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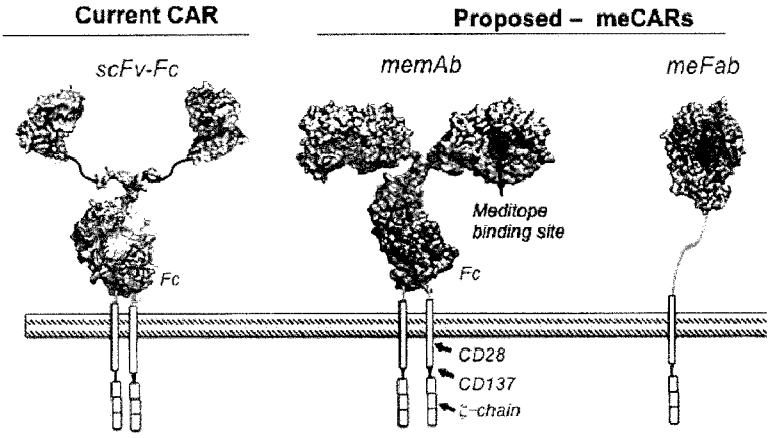
(54) Title: CHIMERIC ANTIGEN RECEPTOR COMPOSITIONS

FIG. 1A



(57) Abstract: Provided herein are compositions which exhibit novel diagnostic capabilities and allow to rapidly add functionality to adoptive immunotherapy. The compositions include isolated nucleic acids encoding proteins including antibody regions capable of binding compounds including a peptidyl moiety (e.g., a meditope). The recombinant proteins provided herein are useful, inter alia, for a broad variety of therapeutic and diagnostic purposes. For example, the recombinant proteins provided herein including embodiments thereof may be used as non-invasive means to characterize chimeric antigen receptor (CAR) T cells before and/or during treatment of diseases (e.g., cancer).

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CHIMERIC ANTIGEN RECEPTOR COMPOSITIONS

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims priority to US Provisional Application No. 62/162,599, filed May 15, 2015, the disclosure of which is incorporated herein in its entirety and for all purposes.

REFERENCE TO A "SEQUENCE LISTING," A TABLE, OR A COMPUTER PROGRAM LISTING APPENDIX SUBMITTED AS AN ASCII TEXT FILE

[0002] The Sequence Listing written in file 48440-558001WO_ST25.TXT, created on May 12, 2016, 169,534 bytes, machine format IBM-PC, MS-Windows operating system, is hereby incorporated by reference in its entirety for all purposes.

BACKGROUND OF THE INVENTION

[0003] Cancer is the second leading cause of death in the United States, killing more people than the next five causes combined including chronic respiratory disease, Alzheimer's disease and diabetes. While extraordinary strides have been made in the detection, prevention and treatment of cancer, there remains an urgent need, especially in advanced cases, to produce therapies that not only halt tumor progression but effectively eliminate all tumor cells. One approach is adoptive T cell immunotherapy (5-7). This method requires the harvesting of the patient's T cells, engineering of these cells with a chimeric antigen receptor (CAR) that recognizes a tumor antigen, and subsequent re-introduction of the modified cells to the patient. The re-programmed T cells then directly target antigen-expressing tumor cells, bypassing the requirement for MHC peptide, and elicit a powerful but localized immune response. This method of treatment (8) has produced some positive results in early clinical trials for a handful, but not for all patients. There is a need in the art to better understand CAR T cell therapy's success and failure. For example, there is a need in the art for the ability to characterize the density of the CARs on the transformed cells, to track administered CAR T cells at any point during therapy and correlate this distribution to therapeutic outcomes, to rapidly functionalize CAR T cells, monitoring the number, location and viability of the transplanted CAR T cells *in situ* and to selectively eliminate CAR T cells if necessary. Meaningful correlations would aid clinicians in determining the best treatment options and give researchers important clues to modify and improve this therapeutic approach. Provided herein are compositions and methods addressing these and other needs in the art.

BRIEF SUMMARY OF THE INVENTION

[0004] In one aspect, an isolated nucleic acid is provided. The nucleic acid encodes a protein including (i) an antibody region including a central cavity formed by a heavy chain variable (VH) region, a light chain variable (VL) region, a heavy chain constant region (CH) and a light chain constant region (CL), wherein the central cavity forms a peptide binding site including framework region amino acid residues; and (ii) a transmembrane domain.

[0005] In one aspect, an isolated nucleic acid is provided. The nucleic acid encodes a protein including (i) an antibody region including a central cavity formed by a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the central cavity forms a peptide binding site including framework region amino acid residues; and (ii) a transmembrane domain.

[0006] In another aspect, an isolated nucleic acid is provided. The isolated nucleic acid encodes a protein including a first portion including an antibody heavy chain variable domain and a second portion including an antibody light chain variable domain and an antibody light chain constant domain, wherein the first portion further includes a transmembrane domain.

[0007] In another aspect, an expression vector including a nucleic acid provided herein including embodiments thereof is provided.

[0008] In another aspect, a T lymphocyte including the expression vector provided herein including embodiments thereof is provided.

[0009] In another aspect, a mammalian cell including the expression vector provided herein including embodiments thereof is provided.

[0010] In another aspect, a recombinant protein is provided. The recombinant protein includes (i) an antibody region including a central cavity formed by a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the central cavity forms a peptide binding site including framework region amino acid residues; and (ii) a transmembrane domain.

[0011] In another aspect, a recombinant protein is provided. The recombinant protein includes a first portion including an antibody heavy chain variable domain and a second portion including an antibody light chain variable domain and an antibody light chain constant domain, wherein the first portion further includes a transmembrane domain, and wherein the antibody heavy chain variable domain, the antibody light chain variable domain and the antibody light chain constant domain together form an antibody region.

[0012] In another aspect, a mammalian cell including the recombinant protein provided herein including embodiments thereof is provided, wherein the transmembrane domain is within the cell membrane of the mammalian cell.

[0013] In another aspect, a T lymphocyte including the recombinant protein provided herein including embodiments thereof is provided, wherein the transmembrane domain is within the cell membrane of the T lymphocyte.

[0014] In another aspect, a method of treating cancer is provided. The method includes administering to a subject in need thereof an effective amount of a mammalian cell provided herein including embodiments thereof, wherein the antibody region is an anti-cancer antibody region.

[0015] In another aspect, a method of treating cancer is provided. The method includes administering to a subject in need thereof an effective amount of the T-lymphocyte provided herein including embodiments thereof, wherein the antibody region is an anti-cancer antibody region.

[0016] In another aspect, a method of reprogramming a T lymphocyte is provided. The method includes contacting a T lymphocyte with the expression vector provided herein including embodiments thereof.

[0017] In another aspect, a method of detecting a cancer is provided. The method includes (i) administering to a cancer patient an effective amount of a T lymphocyte including the recombinant protein provided herein including embodiments thereof and a compound including a peptidyl moiety capable of binding to the peptide binding site, wherein the compound further includes a detectable label, and wherein the antibody region is an anti-cancer antibody region. The method includes (ii) allowing the compound to bind to the peptide binding site thereby forming a recombinant protein-compound complex. And (iii) the recombinant protein-compound complex is detected within the cancer patient thereby detecting the cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] FIG. 1A-1B. FIG.1A: Schematic representation of chimeric antigen receptors. To specifically target tumors, full mAbs or Fab fragments that recognize tumor associated antigens are directly fused to the transmembrane domain and zeta chain. In addition, the meditope binding site can be grafted onto the mAB/Fabs, providing an additional means of adding functionality to CAR T cells. FIG.1B. Since two chains have to be expressed (e.g., the light and

heavy chains of the mAb plus the transmembrane and intracellular signaling segments), there are two different possibility to express the different chains. Specifically, the light chain followed by the heavy chain or the heavy chain followed by the light chain.

[0019] FIG.2A-FIG.2B: HER2-specific CAR. FIG.2A. Diagram of the lentiviral CAR cassette for expressing the HER2-specific CAR, including the 2A ribosomal skip sequence and truncated CD19 (CD19t) which serves as an inert immunogenic marker of cell transduction. FIG.2B. Tcm lentivirally transduced to express HER2R:28ζ display stable cell surface expression of both the CAR and CD19 proteins.

[0020] FIG.3A-3B. HER2-28ζ Tcm kill both high and low expressing HER2 targets. FIG.3A. Flow cytometry analysis evaluating HER2 surface expression in a panel of breast tumor lines, and the HER2-negative U87 glioblastoma cell line. FIG.3B. 4-hour chromium release assay evaluating killing by HER2-28z CAR T cells demonstrates that both high and low-expressing HER2+ tumor lines are killed.

[0021] FIG.4A-4D. Meditope Studies: FIG.4A *Crystal structure*. 5-Diphenyl-meditope and protein L are bound to trastuzumab memAb. Extracellular domain of HER2 defining the antigen binding site, was superimposed using pdb 1n8z. Note: protein L and the meditope are distinct and distant from the antigen binding site. FIG.4B *FACS*. SKBR3 cells were treated with labeled trastuzumab (parental; memAb) and either sequentially or pre-mixed with labeled meditope-protein L (MPL6). Only the trastuzumab memAb shifts the meditope-Protein L, indicating that antigen binding does not preclude meditope binding. FIG.4C *Fluorescence microscopy*. GFP fused to meditope-Protein L (MPL6-GFP) colocalizes with trastuzumab memAb using SKBR3 cells (top row) but not with parental trastuzumab (bottom row). This indicates a bulky biologics does not impair antigen binding. FIG.4D *Super resolution microscopy*. Individual HER2 receptors can be visualized and quantified using paGFP fused to meditope-Protein L (MPL6-GFP) and trastuzumab memAb. Left panel shows entire cell. Right panel shows individual receptors.

[0022] FIG 5. HER2-scFv-CAR Tcm anti-tumor activity against intracranial engrafted breast tumors. Representative therapeutic responses to i.c. engrafted BT474 EGFP-ffLuc^{+} tumors (1×10^{5} cells) following intratumoral i.c. injection of HER2R-CAR T cells in NSG mice. On day 11, mice received either $1x10^{6}$ HER2-CAR Tcm, mock Tcm or PBS.

[0023] FIG 6. meHER2R-CAR T cells. Primary human T cells were lentivirally transduced to express either the HER2-scFv-CAR (HER2R-EQ-28Z) or the meditope enabled HER2-CAR

(meHER2R-dCH2-28Z). Cell surface expression of the meHER2-dCH2-28Z is confirmed by flow cytometry using anti-Fc antibody.

[0024] FIG 7. Expression of murine T84.66 and humanized M5A derived scFv-CEA-specific CARs in Primary Human T cells. Both muT84.66 and M5A derived CEA-scFv-CARs are stably expressed by engineered cells.

[0025] FIG 8A-8B. Comparing murine T84.66 versus humanized M5A scFv-CEA-specific CAR T cells for killing of CEA+ targets. FIG.8A: Target Cell Expression of CEA. FIG.8B: 4-hour Chromium Release Assay. muT84.66 derived CEA-scFv-CAR T cells recognize and kill CEA+ target cells in a 4-hour chromium release assay. By comparison the humanized M5A derived CEA-scFv-CAR T cells do not kill CEA+ Target cells.

[0026] FIG 9. Illustration of exemplary Fab and linker configuration.

[0027] FIG 10. CHO-S cells were transfected with either memAb trastuzumab Fab CAR (meCAR) or trastuzumab scFv-CAR (HER2CAR) plasmid for two days. The meCAR and HER2CAR construct differ only in the HER2-targeting component, each linked to CH3, CD28 transmembrane domain and CD3 zeta. A truncated CD19 can also be expressed from the plasmids and serves as a transfection control. The transfected CHO-S cells were filtered with 40 μm filter to remove debris and were washed once with 0.3% BSA-PBS. The cells were then labeled on ice for 1 h with: PE-anti-CD19 or isotype control, biotinylated anti-Fcγ followed by streptavidin-PE, or double-labeled with Pacific Blue-soluble HER2 and Alexa Fluor 488-meditope Fc (MFC). At the end of the incubations, the cells were washed once and resuspended in 800 μl of wash buffer. Sytox blue was added to the unlabeled cells for viability and membrane permeability assessments. Cells were gated with forward and side scatter only. Sytox Blue and Pacific Blue have considerable overlap in their spectra, thus the Sytox Blue signal of the unlabeled samples have "leaked" into the Pacific Blue channel. It has been noted that when CHO-S cells express membrane-bound proteins, their membrane becomes more permeable to Sytox Blue dye as shown with the group of cells in yellow oval. Both meCAR- and HER2CAR-transfected cells are CD19, CH3 and sHER2 positive, but only the meCAR is also positive for MFC binding.

