccgcct ctgtgggcga tagggtcacc 60
atcacctgcc gtgccagtca ggatgtgaat actgctgtag cctggtatca acagaaacca 120
ggaaaagctc cgaaactact gatttactcg gcatccttcc tctactctgg agtcccttct 180
cgcttctctg gttccagatc tgggacggat ttcactctga ccatcagcag tctgcagccg 240
gaagacttcg caacttatta ctgtcagcaa cattatacta ctcctcccac gttcggacag 300
ggtaccaagg tggagatcaa aggcagtact agcggcggtg gctccggggg cggatccggt 360
gggggcggca gcagcgaggt tcagctggtg gagtctggcg gtggcctggt gcagccaggg 420
ggctcactcc gtttgtcctg tgcagcttct ggcttcaaca ttaaagacac ctatatacac 480
tgggtgcgtc aggccccggg taagggcctg gaatgggttg caaggattta tcctacgaat 540
ggttatacta gatatgccga tagcgtcaag ggccgtttca ctataagcgc agacacatcc 600
aaaaacacag cctacctgca gatgaacagc ctgcgtgctg aggacactgc cgtctattat 660
tgttctagat ggggagggga cggcttctat gctatggact actggggtca aggaaccctg 720
gtcaccgtct cgag 734

<210> 82
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Hinge spacer

<400> 82
gagagcaagt acggaccgcc ctgcccccct tgccct 36

<210> 83
<211> 84
<212> DNA
<213> Artificial Sequence

<220>
<223> CD28tm

<400> 83

atgttctggg tgctggtggt ggtcggaggc gtgctggcct gctacagcct gctggtcacc 60
gtggccttca tcatcttttg ggtg 84

<210> 84
<211> 126
<212> DNA
<213> Artificial Sequence

<220>
<223> 4-1BB

<400> 84
aaacggggca gaaagaaact cctgtatata ttcaaacaac catttatgag accagtacaa 60
actactcaag aggaagatgg ctgtagctgc cgatttccag aagaagaaga aggaggatgt 120
gaactg 126

<210> 85
<211> 336
<212> DNA
<213> Artificial Sequence

<220>
<223> CD3 zeta

<400> 85
cgggtgaagt tcagcagaag cgccgacgcc cctgcctacc agcagggcca gaatcagctg 60
tacaacgagc tgaacctggg cagaagggaa gagtacgacg tcctggataa gcggagaggc 120
cgggaccctg agatgggcgg caagcctcgg cggaagaacc cccaggaagg cctgtataac 180
gaactgcaga aagacaagat ggccgaggcc tacagcgaga tcggcatgaa gggcgagcgg 240
aggcggggca agggccacga cggcctgtat cagggcctgt ccaccgccac caaggatacc 300
tacgacgccc tgcacatgca ggccctgccc ccaagg 336

<210> 86
<211> 72
<212> DNA
<213> Artificial Sequence

<220>
<223> T2A

<400> 86
ctcgagggcg gcggagaggg cagaggaagt cttctaacat gcggtgacgt ggaggagaat 60
cccggcccta gg 72

<210> 87
<211> 1074
<212> DNA
<213> Artificial Sequence

<220>
<223> tEGFR

<400> 87
atgcttctcc tggtgacaag ccttctgctc tgtgagttac cacacccagc attcctcctg 60
atcccacgca aagtgtgtaa cggaataggt attggtgaat ttaaagactc actctccata 120
aatgctacga atattaaaca cttcaaaaac tgcacctcca tcagtggcga tctccacatc 180
ctgccggtgg catttagggg tgactccttc acacatactc ctcctctgga tccacaggaa 240
ctggatattc tgaaaaccgt aaaggaaatc acagggtttt tgctgattca ggcttggcct 300
gaaaacagga cggacctcca tgcctttgag aacctagaaa tcatacgcgg caggaccaag 360
caacatggtc agttttctct tgcagtcgtc agcctgaaca taacatcctt gggattacgc 420
tccctcaagg agataagtga tggagatgtg ataatttcag gaaacaaaaa tttgtgctat 480
gcaaatacaa taaactggaa aaaactgttt gggacctccg gtcagaaaac caaaattata 540
agcaacagag gtgaaaacag ctgcaaggcc acaggccagg tctgccatgc cttgtgctcc 600
cccgagggct gctggggccc ggagcccagg gactgcgtct cttgccggaa tgtcagccga 660
ggcagggaat gcgtggacaa gtgcaacctt ctggagggtg agccaaggga gtttgtggag 720
aactctgagt gcatacagtg ccacccagag tgcctgcctc aggccatgaa catcacctgc 780
acaggacggg gaccagacaa ctgtatccag tgtgcccact acattgacgg cccccactgc 840
gtcaagacct gcccggcagg agtcatggga gaaaacaaca ccctggtctg gaagtacgca 900

gacgccggcc atgtgtgcca cctgtgccat ccaaactgca cctacggatg cactgggcca 960
ggtcttgaag gctgtccaac gaatgggcct aagatcccgt ccatcgccac tgggatggtg 1020
ggggccctcc tcttgctgct ggtggtggcc ctggggatcg gcctcttcat gtga 1074

<210> 88
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Humanized ScFv comprising a variable light chain
comprising a CDRL1 sequence

<400> 88
Arg Ala Ser Gln Asp Ile Ser Lys Tyr Leu Asn
1 5 10

<210> 89
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> CDRL2

<400> 89
Ser Arg Leu His Ser Gly Val
1 5

<210> 90
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> CDRL3

<400> 90
Gly Asn Thr Leu Pro Tyr Thr Phe Gly
1 5

<210> 91
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> CDRH1

<400> 91
Asp Tyr Gly Val Ser
1 5

<210> 92
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> CDRH2

<400> 92
Val Ile Trp Gly Ser Glu Thr Thr Tyr Tyr Asn Ser Ala Leu Lys Ser
1 5 10 15

<210> 93
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> CDRH3

<400> 93
Tyr Ala Met Asp Tyr Trp Gly
 1   5

<210> 94
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Flexible linker between the VH and VL chains in
      the scFv

<400> 94
Gly Ser Thr Ser Gly Ser Gly Lys Pro Gly Ser Gly Glu Gly Ser Thr
 1   5   10   15
Lys Gly