[0028] FIG 11A-11C. Expression of meditope-enabled Fab-CARs in primary human T cells. FIG.11A , Schematic of meditope (me)-enabled trastuzumab Fab-CAR cassette (meHER2), with the T2A ribosomal skip sequence separating the antibody light chain (meLc) and the heavy chain fused to the IgG4-CH3-Fc linker, CD28 trasmembrane domain (Tm) and the CD28 and CD3ζ

cytoplasmic signaling domains (Hc28ζ). Expression is driven by the EF1α promoter and was tested in two orientations: Lc-Hc28ζ and Hc28ζ-Lc. FIG.11B, Primary human T cells were lentivirally transduced and expression of the meHER2-CARs was evaluated by flow cytometry. Protein L staining, which binds the Fab light chain, confirms cell surface expression of both CAR orientations, with higher expression levels (MFI) observed for the Hc28 ζ –Lc (MFI 5357) vs Lc-Hc28 ζ (MFI 2592) orientation. Meditope-AF647 staining confirms the functional formation of the CAR meditope pocket, with greater binding to the Hc28 ζ –Lc (MFI 6042) vs Lc-Hc28 ζ (MFI 2121) orientation. FIG.11C, meHER2-CAR T cells pre-bound to meditope peptide retain the ability to bind protein L and soluble HER2-antigen, suggesting that meditope binding does not alter antigen binding properties and structural components of the Fab.

[0029] FIG 12A-12D. Meditope-enabled HER2-CAR (meHER2) T cells degranulate at comparable levels to scFvHER2-CAR T cells in response to HER2+ targets and meditope peptide does not negatively impact T cell degranulation. FIG.12A, HER2+ breast cancer lines MCF-7 and SK-BR-3 were assessed for cell surface expression of HER2 by flow cytometry (Biolegend; Cat#324413). MCF-7 expresses relatively low levels of HER2 as compared to SK-BR-3 which over-expresses HER2. FIG.12B-12D CD107a degranulation assay. HD187.2 T cells were engineered to express either meHER2(Hc-Lc):28ζ CAR, meHER2(Lc-Hc):28ζ CAR, scFvHER2:28ζ CAR or no CAR (mock). Versions of the meHER2:28ζ-CAR differ only in the orientation of the heavy chain (Hc) and light chain (Lc), see Fig. 11. FIG.12B Representative FACs showing CD107a degranulation for mock T cells (negative control) and scFvHER2-CAR T cells (positive control) following co-culture at a 1:1 effector to MCF-7 ratio (based on CAR expression) for 5 hours. CD107a degranulation (BD Pharmingen™; Cat# 555800), gated on CAR+ CD8+ cells, was detected by flow cytometry (Miltenyi Biotec; MACSQuant) and analyzed using FCS Express (De Novo Software). FIG.12C meHER2(Hc-Lc):28ζ and meHER2(Lc-Hc):28ζ T cells were incubated with and without meditope-AF647 (ME;200nM) and degranulation to MCF-7 targets was evaluated as described in FIG.12B. FIG.12D, Bar graph depicting comparable degranulation of all meHER2 and scFvHER2-CAR T cell lines to either MCF-7 or SK-BR-3. meHER2-CAR T cells incubated with and without meditope peptide show comparable activation as assessed by CD107a degranulation. Plotted are average and standard deviation of three wells per condition. Cells were gated on the $CD8^{+}CAR^{+}$ population.

[0030] FIG 13A-13B. Meditope-enabled HER2-CARs (meHER2) and scFvHER2 CAR-engineered T cells kill HER2+ targets at comparable efficiency and meditope peptide does not negatively impact T cell killing. Long term killing assay to compare killing potency of meHER2

and scFvHER2-CAR T cells. HD187.2 T cells were engineered to express either meHER2(Hc-Lc):28ζ CAR, meHER2(Lc-Hc):28ζ CAR, scFvHER2:28ζ CAR or no CAR (mock). Versions of the meHER2:28ζ-CAR differ only in the orientation of the heavy chain (Hc) and light chain (Lc), see FIG.11. HER2-CAR T cells lines, or mock control were co-cultured with $HER2^+$ breast cancer lines, MCF-7 and SKBR3, for 48-hours at a 1:4 effector to target ratio (based on CAR expression). Killing was assessed by quantifying the number of live tumor cells remaining after co-incubation. A viability stain, DAPI (Molecular Probes™; Cat# D21490) and a human leukocyte antigen, CD45 (BD Biosciences; Cat# 347464), were used to exclude the dead cells and T cells from the live tumor count. meCAR-T cells were incubated with and without meditope-AF647 (200nM) prior to co-culture to evaluate the impact of meditope peptide on meHER2 redirected killing potential, . FIG.13A**,** Bar graph depicts the average live tumor count (DAPI-CD45-) of three replicate wells per combination. FIG.13B**,** Bar graph represents the percent tumor killed per condition when normalized to mock.

DETAILED DESCRIPTION OF THE INVENTION

DEFINITIONS

[0031] While various embodiments and aspects of the present invention are shown and described herein, it will be obvious to those skilled in the art that such embodiments and aspects are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention.

[0032] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited in the application including, without limitation, patents, patent applications, articles, books, manuals, and treatises are hereby expressly incorporated by reference in their entirety for any purpose.

[0033] The abbreviations used herein have their conventional meaning within the chemical and biological arts. The chemical structures and formulae set forth herein are constructed according to the standard rules of chemical valency known in the chemical arts.

[0034] Where substituent groups are specified by their conventional chemical formulae, written from left to right, they equally encompass the chemically identical substituents that would result from writing the structure from right to left, e.g., $-CH_2O-$ is equivalent to $-OCH_2-$.

[0035] The term "alkyl," by itself or as part of another substituent, means, unless otherwise stated, a straight (i.e., unbranched) or branched non-cyclic carbon chain (or carbon), or combination thereof, which may be fully saturated, mono- or polyunsaturated and can include di- and multivalent radicals, having the number of carbon atoms designated (i.e., C_1-C_{10} means one to ten carbons). Examples of saturated hydrocarbon radicals include, but are not limited to, groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, (cyclohexyl)methyl, homologs and isomers of, for example, n-pentyl, n-hexyl, n-heptyl, n-octyl and the like. An unsaturated alkyl group is one having one or more double bonds or triple bonds. Examples of unsaturated alkyl groups include, but are not limited to, vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butynyl, and the higher homologs and isomers. An alkoxy is an alkyl attached to the remainder of the molecule via an oxygen linker (-O-). An alkyl moiety may be an alkenyl moiety. An alkyl moiety may be an alkynyl moiety. An alkyl moiety may be fully saturated.

[0036] The term "alkylene," by itself or as part of another substituent, means, unless otherwise stated, a divalent radical derived from an alkyl, as exemplified, but not limited by, $-CH_2CH_2CH_2CH_2-$. Typically, an alkyl (or alkylene) group will have from 1 to 24 carbon atoms, with those groups having 10 or fewer carbon atoms being preferred in the present invention. A "lower alkyl" or "lower alkylene" is a shorter chain alkyl or alkylene group, generally having eight or fewer carbon atoms. The term "alkenylene," by itself or as part of another substituent, means, unless otherwise stated, a divalent radical derived from an alkene.

[0037] The term "heteroalkyl," by itself or in combination with another term, means, unless otherwise stated, a stable non-cyclic straight or branched chain, or combinations thereof, including at least one carbon atom and at least one heteroatom (e.g. O, N, P, Si or S) and wherein the nitrogen and sulfur atoms may optionally be oxidized, and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) O, N, P, S, and Si may be placed at any interior position of the heteroalkyl group or at the position at which the alkyl group is attached to the remainder of the molecule. Examples include, but are not limited to: $-CH_2-CH_2-O-CH_3$, $-CH_2-CH_2-NH-CH_3$, $-CH_2-CH_2-N(CH_3)-CH_3$, $-CH_2-S-CH_2-CH_3$, $-CH_2-CH_2$, $-S(O)-CH_3$, $-CH_2-CH_2-S(O)_2-CH_3$, $-CH=CH-O-CH_3$, $-Si(CH_3)_3$, $-CH_2-CH=N-OCH_3$, $-CH=CH-N(CH_3)-CH_3$, $-O-CH_3$, $-O-CH_2-CH_3$, and -CN. Up to two or three heteroatoms may be consecutive, such as, for example, $-CH_2-NH-OCH_3$ and $-CH_2-O-Si(CH_3)_3$. A heteroalkyl moiety may include one heteroatom (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include two optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include three

optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include four optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include five optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include up to 8 optionally different heteroatoms (e.g., O, N, S, Si, or P).

[0038] Similarly, the term "heteroalkylene," by itself or as part of another substituent, means, unless otherwise stated, a divalent radical derived from heteroalkyl, as exemplified, but not limited by, $-CH_2-CH_2-S-CH_2-CH_2-$ and $-CH_2-S-CH_2-CH_2-NH-CH_2-$. For heteroalkylene groups, heteroatoms can also occupy either or both of the chain termini (e.g., alkyleneoxy, alkylenedioxy, alkyleneamino, alkylenediamino, and the like). Still further, for alkylene and heteroalkylene linking groups, no orientation of the linking group is implied by the direction in which the formula of the linking group is written. For example, the formula $-C(O)_2R'-$ represents both $-C(O)_2R'-$ and $-R'C(O)_2-$. As described above, heteroalkyl groups, as used herein, include those groups that are attached to the remainder of the molecule through a heteroatom, such as -C(O)R', -C(O)NR', -NR'R", -OR', -SR', and/or $-SO_2R'$. Where "heteroalkyl" is recited, followed by recitations of specific heteroalkyl groups, such as -NR'R" or the like, it will be understood that the terms heteroalkyl and -NR'R" are not redundant or mutually exclusive. Rather, the specific heteroalkyl groups are recited to add clarity. Thus, the term "heteroalkyl" should not be interpreted herein as excluding specific heteroalkyl groups, such as -NR'R" or the like.

[0039] The terms "cycloalkyl" and "heterocycloalkyl," by themselves or in combination with other terms, mean, unless otherwise stated, non-aromatic cyclic versions of "alkyl" and "heteroalkyl," respectively, wherein the carbons making up the ring or rings do not necessarily need to be bonded to a hydrogen due to all carbon valencies participating in bonds with non-hydrogen atoms. Additionally, for heterocycloalkyl, a heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyl include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, 3-hydroxy-cyclobut-3-enyl-1,2, dione, 1H-1,2,4-triazolyl-5(4H)-one, 4H-1,2,4-triazolyl, and the like. Examples of heterocycloalkyl include, but are not limited to, 1-(1,2,5,6-tetrahydropyridyl), 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-morpholinyl, 3-morpholinyl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1-piperazinyl, 2-piperazinyl , and the like. A "cycloalkylene" and a "heterocycloalkylene," alone or as part of another substituent, means a divalent radical derived from a cycloalkyl and heterocycloalkyl, respectively. A heterocycloalkyl moiety may include one ring heteroatom

(e.g., O, N, S, Si, or P). A heterocycloalkyl moiety may include two optionally different ring heteroatoms (e.g., O, N, S, Si, or P). A heterocycloalkyl moiety may include three optionally different ring heteroatoms (e.g., O, N, S, Si, or P). A heterocycloalkyl moiety may include four optionally different ring heteroatoms (e.g., O, N, S, Si, or P). A heterocycloalkyl moiety may include five optionally different ring heteroatoms (e.g., O, N, S, Si, or P). A heterocycloalkyl moiety may include up to 8 optionally different ring heteroatoms (e.g., O, N, S, Si, or P).

[0040] The terms "halo" or "halogen," by themselves or as part of another substituent, mean, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom. Additionally, terms such as "haloalkyl" are meant to include monohaloalkyl and polyhaloalkyl. For example, the term "halo(C_1-C_4)alkyl" includes, but is not limited to, fluoromethyl, difluoromethyl, trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, and the like.

[0041] The term "acyl" means, unless otherwise stated, -C(O)R where R is a substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl.

[0042] The term "aryl" means, unless otherwise stated, a polyunsaturated, aromatic, hydrocarbon substituent, which can be a single ring or multiple rings (preferably from 1 to 3 rings) that are fused together (i.e., a fused ring aryl) or linked covalently. A fused ring aryl refers to multiple rings fused together wherein at least one of the fused rings is an aryl ring. The term "heteroaryl" refers to aryl groups (or rings) that contain at least one heteroatom such as N, O, or S, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. Thus, the term "heteroaryl" includes fused ring heteroaryl groups (i.e., multiple rings fused together wherein at least one of the fused rings is a heteroaromatic ring). A 5,6-fused ring heteroarylene refers to two rings fused together, wherein one ring has 5 members and the other ring has 6 members, and wherein at least one ring is a heteroaryl ring. Likewise, a 6,6-fused ring heteroarylene refers to two rings fused together, wherein one ring has 6 members and the other ring has 6 members, and wherein at least one ring is a heteroaryl ring. And a 6,5-fused ring heteroarylene refers to two rings fused together, wherein one ring has 6 members and the other ring has 5 members, and wherein at least one ring is a heteroaryl ring. A heteroaryl group can be attached to the remainder of the molecule through a carbon or heteroatom. Non-limiting examples of aryl and heteroaryl groups include phenyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-

isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxalinyl, 5-quinoxalinyl, 3-quinolyl, and 6-quinolyl. Substituents for each of the above noted aryl and heteroaryl ring systems are selected from the group of acceptable substituents described below. An "arylene" and a "heteroarylene," alone or as part of another substituent, mean a divalent radical derived from an aryl and heteroaryl, respectively. Non-limiting examples of aryl and heteroaryl groups include pyridinyl, pyrimidinyl, thiophenyl, thienyl, furanyl, indolyl, benzoxadiazolyl, benzodioxolyl, benzodioxanyl, thianaphthanyl, pyrrolopyridinyl, indazolyl, quinolinyl, quinoxalinyl, pyridopyrazinyl, quinazolinonyl, benzoisoxazolyl, imidazopyridinyl, benzofuranyl, benzothienyl, benzothiophenyl, phenyl, naphthyl, biphenyl, pyrrolyl, pyrazolyl, imidazolyl, pyrazinyl, oxazolyl, isoxazolyl, thiazolyl, furylthienyl, pyridyl, pyrimidyl, benzothiazolyl, purinyl, benzimidazolyl, isoquinolyl, thiadiazolyl, oxadiazolyl, pyrrolyl, diazolyl, triazolyl, tetrazolyl, benzothiadiazolyl, isothiazolyl, pyrazolopyrimidinyl, pyrrolopyrimidinyl, benzotriazolyl, benzoxazolyl, or quinolyl. The examples above may be substituted or unsubstituted and divalent radicals of each heteroaryl example above are non-limiting examples of heteroarylene. A heteroaryl moiety may include one ring heteroatom (e.g., O, N, or S). A heteroaryl moiety may include two optionally different ring heteroatoms (e.g., O, N, or S). A heteroaryl moiety may include three optionally different ring heteroatoms (e.g., O, N, or S). A heteroaryl moiety may include four optionally different ring heteroatoms (e.g., O, N, or S). A heteroaryl moiety may include five optionally different ring heteroatoms (e.g., O, N, or S). An aryl moiety may have a single ring. An aryl moiety may have two optionally different rings. An aryl moiety may have three optionally different rings. An aryl moiety may have four optionally different rings. A heteroaryl moiety may have one ring. A heteroaryl moiety may have two optionally different rings. A heteroaryl moiety may have three optionally different rings. A heteroaryl moiety may have four optionally different rings. A heteroaryl moiety may have five optionally different rings.

[0043] A fused ring heterocycloalkyl-aryl is an aryl fused to a heterocycloalkyl. A fused ring heterocycloalkyl-heteroaryl is a heteroaryl fused to a heterocycloalkyl. A fused ring heterocycloalkyl-cycloalkyl is a heterocycloalkyl fused to a cycloalkyl. A fused ring heterocycloalkyl-heterocycloalkyl is a heterocycloalkyl fused to another heterocycloalkyl. Fused ring heterocycloalkyl-aryl, fused ring heterocycloalkyl-heteroaryl, fused ring heterocycloalkyl-cycloalkyl, or fused ring heterocycloalkyl-heterocycloalkyl may each independently be unsubstituted or substituted with one or more of the substituents described herein.

[0044] The term "oxo," as used herein, means an oxygen that is double bonded to a carbon atom.

[0045] The term "alkylsulfonyl," as used herein, means a moiety having the formula -S(O_2)-R', where R' is a substituted or unsubstituted alkyl group as defined above. R' may have a specified number of carbons (e.g., "C_1-C_4 alkylsulfonyl").

[0046] Each of the above terms (e.g., "alkyl," "heteroalkyl,", "cycloalkyl", "heterocycloalkyl", "aryl," and "heteroaryl") includes both substituted and unsubstituted forms of the indicated radical. Preferred substituents for each type of radical are provided below.

[0047] Substituents for the alkyl and heteroalkyl radicals (including those groups often referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) can be one or more of a variety of groups selected from, but not limited to, -OR', =O, =NR', =N-OR', -NR'R", -SR', -halogen, -SiR'R"R'", -OC(O)R', -C(O)R', -CO_2R', -CONR'R", -OC(O)NR'R", -NR"C(O)R', -NR'-C(O)NR"R'", -NR"C(O_2)R', -NR-C(NR'R")=NR'", -S(O)R', -S(O_2)R', -S(O_2)N(R)('R"-NRSO_2R'), -CN, and -NO_2 in a number ranging from zero to (2m'+1), where m' is the total number of carbon atoms in such radical. R', R", R'", and R"" each preferably independently refer to hydrogen, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl (e.g., aryl substituted with 1-3 halogens), substituted or unsubstituted alkyl, alkoxy, or thioalkoxy groups, or arylalkyl groups. When a compound of the invention includes more than one R group, for example, each of the R groups is independently selected as are each R', R", R'", and R"" group when more than one of these groups is present. When R' and R" are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 4-, 5-, 6-, or 7-membered ring. For example, -NR'R" includes, but is not limited to, 1-pyrrolidinyl and 4-morpholinyl. From the above discussion of substituents, one of skill in the art will understand that the term "alkyl" is meant to include groups including carbon atoms bound to groups other than hydrogen groups, such as haloalkyl (e.g., -CF_3 and -CH_2CF_3) and acyl (e.g., -C(O)CH_3, -C(O)CF_3, -C(O)CH_2OCH_3, and the like).

[0048] Similar to the substituents described for the alkyl radical, substituents for the aryl and heteroaryl groups are varied and are selected from, for example: -OR', -NR'R", -SR', -halogen, -SiR'R"R'", -OC(O)R', -C(O)R', -CO_2R', -CONR'R", -OC(O)NR'R", -NR"C(O)R', -NR'-C(O)NR"R'", NR"C(O_2)R', NRC(NR'R")=NR'", S(O)R', -S(O_2)R',

-$S(O)_2N(R')(R''$, -$NRSO_2R'$), -CN, -NO_2, -R', -N_3, -$CH(Ph)_2$, fluoro(C_1-C_4)alkoxy, and fluoro(C_1-C_4)alkyl, in a number ranging from zero to the total number of open valences on the aromatic ring system; and where R', R'', R''', and R'''' are preferably independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl. When a compound of the invention includes more than one R group, for example, each of the R groups is independently selected as are each R', R'', R''', and R'''' groups when more than one of these groups is present.

[0049] Where a moiety is substituted with an R substituent, the group may be referred to as "R-substituted." Where a moiety is R-substituted, the moiety is substituted with at least one R substituent and each R substituent is optionally different. For example, where a moiety herein is R^{1A}-substituted or unsubstituted alkyl, a plurality of R^{1A} substituents may be attached to the alkyl moiety wherein each R^{1A} substituent is optionally different. Where an R-substituted moiety is substituted with a plurality R substituents, each of the R-substituents may be differentiated herein using a prime symbol (') such as R', R'', etc. For example, where a moiety is R^{3A}-substituted or unsubstituted alkyl, and the moiety is substituted with a plurality of R^{3A} substituents, the plurality of R^{3A} substituents may be differentiated as $R^{3A'}$, $R^{3A''}$, $R^{3A'''}$, etc. In some embodiments, the plurality of R substituents is 3. In some embodiments, the plurality of R substituents is 2.

[0050] Two or more substituents may optionally be joined to form aryl, heteroaryl, cycloalkyl, or heterocycloalkyl groups. Such so-called ring-forming substituents are typically, though not necessarily, found attached to a cyclic base structure. In one embodiment, the ring-forming substituents are attached to adjacent members of the base structure. For example, two ring-forming substituents attached to adjacent members of a cyclic base structure create a fused ring structure. In another embodiment, the ring-forming substituents are attached to a single member of the base structure. For example, two ring-forming substituents attached to a single member of a cyclic base structure create a spirocyclic structure. In yet another embodiment, the ring-forming substituents are attached to non-adjacent members of the base structure.

[0051] Two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally form a ring of the formula -T-C(O)-$(CRR')_q$-U-, wherein T and U are independently -NR-, -O-, -CRR'-, or a single bond, and q is an integer of from 0 to 3. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -A-$(CH_2)_r$-B-, wherein A and B are

independently -CRR'-, -O-, -NR-, -S-, -S(O) -, -$S(O)_2$-, -$S(O)_2$NR'-, or a single bond, and r is an integer of from 1 to 4. One of the single bonds of the new ring so formed may optionally be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -$(CRR')_s$-X'- $(C''R''R''')_d$-, where variables s and d are independently integers of from 0 to 3, and X' is -O-, -NR'-, -S-, -S(O)-, -$S(O)_2$-, or -$S(O)_2$NR'-. The substituents R, R', R'', and R''' are preferably independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl.

[0052] As used herein, the terms "heteroatom" or "ring heteroatom" are meant to include, oxygen (O), nitrogen (N), sulfur (S), phosphorus (P), and silicon (Si).

[0053] A "substituent group," as used herein, means a group selected from the following moieties:

(A) oxo, halogen, -CF_3, -CN, -OH, -NH_2, -COOH, -$CONH_2$, -NO_2, -SH, -SO_2Cl, -SO_3H, -SO_4H, -SO_2NH_2, –$NHNH_2$, –ONH_2, –NHC=(O)$NHNH_2$, –NHC=(O) NH_2, -$NHSO_2H$, -NHC= (O)H, -NHC(O)-OH, -NHOH, -OCF_3, -$OCHF_2$, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl, and

(B) alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, substituted with at least one substituent selected from:

(i) oxo, halogen, -CF_3, -CN, -OH, -NH_2, -COOH, -$CONH_2$, -NO_2, -SH, -SO_2Cl, -SO_3H, -SO_4H, -SO_2NH_2, –$NHNH_2$, –ONH_2, –NHC=(O)$NHNH_2$, –NHC=(O) NH_2, -$NHSO_2H$, -NHC= (O)H, -NHC(O)-OH, -NHOH, -OCF_3, -$OCHF_2$, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl, and

(ii) alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, substituted with at least one substituent selected from:

(a) oxo, halogen, -CF_3, -CN, -OH, -NH_2, -COOH, -$CONH_2$, -NO_2, -SH, -SO_2Cl, -SO_3H, -SO_4H, -SO_2NH_2, –$NHNH_2$, –ONH_2, –NHC=(O)$NHNH_2$, –NHC=(O) NH_2, -$NHSO_2H$, -NHC= (O)H, -NHC(O)-OH, -NHOH, -OCF_3, -$OCHF_2$, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl, and

(b) alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, substituted with at least one substituent selected from: oxo, halogen, $-CF_3$, -CN, -OH, $-NH_2$, -COOH, $-CONH_2$, $-NO_2$, -SH, $-SO_2Cl$, $-SO_3H$, $-SO_4H$, $-SO_2NH_2$, $-NHNH_2$, $-ONH_2$, $-NHC=(O)NHNH_2$, $-NHC=(O)\ NH_2$, $-NHSO_2H$, -NHC= (O)H, -NHC(O)-OH, -NHOH, $-OCF_3$, $-OCHF_2$, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl.

[0054] A "size-limited substituent" or "size-limited substituent group," as used herein, means a group selected from all of the substituents described above for a "substituent group," wherein each substituted or unsubstituted alkyl is a substituted or unsubstituted C_1-C_{20} alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 20 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C_3-C_8 cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 8 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C_6-C_{10} aryl, and each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 10 membered heteroaryl.

[0055] A "lower substituent" or "lower substituent group," as used herein, means a group selected from all of the substituents described above for a "substituent group," wherein each substituted or unsubstituted alkyl is a substituted or unsubstituted C_1-C_8 alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 8 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C_3-C_7 cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 7 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C_6-C_{10} aryl, and each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 9 membered heteroaryl.

[0056] In some embodiments, each substituted group described in the compounds herein is substituted with at least one substituent group. More specifically, in some embodiments, each substituted alkyl, substituted heteroalkyl, substituted cycloalkyl, substituted heterocycloalkyl, substituted aryl, substituted heteroaryl, substituted alkylene, substituted heteroalkylene, substituted cycloalkylene, substituted heterocycloalkylene, substituted arylene, and/or substituted heteroarylene described in the compounds herein are substituted with at least one substituent group. In other embodiments, at least one or all of these groups are substituted with at least one

size-limited substituent group. In other embodiments, at least one or all of these groups are substituted with at least one lower substituent group.

[0057] In other embodiments of the compounds herein, each substituted or unsubstituted alkyl may be a substituted or unsubstituted C_1-C_{20} alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 20 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C_3-C_8 cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 8 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C_6-C_{10} aryl, and/or each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 10 membered heteroaryl. In some embodiments of the compounds herein, each substituted or unsubstituted alkylene is a substituted or unsubstituted C_1-C_{20} alkylene, each substituted or unsubstituted heteroalkylene is a substituted or unsubstituted 2 to 20 membered heteroalkylene, each substituted or unsubstituted cycloalkylene is a substituted or unsubstituted C_3-C_8 cycloalkylene, each substituted or unsubstituted heterocycloalkylene is a substituted or unsubstituted 3 to 8 membered heterocycloalkylene, each substituted or unsubstituted arylene is a substituted or unsubstituted C_6-C_{10} arylene, and/or each substituted or unsubstituted heteroarylene is a substituted or unsubstituted 5 to 10 membered heteroarylene.

[0058] In some embodiments, each substituted or unsubstituted alkyl is a substituted or unsubstituted C_1-C_8 alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 8 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C_3-C_7 cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 7 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C_6-C_{10} aryl, and/or each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 9 membered heteroaryl. In some embodiments, each substituted or unsubstituted alkylene is a substituted or unsubstituted C_1-C_8 alkylene, each substituted or unsubstituted heteroalkylene is a substituted or unsubstituted 2 to 8 membered heteroalkylene, each substituted or unsubstituted cycloalkylene is a substituted or unsubstituted C_3-C_7 cycloalkylene, each substituted or unsubstituted heterocycloalkylene is a substituted or unsubstituted 3 to 7 membered heterocycloalkylene, each substituted or unsubstituted arylene is a substituted or unsubstituted C_6-C_{10} arylene, and/or each substituted or unsubstituted heteroarylene is a substituted or unsubstituted 5 to 9 membered heteroarylene. In some embodiments, the compound is a chemical species set forth in the Examples section, figures, or tables below.

[0059] As used herein, the term "conjugate" refers to the association between atoms or molecules. The association can be direct or indirect. For example, a conjugate between a nucleic acid and a protein can be direct, e.g., by covalent bond, or indirect, e.g., by non-covalent bond (e.g. electrostatic interactions (e.g. ionic bond, hydrogen bond, halogen bond), van der Waals interactions (e.g. dipole-dipole, dipole-induced dipole, London dispersion), ring stacking (pi effects), hydrophobic interactions and the like). In embodiments, conjugates are formed using conjugate chemistry including, but are not limited to nucleophilic substitutions (e.g., reactions of amines and alcohols with acyl halides, active esters), electrophilic substitutions (e.g., enamine reactions) and additions to carbon-carbon and carbon-heteroatom multiple bonds (e.g., Michael reaction, Diels-Alder addition). These and other useful reactions are discussed in, for example, March, ADVANCED ORGANIC CHEMISTRY, 3rd Ed., John Wiley & Sons, New York, 1985; Hermanson, BIOCONJUGATE TECHNIQUES, Academic Press, San Diego, 1996; and Feeney et al., MODIFICATION OF PROTEINS; Advances in Chemistry Series, Vol. 198, American Chemical Society, Washington, D.C., 1982. In embodiments, the microparticle is non-covalently attached to solid support through a non-covalent chemical reaction between a component of the microparticle and a component of solid support. In other embodiments, the microparticle includes one or more reactive moieties, e.g., a covalent reactive moiety, as described herein (e.g., an amine reactive moiety). In other embodiments, the microparticle includes a linker with one or more reactive moieties, e.g., a covalent reactive moiety, as described herein (e.g., an amine reactive moiety).

[0060] Useful reactive moieties or functional groups used for conjugate chemistries (including "click chemistries" as known in the art) herein include, for example:

(a) carboxyl groups and various derivatives thereof including, but not limited to, N-hydroxysuccinimide esters, N-hydroxybenztriazole esters, acid halides, acyl imidazoles, thioesters, p-nitrophenyl esters, alkyl, alkenyl, alkynyl and aromatic esters;

(b) hydroxyl groups which can be converted to esters, ethers, aldehydes, etc.

(c) haloalkyl groups wherein the halide can be later displaced with a nucleophilic group such as, for example, an amine, a carboxylate anion, thiol anion, carbanion, or an alkoxide ion, thereby resulting in the covalent attachment of a new group at the site of the halogen atom;

(d) dienophile groups which are capable of participating in Diels-Alder reactions such as, for example, maleimido groups;

(e) aldehyde or ketone groups such that subsequent derivatization is possible via formation of carbonyl derivatives such as, for example, imines, hydrazones, semicarbazones or oximes, or via such mechanisms as Grignard addition or alkyllithium addition;

(f) sulfonyl halide groups for subsequent reaction with amines, for example, to form sulfonamides;

(g) thiol groups, which can be converted to disulfides, reacted with acyl halides, or bonded to metals such as gold;

(h) amine or sulfhydryl groups, which can be, for example, acylated, alkylated or oxidized;

(i) alkenes, which can undergo, for example, cycloadditions, acylation, Michael addition, etc.;

(j) epoxides, which can react with, for example, amines and hydroxyl compounds;

(k) phosphoramidites and other standard functional groups useful in nucleic acid synthesis;

(l) metal silicon oxide bonding;

(m) metal bonding to reactive phosphorus groups (e.g. phosphines) to form, for example, phosphate diester bonds; and

(n) sulfones, for example, vinyl sulfone.

[0061] Chemical synthesis of compositions by joining small modular units using conjugate ("click") chemistry is well known in the art and described, for example, in H. C. Kolb, M. G. Finn and K. B. Sharpless ((2001). "Click Chemistry: Diverse Chemical Function from a Few Good Reactions". Angewandte Chemie International Edition 40 (11): 2004–2021); R. A. Evans ((2007). "The Rise of Azide–Alkyne 1,3-Dipolar 'Click' Cycloaddition and its Application to Polymer Science and Surface Modification". Australian Journal of Chemistry 60 (6): 384–395; W.C. Guida et al. Med. Res. Rev. p 3 1996; Spiteri, Christian and Moses, John E. ((2010). "Copper-Catalyzed Azide–Alkyne Cycloaddition: Regioselective Synthesis of 1,4,5-Trisubstituted 1,2,3-Triazoles". Angewandte Chemie International Edition 49 (1): 31–33); Hoyle, Charles E. and Bowman, Christopher N. ((2010). "Thiol–Ene Click Chemistry". Angewandte Chemie International Edition 49 (9): 1540–1573); Blackman, Melissa L. and Royzen, Maksim and Fox, Joseph M. ((2008). "Tetrazine Ligation: Fast Bioconjugation Based on Inverse-Electron-Demand Diels−Alder Reactivity". Journal of the American Chemical Society 130 (41): 13518–13519); Devaraj, Neal K. and Weissleder, Ralph and Hilderbrand, Scott A. ((2008). "Tetrazine Based Cycloadditions: Application to Pretargeted Live Cell Labeling". Bioconjugate Chemistry 19 (12): 2297–2299); Stöckmann, Henning; Neves, Andre; Stairs, Shaun; Brindle, Kevin; Leeper, Finian ((2011). "Exploring isonitrile-based click chemistry for ligation with biomolecules". Organic & Biomolecular Chemistry), all of which are hereby incorporated by reference in their entirety and for all purposes.

[0062] The reactive functional groups can be chosen such that they do not participate in, or interfere with, the chemical stability of the proteins or nucleic acids described herein. By way of example, the nucleic acids can include a vinyl sulfone or other reactive moiety (e.g., maleimide). Optionally, the nucleic acids can include a reactive moiety having the formula -S-S-R. R can be, for example, a protecting group. Optionally, R is hexanol. As used herein, the term hexanol includes compounds with the formula $C_6H_{13}OH$ and includes, 1-hexanol, 2-hexanol, 3-hexanol, 2-methyl-1-pentanol, 3-methyl-1-pentanol, 4-methyl-1-pentanol, 2-methyl-2-pentanol, 3-methyl-2-pentanol, 4-methyl-2-pentanol, 2-methyl-3-pentanol, 3-methyl-3-pentanol, 2,2-dimethyl-1-butanol, 2,3-dimethyl-1-butanol, 3,3-dimethyl-1-butanol, 2,3-dimethyl-2-butanol, 3,3-dimethyl-2-butanol, and 2-ethyl-1-butanol. Optionally, R is 1-hexanol.

[0063] As used herein, the term "about" means a range of values including the specified value, which a person of ordinary skill in the art would consider reasonably similar to the specified value. In embodiments, the term "about" means within a standard deviation using measurements generally acceptable in the art. In embodiments, about means a range extending to +/- 10% of the specified value. In embodiments, about means the specified value.

[0064] The terms "a" or "an," as used in herein means one or more. In addition, the phrase "substituted with a[n]," as used herein, means the specified group may be substituted with one or more of any or all of the named substituents. For example, where a group, such as an alkyl or heteroaryl group, is "substituted with an unsubstituted C_1-C_{20} alkyl, or unsubstituted 2 to 20 membered heteroalkyl," the group may contain one or more unsubstituted C_1-C_{20} alkyls, and/or one or more unsubstituted 2 to 20 membered heteroalkyls. Moreover, where a moiety is substituted with an R substituent, the group may be referred to as “R-substituted.” Where a moiety is R-substituted, the moiety is substituted with at least one R substituent and each R substituent is optionally different.

[0065] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by a person of ordinary skill in the art. See, e.g., Singleton et al., DICTIONARY OF MICROBIOLOGY AND MOLECULAR BIOLOGY 2nd ed., J. Wiley & Sons (New York, NY 1994); Sambrook et al., MOLECULAR CLONING, A LABORATORY MANUAL, Cold Springs Harbor Press (Cold Springs Harbor, NY 1989). Any methods, devices and materials similar or equivalent to those described herein can be used in the practice of this invention. The following definitions are provided to facilitate understanding of certain terms used frequently herein and are not meant to limit the scope of the present disclosure.

[0066] "Biological sample" or "sample" refer to materials obtained from or derived from a subject or patient. A biological sample includes sections of tissues such as biopsy and autopsy samples, and frozen sections taken for histological purposes. Such samples include bodily fluids such as blood and blood fractions or products (e.g., serum, plasma, platelets, red blood cells, and the like), sputum, tissue, cultured cells (e.g., primary cultures, explants, and transformed cells) stool, urine, synovial fluid, joint tissue, synovial tissue, synoviocytes, fibroblast-like synoviocytes, macrophage-like synoviocytes, immune cells, hematopoietic cells, fibroblasts, macrophages, T cells, etc. A biological sample is typically obtained from a eukaryotic organism, such as a mammal such as a primate e.g., chimpanzee or human; cow; dog; cat; a rodent, e.g., guinea pig, rat, mouse; rabbit; or a bird; reptile; or fish.

[0067] A "cell" as used herein, refers to a cell carrying out metabolic or other functions sufficient to preserve or replicate its genomic DNA. A cell can be identified by well-known methods in the art including, for example, presence of an intact membrane, staining by a particular dye, ability to produce progeny or, in the case of a gamete, ability to combine with a second gamete to produce a viable offspring. Cells may include prokaryotic and eukaryotic cells. Prokaryotic cells include but are not limited to bacteria. Eukaryotic cells include but are not limited to yeast cells and cells derived from plants and animals, for example mammalian, insect (*e.g.*, spodoptera) and human cells. Cells may be useful when they are naturally nonadherent or have been treated not to adhere to surfaces, for example by trypsinization.

[0068] The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues, wherein the polymer may optionally be conjugated to a moiety that does not consist of amino acids. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers. A "fusion protein" refers to a chimeric protein encoding two or more separate protein sequences that are recombinantly expressed as a single moiety.

[0069] The term "peptidyl" and "peptidyl moiety" refers to a monovalent peptide.

[0070] A "label" or a "detectable moiety" is a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, chemical, or other physical means. For example, useful labels include ^{32}P, fluorescent dyes, electron-dense reagents, enzymes (e.g., as commonly used in an ELISA), biotin, digoxigenin, or haptens and proteins or other entities which can be made detectable, e.g., by incorporating a radiolabel into a peptide or antibody specifically

reactive with a target peptide. Any appropriate method known in the art for conjugating an antibody to the label may be employed, e.g., using methods described in Hermanson, Bioconjugate Techniques 1996, Academic Press, Inc., San Diego.

[0071] A "labeled protein or polypeptide" is one that is bound, either covalently, through a linker or a chemical bond, or noncovalently, through ionic, van der Waals, electrostatic, or hydrogen bonds to a label such that the presence of the labeled protein or polypeptide may be detected by detecting the presence of the label bound to the labeled protein or polypeptide. Alternatively, methods using high affinity interactions may achieve the same results where one of a pair of binding partners binds to the other, *e.g.*, biotin, streptavidin.

[0072] The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that function in a manner similar to a naturally occurring amino acid.

[0073] Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0074] An amino acid or nucleotide base "position" is denoted by a number that sequentially identifies each amino acid (or nucleotide base) in the reference sequence based on its position relative to the N-terminus (or 5'-end). Due to deletions, insertions, truncations, fusions, and the like that may be taken into account when determining an optimal alignment, in general the amino acid residue number in a test sequence determined by simply counting from the N-terminus will not necessarily be the same as the number of its corresponding position in the reference sequence. For example, in a case where a variant has a deletion relative to an aligned reference

sequence, there will be no amino acid in the variant that corresponds to a position in the reference sequence at the site of deletion. Where there is an insertion in an aligned reference sequence, that insertion will not correspond to a numbered amino acid position in the reference sequence. In the case of truncations or fusions there can be stretches of amino acids in either the reference or aligned sequence that do not correspond to any amino acid in the corresponding sequence.

[0075] The terms "numbered with reference to" or "corresponding to," when used in the context of the numbering of a given amino acid or polynucleotide sequence, refers to the numbering of the residues of a specified reference sequence when the given amino acid or polynucleotide sequence is compared to the reference sequence. An amino acid residue in a protein "corresponds" to a given residue when it occupies the same essential structural position within the protein as the given residue. For example, a selected residue in a selected antibody (or Fab domain) corresponds to light chain threonine at Kabat position 40, when the selected residue occupies the same essential spatial or other structural relationship as a light chain threonine at Kabat position 40. In some embodiments, where a selected protein is aligned for maximum homology with the light chain of an antibody (or Fab domain), the position in the aligned selected protein aligning with threonine 40 is said to correspond to threonine 40. Instead of a primary sequence alignment, a three dimensional structural alignment can also be used, e.g., where the structure of the selected protein is aligned for maximum correspondence with the light chain threonine at Kabat position 40, and the overall structures compared. In this case, an amino acid that occupies the same essential position as threonine 40 in the structural model is said to correspond to the threonine 40 residue.

[0076] "Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids sequences encode any given amino acid residue. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible

silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence with respect to the expression product, but not with respect to actual probe sequences.

[0077] As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

[0078] The following eight groups each contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (see, e.g., Creighton, Proteins (1984)).

[0079] "Nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form, and complements thereof. The term "polynucleotide" refers to a linear sequence of nucleotides. The term "nucleotide" typically refers to a single unit of a polynucleotide, *i.e.*, a monomer. Nucleotides can be ribonucleotides, deoxyribonucleotides, or modified versions thereof. Examples of polynucleotides contemplated herein include single and double stranded DNA, single and double stranded RNA (including siRNA), and hybrid molecules having mixtures of single and double stranded DNA and RNA. Nucleic acid as used herein also refers to nucleic acids that have the same basic chemical structure as a naturally occurring nucleic acid. Such analogues have modified sugars and/or modified ring substituents, but retain the same basic chemical structure as the naturally occurring nucleic acid. A nucleic acid mimetic refers to chemical compounds that have a structure that is different the general chemical structure of a nucleic acid, but that functions in a manner similar to a naturally occurring nucleic acid. Examples of such analogues include, without limitation,

phosphorothiolates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, and peptide-nucleic acids (PNAs).

[0080] "Percentage of sequence identity" is determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

[0081] The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (*i.e.*, 60% identity, optionally 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% identity over a specified region, e.g., of the entire polypeptide sequences of the invention or individual domains of the polypeptides of the invention), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Such sequences are then said to be "substantially identical." This definition also refers to the complement of a test sequence. Optionally, the identity exists over a region that is at least about 50 nucleotides in length, or more preferably over a region that is 100 to 500 or 1000 or more nucleotides in length. The present invention includes polypeptides that are substantially identical to any of SEQ ID NOs:1-35.

[0082] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

[0083] A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of, e.g., a full length sequence or from 20 to 600, about 50 to about 200, or about 100 to about 150 amino acids or nucleotides in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith and Waterman (1970) *Adv. Appl. Math.* 2:482c, by the homology alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443, by the search for similarity method of Pearson and Lipman (1988) *Proc. Nat'l. Acad. Sci. USA* 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (*see, e.g.,* Ausubel *et al.*, *Current Protocols in Molecular Biology* (1995 supplement)).

[0084] An example of an algorithm that is suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.* (1977) *Nuc. Acids Res.* 25:3389-3402, and Altschul *et al.* (1990) *J. Mol. Biol.* 215:403-410, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al.*, *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an

expectation (E) or 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff and Henikoff (1989) *Proc. Natl. Acad. Sci. USA* 89:10915) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

[0085] The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see, e.g.*, Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5787). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

[0086] An indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross-reactive with the antibodies raised against the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules or their complements hybridize to each other under stringent conditions, as described below. Yet another indication that two nucleic acid sequences are substantially identical is that the same primers can be used to amplify the sequence.

[0087] The word "expression" or "expressed" as used herein in reference to a gene means the transcriptional and/or translational product of that gene. The level of expression of a DNA molecule in a cell may be determined on the basis of either the amount of corresponding mRNA that is present within the cell or the amount of protein encoded by that DNA produced by the cell. The level of expression of non-coding nucleic acid molecules (e.g., siRNA) may be detected by standard PCR or Northern blot methods well known in the art. *See*, Sambrook *et al.*, 1989 *Molecular Cloning: A Laboratory Manual*, 18.1-18.88.

[0088] Expression of a transfected gene can occur transiently or stably in a cell. During "transient expression" the transfected gene is not transferred to the daughter cell during cell division. Since its expression is restricted to the transfected cell, expression of the gene is lost

over time. In contrast, stable expression of a transfected gene can occur when the gene is co-transfected with another gene that confers a selection advantage to the transfected cell. Such a selection advantage may be a resistance towards a certain toxin that is presented to the cell. Expression of a transfected gene can further be accomplished by transposon-mediated insertion into to the host genome. During transposon-mediated insertion, the gene is positioned in a predictable manner between two transposon linker sequences that allow insertion into the host genome as well as subsequent excision. Stable expression of a transfected gene can further be accomplished by infecting a cell with a lentiviral vector, which after infection forms part of (integrates into) the cellular genome thereby resulting in stable expression of the gene.

[0089] The terms "plasmid", "vector" or "expression vector" refer to a nucleic acid molecule that encodes for genes and/or regulatory elements necessary for the expression of genes. Expression of a gene from a plasmid can occur in cis or in trans. If a gene is expressed in cis, the gene and the regulatory elements are encoded by the same plasmid. Expression in trans refers to the instance where the gene and the regulatory elements are encoded by separate plasmids.

[0090] The terms "transfection", "transduction", "transfecting" or "transducing" can be used interchangeably and are defined as a process of introducing a nucleic acid molecule or a protein to a cell. Nucleic acids are introduced to a cell using non-viral or viral-based methods. The nucleic acid molecules may be gene sequences encoding complete proteins or functional portions thereof. Non-viral methods of transfection include any appropriate transfection method that does not use viral DNA or viral particles as a delivery system to introduce the nucleic acid molecule into the cell. Exemplary non-viral transfection methods include calcium phosphate transfection, liposomal transfection, nucleofection, sonoporation, transfection through heat shock, magnetifection and electroporation. In some embodiments, the nucleic acid molecules are introduced into a cell using electroporation following standard procedures well known in the art. For viral-based methods of transfection any useful viral vector may be used in the methods described herein. Examples for viral vectors include, but are not limited to retroviral, adenoviral, lentiviral and adeno-associated viral vectors. In some embodiments, the nucleic acid molecules are introduced into a cell using a retroviral vector following standard procedures well known in the art. The terms "transfection" or "transduction" also refer to introducing proteins into a cell from the external environment. Typically, transduction or transfection of a protein relies on attachment of a peptide or protein capable of crossing the cell membrane to the protein of interest. *See, e.g.*, Ford *et al.* (2001) *Gene Therapy* 8:1-4 and Prochiantz (2007) *Nat. Methods* 4:119-20.

[0091] "Antibody" refers to a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. Typically, the antigen-binding region of an antibody plays a significant role in determining the specificity and affinity of binding. In some embodiments, antibodies or fragments of antibodies may be derived from different organisms, including humans, mice, rats, hamsters, camels, etc. Antibodies of the invention may include antibodies that have been modified or mutated at one or more amino acid positions to improve or modulate a desired function of the antibody (e.g. glycosylation, expression, antigen recognition, effector functions, antigen binding, specificity, etc.).

[0092] Antibodies are large, complex molecules (molecular weight of ~150,000 or about 1320 amino acids) with intricate internal structure. A natural antibody molecule contains two identical pairs of polypeptide chains, each pair having one light chain and one heavy chain. Each light chain and heavy chain in turn consists of two regions: a variable ("V") region involved in binding the target antigen, and a constant ("C") region that interacts with other components of the immune system. The light and heavy chain variable regions come together in 3-dimensional space to form a variable region that binds the antigen (for example, a receptor on the surface of a cell). Within each light or heavy chain variable region, there are three short segments (averaging 10 amino acids in length) called the complementarity determining regions ("CDRs"). The six CDRs in an antibody variable domain (three from the light chain and three from the heavy chain) fold up together in 3-dimensional space to form the actual antibody binding site which docks onto the target antigen. The position and length of the CDRs have been precisely defined by Kabat, E. et al., Sequences of Proteins of Immunological Interest, U.S. Department of Health and Human Services, 1983, 1987. The part of a variable region not contained in the CDRs is called the framework ("FR"), which forms the environment for the CDRs.

[0093] An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one “light” (about 25 kD) and one “heavy” chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (VL) or light chain variable region and variable

heavy chain (VH) or heavy chain variable region refer to these light and heavy chain regions, respectively. The terms variable light chain (VL) and light chain variable region as referred to herein may be used interchangeably. The terms variable heavy chain (VH) and heavy chain variable region as referred to herein may be used interchangeably. The Fc (i.e. fragment crystallizable region) is the "base" or "tail" of an immunoglobulin and is typically composed of two heavy chains that contribute two or three constant domains depending on the class of the antibody. By binding to specific proteins the Fc region ensures that each antibody generates an appropriate immune response for a given antigen. The Fc region also binds to various cell receptors, such as Fc receptors, and other immune molecules, such as complement proteins.

[0094] The term "antigen" as provided herein refers to molecules capable of binding to the antibody region provided herein, wherein the binding site is not the peptide binding site.

[0095] Antibodies exist, for example, as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce F(ab)'2, a dimer of Fab which itself is a light chain joined to VH-CH1 by a disulfide bond. The F(ab)'2 may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the F(ab)'2 dimer into an Fab' monomer. The Fab' monomer is essentially the antigen binding portion with part of the hinge region (see Fundamental Immunology (Paul ed., 3d ed. 1993). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized de novo either chemically or by using recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized de novo using recombinant DNA methodologies (e.g., single chain Fv) or those identified using phage display libraries (see, e.g., McCafferty et al., Nature 348:552-554 (1990)).

[0096] A single-chain variable fragment (scFv) is typically a fusion protein of the variable regions of the heavy (VH) and light chains (VL) of immunoglobulins, connected with a short linker peptide of 10 to about 25 amino acids. The linker may usually be rich in glycine for flexibility, as well as serine or threonine for solubility. The linker can either connect the N-terminus of the VH with the C-terminus of the VL, or vice versa.

[0097] The epitope of a mAb is the region of its antigen to which the mAb binds. Two antibodies bind to the same or overlapping epitope if each competitively inhibits (blocks)

binding of the other to the antigen. That is, a 1x, 5x, 10x, 20x or 100x excess of one antibody inhibits binding of the other by at least 30% but preferably 50%, 75%, 90% or even 99% as measured in a competitive binding assay (see, e.g., Junghans *et al.*, Cancer Res. 50:1495, 1990). Alternatively, two antibodies have the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[0098] For preparation of suitable antibodies of the invention and for use according to the invention, e.g., recombinant, monoclonal, or polyclonal antibodies, many techniques known in the art can be used (see, e.g., Kohler & Milstein, Nature 256:495-497 (1975); Kozbor et al., Immunology Today 4: 72 (1983); Cole et al., pp. 77-96 in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc. (1985); Coligan, Current Protocols in Immunology (1991); Harlow & Lane, Antibodies, A Laboratory Manual (1988); and Goding, Monoclonal Antibodies: Principles and Practice (2d ed. 1986)). The genes encoding the heavy and light chains of an antibody of interest can be cloned from a cell, e.g., the genes encoding a monoclonal antibody can be cloned from a hybridoma and used to produce a recombinant monoclonal antibody. Gene libraries encoding heavy and light chains of monoclonal antibodies can also be made from hybridoma or plasma cells. Random combinations of the heavy and light chain gene products generate a large pool of antibodies with different antigenic specificity (see, e.g., Kuby, Immunology (3rd ed. 1997)). Techniques for the production of single chain antibodies or recombinant antibodies (U.S. Patent 4,946,778, U.S. Patent No. 4,816,567) can be adapted to produce antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized or human antibodies (see, e.g., U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, Marks et al., Bio/Technology 10:779-783 (1992); Lonberg et al., Nature 368:856-859 (1994); Morrison, Nature 368:812-13 (1994); Fishwild et al., Nature Biotechnology 14:845-51 (1996); Neuberger, Nature Biotechnology 14:826 (1996); and Lonberg & Huszar, Intern. Rev. Immunol. 13:65-93 (1995)). Alternatively, phage display technology can be used to identify antibodies and heteromeric Fab fragments that specifically bind to selected antigens (see, e.g., McCafferty et al., Nature 348:552-554 (1990); Marks et al., Biotechnology 10:779-783 (1992)). Antibodies can also be made bispecific, i.e., able to recognize two different antigens (see, e.g., WO 93/08829, Traunecker et al., EMBO J. 10:3655-3659 (1991); and Suresh et al., Methods in Enzymology 121:210 (1986)). Antibodies can also be heteroconjugates, e.g., two covalently joined

antibodies, or immunotoxins (see, e.g., U.S. Patent No. 4,676,980 , WO 91/00360; WO 92/200373; and EP 03089).

[0099] Methods for humanizing or primatizing non-human antibodies are well known in the art (e.g., U.S. Patent Nos. 4,816,567; 5,530,101; 5,859,205; 5,585,089; 5,693,761; 5,693,762; 5,777,085; 6,180,370; 6,210,671; and 6,329,511; WO 87/02671; EP Patent Application 0173494; Jones et al. (1986) Nature 321:522; and Verhoyen et al. (1988) Science 239:1534). Humanized antibodies are further described in, e.g., Winter and Milstein (1991) Nature 349:293. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as import residues, which are typically taken from an import variable domain. Humanization can be essentially performed following the method of Winter and co-workers (see, e.g., Morrison et al., PNAS USA, 81:6851-6855 (1984), Jones et al., Nature 321:522-525 (1986); Riechmann et al., Nature 332:323-327 (1988); Morrison and Oi, Adv. Immunol., 44:65-92 (1988), Verhoeyen et al., Science 239:1534-1536 (1988) and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992), Padlan, Molec. Immun., 28:489-498 (1991); Padlan, Molec. Immun., 31(3):169-217 (1994)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such humanized antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies. For example, polynucleotides comprising a first sequence coding for humanized immunoglobulin framework regions and a second sequence set coding for the desired immunoglobulin complementarity determining regions can be produced synthetically or by combining appropriate cDNA and genomic DNA segments. Human constant region DNA sequences can be isolated in accordance with well known procedures from a variety of human cells.

[0100] A "chimeric antibody" is an antibody molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, e.g., an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered

antigen specificity. The preferred antibodies of, and for use according to the invention include humanized and/or chimeric monoclonal antibodies.

[0101] A "therapeutic antibody" as provided herein refers to any antibody or functional fragment thereof that is used to treat cancer, autoimmune diseases, transplant rejection, cardiovascular disease or other diseases or conditions such as those described herein. Non-limiting examples of therapeutic antibodies include murine antibodies, murinized or humanized chimera antibodies or human antibodies including, but not limited to, Erbitux (cetuximab), ReoPro (abciximab), Simulect (basiliximab), Remicade (infliximab); Orthoclone OKT3 (muromonab-CD3); Rituxan (rituximab), Bexxar (tositumomab) Humira (adalimumab), Campath (alemtuzumab), Simulect (basiliximab), Avastin (bevacizumab), Cimzia (certolizumab pegol), Zenapax (daclizumab), Soliris (eculizumab), Raptiva (efalizumab), Mylotarg (gemtuzumab), Zevalin (ibritumomab tiuxetan), Tysabri (natalizumab), Xolair (omalizumab), Synagis (palivizumab), Vectibix (panitumumab), Lucentis (ranibizumab), and Herceptin (trastuzumab).

[0102] Techniques for conjugating therapeutic agents to antibodies are well known (see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery"in Controlled Drug Delivery (2^{nd} Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review" in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982)). As used herein, the term "antibody-drug conjugate" or "ADC" refers to a therapeutic agent conjugated or otherwise covalently bound to to an antibody. A "therapeutic agent" as referred to herein, is a composition useful in treating or preventing a disease such as cancer.

[0103] The phrase "specifically (or selectively) binds" to an antibody or "specifically (or selectively) immunoreactive with," when referring to a protein or peptide, refers to a binding reaction that is determinative of the presence of the protein, often in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times the background and more typically more than 10 to 100 times background. Specific binding to an antibody under such conditions typically requires an antibody that is selected for its specificity for a particular protein. For example, polyclonal antibodies can be selected to obtain only a subset of antibodies that are

specifically immunoreactive with the selected antigen and not with other proteins. This selection may be achieved by subtracting out antibodies that cross-react with other molecules. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (see, e.g., Harlow & Lane, Using Antibodies, A Laboratory Manual (1998) for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity).

protein).

[0104] A "ligand" refers to an agent, e.g., a polypeptide or other molecule, capable of binding to a receptor.

[0105] The term "recombinant" when used with reference, for example, to a cell, a nucleic acid, a protein, or a vector, indicates that the cell, nucleic acid, protein or vector has been modified by or is the result of laboratory methods. Thus, for example, recombinant proteins include proteins produced by laboratory methods. Recombinant proteins can include amino acid residues not found within the native (non-recombinant) form of the protein or can be include amino acid residues that have been modified, e.g., labeled.

[0106] The term "heterologous" when used with reference to portions of a nucleic acid indicates that the nucleic acid comprises two or more subsequences that are not found in the same relationship to each other in nature. For instance, the nucleic acid is typically recombinantly produced, having two or more sequences from unrelated genes arranged to make a new functional nucleic acid, e.g., a promoter from one source and a coding region from another source. Similarly, a heterologous protein indicates that the protein comprises two or more subsequences that are not found in the same relationship to each other in nature (e.g., a fusion protein).

[0107] The term "isolated", when applied to a nucleic acid or protein, denotes that the nucleic acid or protein is essentially free of other cellular components with which it is associated in the natural state. It can be, for example, in a homogeneous state and may be in either a dry or aqueous solution. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein that is the predominant species present in a preparation is substantially purified.

[0108] "Contacting" is used in accordance with its plain ordinary meaning and refers to the process of allowing at least two distinct species (e.g. chemical compounds including biomolecules or cells) to become sufficiently proximal to react, interact or physically touch. It should be appreciated; however, the resulting reaction product can be produced directly from a reaction between the added reagents or from an intermediate from one or more of the added reagents which can be produced in the reaction mixture.

[0109] The term "contacting" may include allowing two species to react, interact, or physically touch, wherein the two species may be, for example, a compound as described herein and a steric hindering chemical molecule. In embodiments contacting includes, for example, allowing a compound described herein to interact with a steric hindering chemical molecule.

[0110] A "control" sample or value refers to a sample that serves as a reference, usually a known reference, for comparison to a test sample. For example, a test sample can be taken from a test condition, *e.g.*, in the presence of a test compound, and compared to samples from known conditions, *e.g.*, in the absence of the test compound (negative control), or in the presence of a known compound (positive control). A control can also represent an average value gathered from a number of tests or results. One of skill in the art will recognize that controls can be designed for assessment of any number of parameters. For example, a control can be devised to compare therapeutic benefit based on pharmacological data (*e.g.*, half-life) or therapeutic measures (*e.g.*, comparison of side effects). One of skill in the art will understand which controls are valuable in a given situation and be able to analyze data based on comparisons to control values. Controls are also valuable for determining the significance of data. For example, if values for a given parameter are widely variant in controls, variation in test samples will not be considered as significant.

[0111] "Patient" or "subject in need thereof" refers to a living organism suffering from or prone to a disease or condition that can be treated by administration of a composition or pharmaceutical composition as provided herein. Non-limiting examples include humans, other mammals, bovines, rats, mice, dogs, monkeys, goat, sheep, cows, deer, and other non-mammalian animals. In some embodiments, a patient is human.

[0112] The terms "disease" or "condition" refer to a state of being or health status of a patient or subject capable of being treated with a compound, pharmaceutical composition, or method provided herein. In embodiments, the disease is cancer (e.g. lung cancer, ovarian cancer, osteosarcoma, bladder cancer, cervical cancer, liver cancer, kidney cancer, skin cancer (e.g.,

Merkel cell carcinoma), testicular cancer, leukemia, lymphoma, head and neck cancer, colorectal cancer, prostate cancer, pancreatic cancer, melanoma, breast cancer, neuroblastoma).

[0113] The terms "treating", or "treatment" refers to any indicia of success in the treatment or amelioration of an injury, disease, pathology or condition, including any objective or subjective parameter such as abatement; remission; diminishing of symptoms or making the injury, pathology or condition more tolerable to the patient; slowing in the rate of degeneration or decline; making the final point of degeneration less debilitating; improving a patient's physical or mental well-being. The treatment or amelioration of symptoms can be based on objective or subjective parameters; including the results of a physical examination, neuropsychiatric exams, and/or a psychiatric evaluation. The term "treating" and conjugations thereof, include prevention of an injury, pathology, condition, or disease. In embodiments, "treating" refers to treatment of cancer.

[0114] An "effective amount" is an amount sufficient for a compound to accomplish a stated purpose relative to the absence of the compound (e.g. achieve the effect for which it is administered, treat a disease, reduce enzyme activity, increase enzyme activity, reduce a signaling pathway, or reduce one or more symptoms of a disease or condition). An example of an "therapeutically effective amount" is an amount sufficient to contribute to the treatment, prevention, or reduction of a symptom or symptoms of a disease, which could also be referred to as a "therapeutically effective amount." A "reduction" of a symptom or symptoms (and grammatical equivalents of this phrase) means decreasing of the severity or frequency of the symptom(s), or elimination of the symptom(s). The exact amounts will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (*see*, *e.g.*, Lieberman, *Pharmaceutical Dosage Forms* (vols. 1-3, 1992); Lloyd, *The Art, Science and Technology of Pharmaceutical Compounding* (1999); Pickar, *Dosage Calculations* (1999); and *Remington: The Science and Practice of Pharmacy*, 20th Edition, 2003, Gennaro, Ed., Lippincott, Williams & Wilkins).

[0115] As used herein, the term "cancer" refers to all types of cancer, neoplasm or malignant tumors found in mammals, including leukemias, lymphomas, melanomas, neuroendocrine tumors, carcinomas and sarcomas. Exemplary cancers that may be treated with a compound, pharmaceutical composition, or method provided herein include lymphoma, sarcoma, bladder cancer, bone cancer, brain tumor, cervical cancer, colon cancer, esophageal cancer, gastric cancer, head and neck cancer, kidney cancer, myeloma, thyroid cancer, leukemia, prostate cancer, breast cancer (e.g. triple negative, ER positive, ER negative, chemotherapy resistant,

herceptin resistant, HER2 positive, doxorubicin resistant, tamoxifen resistant, ductal carcinoma, lobular carcinoma, primary, metastatic), ovarian cancer, pancreatic cancer, liver cancer (e.g., hepatocellular carcinoma) , lung cancer (e.g. non-small cell lung carcinoma, squamous cell lung carcinoma, adenocarcinoma, large cell lung carcinoma, small cell lung carcinoma, carcinoid, sarcoma), glioblastoma multiforme, glioma, melanoma, prostate cancer, castration-resistant prostate cancer, breast cancer, triple negative breast cancer, glioblastoma, ovarian cancer, lung cancer, squamous cell carcinoma (e.g., head, neck, or esophagus), colorectal cancer, leukemia, acute myeloid leukemia, lymphoma, B cell lymphoma, or multiple myeloma. Additional examples include, cancer of the thyroid, endocrine system, brain, breast, cervix, colon, head & neck, esophagus, liver, kidney, lung, non-small cell lung, melanoma, mesothelioma, ovary, sarcoma, stomach, uterus or Medulloblastoma, Hodgkin's Disease, Non-Hodgkin's Lymphoma, multiple myeloma, neuroblastoma, glioma, glioblastoma multiforme, ovarian cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, primary brain tumors, cancer, malignant pancreatic insulanoma, malignant carcinoid, urinary bladder cancer, premalignant skin lesions, testicular cancer, lymphomas, thyroid cancer, neuroblastoma, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, endometrial cancer, adrenal cortical cancer, neoplasms of the endocrine or exocrine pancreas, medullary thyroid cancer, medullary thyroid carcinoma, melanoma, colorectal cancer, papillary thyroid cancer, hepatocellular carcinoma, Paget's Disease of the Nipple, Phyllodes Tumors, Lobular Carcinoma, Ductal Carcinoma, cancer of the pancreatic stellate cells, cancer of the hepatic stellate cells, or prostate cancer.

[0116] The term "leukemia" refers broadly to progressive, malignant diseases of the blood-forming organs and is generally characterized by a distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow. Leukemia is generally clinically classified on the basis of (1) the duration and character of the disease-acute or chronic; (2) the type of cell involved; myeloid (myelogenous), lymphoid (lymphogenous), or monocytic; and (3) the increase or non-increase in the number abnormal cells in the blood-leukemic or aleukemic (subleukemic). Exemplary leukemias that may be treated with a compound, pharmaceutical composition, or method provided herein include, for example, acute nonlymphocytic leukemia, chronic lymphocytic leukemia, acute granulocytic leukemia, chronic granulocytic leukemia, acute promyelocytic leukemia, adult T-cell leukemia, aleukemic leukemia, a leukocythemic leukemia, basophylic leukemia, blast cell leukemia, bovine leukemia, chronic myelocytic leukemia, leukemia cutis, embryonal leukemia, eosinophilic leukemia, Gross' leukemia, hairy-cell leukemia, hemoblastic leukemia, hemocytoblastic leukemia, histiocytic leukemia, stem cell

leukemia, acute monocytic leukemia, leukopenic leukemia, lymphatic leukemia, lymphoblastic leukemia, lymphocytic leukemia, lymphogenous leukemia, lymphoid leukemia, lymphosarcoma cell leukemia, mast cell leukemia, megakaryocytic leukemia, micromyeloblastic leukemia, monocytic leukemia, myeloblastic leukemia, myelocytic leukemia, myeloid granulocytic leukemia, myelomonocytic leukemia, Naegeli leukemia, plasma cell leukemia, multiple myeloma, plasmacytic leukemia, promyelocytic leukemia, Rieder cell leukemia, Schilling's leukemia, stem cell leukemia, subleukemic leukemia, or undifferentiated cell leukemia.

[0117] The term "sarcoma" generally refers to a tumor which is made up of a substance like the embryonic connective tissue and is generally composed of closely packed cells embedded in a fibrillar or homogeneous substance. Sarcomas that may be treated with a compound, pharmaceutical composition, or method provided herein include a chondrosarcoma, fibrosarcoma, lymphosarcoma, melanosarcoma, myxosarcoma, osteosarcoma, Abemethy's sarcoma, adipose sarcoma, liposarcoma, alveolar soft part sarcoma, ameloblastic sarcoma, botryoid sarcoma, chloroma sarcoma, chorio carcinoma, embryonal sarcoma, Wilms' tumor sarcoma, endometrial sarcoma, stromal sarcoma, Ewing's sarcoma, fascial sarcoma, fibroblastic sarcoma, giant cell sarcoma, granulocytic sarcoma, Hodgkin's sarcoma, idiopathic multiple pigmented hemorrhagic sarcoma, immunoblastic sarcoma of B cells, lymphoma, immunoblastic sarcoma of T-cells, Jensen's sarcoma, Kaposi's sarcoma, Kupffer cell sarcoma, angiosarcoma, leukosarcoma, malignant mesenchymoma sarcoma, parosteal sarcoma, reticulocytic sarcoma, Rous sarcoma, serocystic sarcoma, synovial sarcoma, or telangiectaltic sarcoma.

[0118] The term "melanoma" is taken to mean a tumor arising from the melanocytic system of the skin and other organs. Melanomas that may be treated with a compound, pharmaceutical composition, or method provided herein include, for example, acral-lentiginous melanoma, amelanotic melanoma, benign juvenile melanoma, Cloudman's melanoma, S91 melanoma, Harding-Passey melanoma, juvenile melanoma, lentigo maligna melanoma, malignant melanoma, nodular melanoma, subungal melanoma, or superficial spreading melanoma.

[0119] The term "carcinoma" refers to a malignant new growth made up of epithelial cells tending to infiltrate the surrounding tissues and give rise to metastases. Exemplary carcinomas that may be treated with a compound, pharmaceutical composition, or method provided herein include, for example, medullary thyroid carcinoma, familial medullary thyroid carcinoma, acinar carcinoma, acinous carcinoma, adenocystic carcinoma, adenoid cystic carcinoma, carcinoma adenomatosum, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma, basal cell carcinoma, carcinoma basocellulare, basaloid carcinoma, basosquamous cell carcinoma,

bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebriform carcinoma, cholangiocellular carcinoma, chorionic carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct carcinoma, ductal carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epiermoid carcinoma, carcinoma epitheliale adenoides, exophytic carcinoma, carcinoma ex ulcere, carcinoma fibrosum, gelatiniforni carcinoma, gelatinous carcinoma, giant cell carcinoma, carcinoma gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix carcinoma, hematoid carcinoma, hepatocellular carcinoma, Hurthle cell carcinoma, hyaline carcinoma, hypernephroid carcinoma, infantile embryonal carcinoma, carcinoma in situ, intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, large-cell carcinoma, lenticular carcinoma, carcinoma lenticulare, lipomatous carcinoma, lobular carcinoma, lymphoepithelial carcinoma, carcinoma medullare, medullary carcinoma, melanotic carcinoma, carcinoma molle, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucoepidermoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, nasopharyngeal carcinoma, oat cell carcinoma, carcinoma ossificans, osteoid carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prickle cell carcinoma, pultaceous carcinoma, renal cell carcinoma of kidney, reserve cell carcinoma, carcinoma sarcomatodes, schneiderian carcinoma, scirrhous carcinoma, carcinoma scroti, signet-ring cell carcinoma, carcinoma simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squamous carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes, transitional cell carcinoma, carcinoma tuberosum, tubular carcinoma, tuberous carcinoma, verrucous carcinoma, or carcinoma villosum.

[**0120**] As used herein, the terms "metastasis," "metastatic," and "metastatic cancer" can be used interchangeably and refer to the spread of a proliferative disease or disorder, e.g., cancer, from one organ or another non-adjacent organ or body part. Cancer occurs at an originating site, e.g., breast, which site is referred to as a primary tumor, e.g., primary breast cancer. Some cancer cells in the primary tumor or originating site acquire the ability to penetrate and infiltrate surrounding normal tissue in the local area and/or the ability to penetrate the walls of the lymphatic system or vascular system circulating through the system to other sites and tissues in the body. A second clinically detectable tumor formed from cancer cells of a primary tumor is referred to as a metastatic or secondary tumor. When cancer cells metastasize, the metastatic tumor and its cells are presumed to be similar to those of the original tumor. Thus, if lung cancer

metastasizes to the breast, the secondary tumor at the site of the breast consists of abnormal lung cells and not abnormal breast cells. The secondary tumor in the breast is referred to a metastatic lung cancer. Thus, the phrase metastatic cancer refers to a disease in which a subject has or had a primary tumor and has one or more secondary tumors. The phrases non-metastatic cancer or subjects with cancer that is not metastatic refers to diseases in which subjects have a primary tumor but not one or more secondary tumors. For example, metastatic lung cancer refers to a disease in a subject with or with a history of a primary lung tumor and with one or more secondary tumors at a second location or multiple locations, e.g., in the breast.

[0121] "Anti-cancer agent" is used in accordance with its plain ordinary meaning and refers to a composition (e.g. compound, drug, antagonist, inhibitor, modulator) having antineoplastic properties or the ability to inhibit the growth or proliferation of cells. In embodiments, an anti-cancer agent is a chemotherapeutic. In embodiments, an anti-cancer agent is an agent identified herein having utility in methods of treating cancer. In embodiments, an anti-cancer agent is an agent approved by the FDA or similar regulatory agency of a country other than the USA, for treating cancer.

[0122] The term "associated" or "associated with" in the context of a substance or substance activity or function associated with a disease (e.g., diabetes, cancer (e.g. prostate cancer, renal cancer, metastatic cancer, melanoma, castration-resistant prostate cancer, breast cancer, triple negative breast cancer, glioblastoma, ovarian cancer, lung cancer, squamous cell carcinoma (e.g., head, neck, or esophagus), colorectal cancer, leukemia, acute myeloid leukemia, lymphoma, B cell lymphoma, or multiple myeloma)) means that the disease (e.g. lung cancer, ovarian cancer, osteosarcoma, bladder cancer, cervical cancer, liver cancer, kidney cancer, skin cancer (e.g., Merkel cell carcinoma), testicular cancer, leukemia, lymphoma, head and neck cancer, colorectal cancer, prostate cancer, pancreatic cancer, melanoma, breast cancer, neuroblastoma) is caused by (in whole or in part), or a symptom of the disease is caused by (in whole or in part) the substance or substance activity or function.

[0123] "Chemotherapeutic" or "chemotherapeutic agent" is used in accordance with its plain ordinary meaning and refers to a chemical composition or compound having antineoplastic properties or the ability to inhibit the growth or proliferation of cells.

[0124] The term "aberrant" as used herein refers to different from normal. When used to describe enzymatic activity, aberrant refers to activity that is greater or less than a normal control or the average of normal non-diseased control samples. Aberrant activity may refer to an amount

of activity that results in a disease, wherein returning the aberrant activity to a normal or non-disease-associated amount (e.g. by using a method as described herein), results in reduction of the disease or one or more disease symptoms.

RECOMBINANT NUCLEIC ACIDS

[0125] Provided herein are compositions which exhibit novel diagnostic capabilities and allow to rapidly add functionality to adoptive immunotherapy. The recombinant proteins provided herein are useful, *inter alia*, for a broad variety of therapeutic and diagnostic purposes. For example, the recombinant proteins provided herein including embodiments thereof may be used as non-invasive means to characterize chimeric antigen receptor (CAR) T cells before and/or during treatment of diseases (e.g., cancer). By adding functionality to the CAR immunoreceptors a population of patients with antigen-positive tumors can be efficiently treated and monitored irrespective of their HLA genotype. Adoptive immunotherapy using T lymphocytes that express these functionally improved tumor-specific CARs can be a powerful therapeutic strategy for the treatment of cancer and other diseases (e.g., infectious diseases (e.g., HIV infection)). Further, using the recombinant proteins provided herein including embodiments thereof allow for testing and improvement of the functionality and safety of CAR T cells.

[0126] In one aspect, an isolated nucleic acid is provided. The nucleic acid encodes a protein including (i) an antibody region including a central cavity formed by a heavy chain variable (VH) region, a light chain variable (VL) region, a heavy chain constant region (CH) and a light chain constant region (CL), wherein the central cavity forms a peptide binding site including framework region amino acid residues; and (ii) a transmembrane domain.

[0127] In another aspect, an isolated nucleic acid is provided. The nucleic acid encodes a protein including (i) an antibody region including a central cavity formed by a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the central cavity forms a peptide binding site including framework region amino acid residues; and (ii) a transmembrane domain.

[0128] An "antibody region" as provided herein refers to a monovalent or multivalent protein moiety that forms part of the protein provided herein including embodiments thereof. A person of ordinary skill in the art would therefor immediately recognize that the antibody region is a protein moiety capable of binding an antigen (epitope). Thus, the antibody region provided herein may include a domain of an antibody or fragment (e.g., Fab) thereof. In embodiments, the

antibody region is a protein conjugate. A "protein conjugate" a provided herein refers to a construct consisting of more than one polypeptide, wherein the polypeptides are bound together covalently or non-covalently. In embodiments, the protein conjugate includes a Fab moiety (a monovalent Fab) covalently attached to an scFv moiety (a monovalent scFv). In embodiments, the protein conjugate includes a plurality (at least two) Fab moieties. In embodiments, the polypeptides of a protein conjugate are encoded by one nucleic acid molecule. In embodiments, the polypeptides of a protein conjugate are encoded by different nucleic acid molecules. In embodiments, the polypeptides are connected through a linker. In embodiments, the polypeptides are connected through a chemical linker.

[0129] In embodiments, the antibody region includes a plurality of variable light chain domains and a plurality of variable heavy chain domains. A "variable light chain domain" as provided herein refers to a polypeptide including a light chain variable (VL) region. In embodiments, the variable light chain domain is a light chain variable (VL) region. A "variable heavy chain domain" as provided herein refers to a polypeptide including a heavy chain variable (VH) region. In embodiments, the variable heavy chain domain is a heavy chain variable (VH) region. In embodiments, each of said plurality of variable light chain domains and plurality of variable heavy chain domains is chemically different. Where the plurality of variable light chain domains and plurality of variable heavy chain domains is chemically different, each of the variable light chain domains and the variable heavy chain domains bind a different antigen (epitope). The antigens bound by chemically different variable light chain domains and different variable heavy chain domains may form part of the same protein or a different protein. In embodiments, the antigen forms part of a cancer cell. In embodiments, the antibody region includes a first variable light chain domain and a first variable heavy chain domain and a second variable light chain domain and a second variable heavy chain domain. The first variable heavy chain domain and the first variable light chain domain form a first paratope binding a first epitope and the second variable heavy chain domain and the second variable light chain domain form a second paratope binding to a second epitope, wherein the first and the second paratope are independently different. The term "paratope" refers to the antigen binding site of an antibody or fragment thereof.

[0130] In embodiments, the antibody region includes a first variable light chain domain and a first variable heavy chain domain, a second variable light chain domain and a second variable heavy chain domain, a third variable light chain domain and a third variable heavy chain domain, and a forth variable light chain domain and a forth variable heavy chain domain. The first

variable heavy chain domain and the first variable light chain domain form a first paratope binding a first epitope, the second variable heavy chain domain and the second variable light chain domain form a second paratope binding to a second epitope, the third variable heavy chain domain and the third variable light chain domain form a third paratope binding a third epitope, the forth variable heavy chain domain and the forth variable light chain domain form a forth paratope binding to a second epitope, wherein the first, the second, the third and the forth paratope are independently different.

[0131] In embodiments, the first, the second, the third and the forth paratope are connected through a chemical linker. In embodiments, the chemical linker is a covalent linker, a non-covalent linker, a peptide linker (a linker including a peptide moiety), a cleavable peptide linker, a substituted or unsubstituted alkylene, substituted or unsubstituted heteroalkylene, substituted or unsubstituted cycloalkylene, substituted or unsubstituted heterocycloalkylene, substituted or unsubstituted arylene or substituted or unsubstituted heteroarylene or any combination thereof. Thus, a chemical linker as provided herein may include a plurality of chemical moieties, wherein each of the plurality of moieties is chemically different. In embodiments, the linker is a peptide linker. In embodiments, the peptide linker has a length of about 5- to about 15 amino acid residues.

[0132] In embodiments, the antibody region is a bispecific antibody. In embodiments, the antibody region is a tetravalent antibody. In embodiments, the antibody region is a tetravalent IgG. In embodiments, the antibody region is a dual-variable domain immunoglobulin as described in Jakob CG et al. (MAbs. 2013 May 1; 5(3): 358–363) and Byrne H et al. (Cell Volume 31, Issue 11, p621–632, November 2013), which are hereby incorporated by reference in their entirety and for all purposes.

[0133] In embodiments, the antibody region includes SEQ ID NO:31 and SEQ ID NO:32. In embodiments, the antibody region includes SEQ ID NO:33 and SEQ ID NO:34. In embodiments, the antibody region includes SEQ ID NO:35 and SEQ ID NO:36. In embodiments, the antibody region includes SEQ ID NO:37 and SEQ ID NO:38. In embodiments, the antibody region includes SEQ ID NO:39 and SEQ ID NO:40. In embodiments, the antibody region includes SEQ ID NO:41 and SEQ ID NO:42. In embodiments, the antibody region includes SEQ ID NO:43 and SEQ ID NO:44. In embodiments, the antibody region includes SEQ ID NO:45 and SEQ ID NO:46. In embodiments, the antibody region includes SEQ ID NO:47 and SEQ ID NO:48. In embodiments, the antibody region includes SEQ ID NO:49 and SEQ ID NO:50. In

embodiments, the antibody region includes SEQ ID NO:51 and SEQ ID NO:52. In embodiments, the antibody region includes SEQ ID NO:53 and SEQ ID NO:54. In embodiments, the antibody region includes SEQ ID NO:55 and SEQ ID NO:56. In embodiments, the antibody region includes SEQ ID NO:57 and SEQ ID NO:58. In embodiments, the antibody region includes SEQ ID NO:59 and SEQ ID NO:60. In embodiments, the antibody region includes SEQ ID NO:61 and SEQ ID NO:62. In embodiments, the antibody region includes SEQ ID NO:63 and SEQ ID NO:64. In embodiments, the antibody region includes SEQ ID NO:65 and SEQ ID NO:66. In embodiments, the antibody region includes SEQ ID NO:67 and SEQ ID NO:68. In embodiments, the antibody region includes SEQ ID NO:69 and SEQ ID NO:70. In embodiments, the antibody region includes SEQ ID NO:71 and SEQ ID NO:72. In embodiments, the antibody region includes SEQ ID NO:73 and SEQ ID NO:74. In embodiments, the antibody region includes SEQ ID NO:75 and SEQ ID NO:76. In embodiments, the antibody region includes SEQ ID NO:77 and SEQ ID NO:78. In embodiments, the antibody region includes SEQ ID NO:79 and SEQ ID NO:80. In embodiments, the antibody region includes SEQ ID NO:81 and SEQ ID NO:82. In embodiments, the antibody region includes SEQ ID NO:111 and SEQ ID NO:112. In embodiments, the antibody region includes SEQ ID NO:113 and SEQ ID NO:114. In embodiments, the antibody region includes SEQ ID NO:115 and SEQ ID NO:116. In embodiments, the antibody region includes SEQ ID NO:117 and SEQ ID NO:118. In embodiments, the antibody region includes SEQ ID NO:119 and SEQ ID NO:120.

[0134] The "heavy chain variable (VH) region" as provided herein is a domain which includes the variable region of a heavy chain of an antibody or a fragment thereof. Likewise, the "light chain variable (VL) region" as provided herein is a domain including the variable region of a light chain of an antibody or a fragment thereof. In embodiments, the heavy chain variable (VH) region is the variable region of the heavy chain of an antibody. In embodiments, the heavy chain variable (VH) region is the variable region of the heavy chain of an antibody fragment. In embodiments, the heavy chain variable (VH) region is the variable region of the heavy chain of a Fab. In embodiments, the light chain variable (VL) region is the variable region of the light chain of an antibody. In embodiments, the light chain variable (VL) region is the variable region of the light chain of an antibody fragment. In embodiments, the light chain variable (VL) region is the variable region of the light chain of a Fab.

[0135] In embodiments, the antibody region further includes a heavy chain constant region (CH) and a light chain constant region (CL). In embodiments, the heavy chain constant region (CH) is the constant region of the heavy chain of an antibody or fragment thereof. In embodiments, the light chain constant region (CL) is the constant region of the light chain of an antibody or fragment thereof. In embodiments, the heavy chain constant region (CH) is the constant region of a Fab. In embodiments, the light chain constant region (CL) is the constant region of the light chain of a Fab. In embodiments, the heavy chain constant region (CH) is the constant region of a F(ab)'2 dimer. In embodiments, the light chain constant region (CL) is the constant region of the light chain of a F(ab)'2 dimer. In embodiments, the antibody region includes an Fc domain. In embodiments, the antibody region is a humanized antibody region. In embodiments, the antibody region is a humanized mouse antibody region. In embodiments, the antibody region does not include an scFV antibody region. Where the antibody region does not include a scFv antibody region, the antibody region does not include a fusion protein of the variable regions of the heavy (VH) and light chains (VL) of immunoglobulins, connected with a short linker peptide.

[0136] The "central cavity" with respect to the three-dimensional structure of a Fab, refers to the internal cavity of the Fab lined by portions of the heavy and light chain variable and constant regions and including amino acids lining a hole within the cavity. In embodiments, the central cavity including the hole has a structure, e.g., as depicted in, or similar to, Fig.4A. In embodiments, where the antibody region includes a Fab, the central cavity thus is lined by residues of the VH, VL, CH1, and CL regions. The central cavity does not include the antigen binding site. Thus, in embodiments the compound that binds to the central cavity does not impact (e.g. measurably impact) the binding of the antibody region to the epitope. In other words, in embodiments, occupancy of this site does not affect antigen binding. In embodiments, the central cavity is lined by amino acid residues capable of interacting with a compound including a peptidyl moiety (e.g. a meditope) provided herein including embodiments thereof (e.g., a peptide of formula (I) or (II)). The amino acids residues capable of interacting with the compound including a peptidyl moiety (e.g. a meditope) may from part of the peptide binding site (also referred to herein as a meditope binding site). The peptide binding site may be engineered into any appropriate antibody thereby forming an antibody or antibody region with the peptide binding site (also referred to herein as a meditope enabled antibody or meditope enabled antibody region).

[0137] In embodiments, the amino acid residues lining the central cavity include a residue at a position corresponding to Kabat position 83, a residue at a position corresponding to Kabat position 30 or a residue at a position corresponding to Kabat position 52. In embodiments, the amino acid residues lining the central cavity include a residue at a position corresponding to Kabat position 40, a residue at a position corresponding to Kabat position 41, a residue at a position corresponding to Kabat position 30, a residue at a position corresponding to Kabat position 52, a residue at a position corresponding to Kabat position 83, or a residue at a position corresponding to Kabat position 85. In embodiments, the amino acid residues lining the central cavity include a residue at a position corresponding to Kabat position 40. In embodiments, the amino acid residues lining the central cavity include a residue at a position corresponding to Kabat position 41. In embodiments, the amino acid residues lining the central cavity include a residue at a position corresponding to Kabat position 30. In embodiments, the amino acid residues lining the central cavity include a residue at a position corresponding to Kabat position 52. In embodiments, the amino acid residues lining the central cavity include a residue at a position corresponding to Kabat 83. In embodiments, the amino acid residues lining the central cavity include a residue at a position corresponding to Kabat position 85.

[0138] In embodiments, the amino acid residues lining the central cavity include a residue at a position corresponding to Kabat position 30. In embodiments, the residue at a position corresponding to Kabat position 30 is a negatively charged amino acid residue. In embodiments, the residue at a position corresponding to Kabat position 30 is aspartic acid. In embodiments, the amino acid residues lining the central cavity include a residue at a position corresponding to Kabat position 52. In embodiments, the residue at a position corresponding to Kabat position 52 is a negatively charged amino acid residue. In embodiments, the residue at a position corresponding to Kabat position 52 is aspartic acid. In embodiments, the amino acid residues lining the central cavity include a residue at a position corresponding to Kabat position 83. In embodiments, the residue at a position corresponding to Kabat position 83 is a negatively charged amino acid residue. In embodiments, the residue at a position corresponding to Kabat position 83 is glutamic acid. In embodiments, the residue at a position corresponding to Kabat position 83 is isoleucine. In embodiments, the amino acid residues lining the central cavity include a residue at a position corresponding to Kabat position 85.

[0139] In embodiments, the central cavity is lined by (formed by) a light chain residue at a position corresponding to Kabat position Gln38, Thr40, Gln41, Gly42, Ser43, Asp 52, Asp85, Ile83, Tyr87, Lys103, Val163, Thr164, or Glu165. A "light chain residue" as provided herein

refers to a residue forming part of a light chain of an antibody or antibody fragment. In embodiments, the central cavity is lined (e.g., formed) by a light chain residue at a position corresponding to Kabat position Gln38. In embodiments, the central cavity is lined (e.g., formed) by a light chain residue at a position corresponding to Kabat position Thr40 In embodiments, the central cavity is lined (e.g., formed) by a light chain residue at a position corresponding to Kabat position Gln41. In embodiments, the central cavity is lined (e.g., formed) by a light chain residue at a position corresponding to Kabat position Gly42. In embodiments, the central cavity is lined (e.g., formed) by a light chain residue at a position corresponding to Kabat position to Ser43. In embodiments, the central cavity is lined (e.g., formed) by a light chain residue at a position corresponding to Kabat position Asp85. In embodiments, the central cavity is lined (e.g., formed) by a light chain residue at a position corresponding to Kabat position Tyr87. In embodiments, the central cavity is lined (e.g., formed) by a light chain residue at a position corresponding to Kabat position Lys103. In embodiments, the central cavity is lined (e.g., formed) by a light chain residue at a position corresponding to Kabat position Val163. In embodiments, the central cavity is lined (e.g., formed) by a light chain residue at a position corresponding to Kabat position Thr164 In embodiments, the central cavity is lined (e.g., formed) by a light chain residue at a position corresponding to Kabat position Glu165.

[0140] In embodiments, the central cavity is lined by (formed by) a heavy chain residue at a position corresponding to Kabat position Asp 30, Gln39, Pro40, Thr91, Ala92, Ile93, Tyr95, Gln112, Leu115, Glu155, Pro156, Pro174, Ala175, or Tyr183. A "heavy chain residue" as provided herein refers to a residue forming part of a heavy chain of an antibody or antibody fragment. In embodiments, the central cavity is lined (e.g., formed) by a heavy chain residue at a position corresponding to Kabat position Gln39. In embodiments, the central cavity is lined (e.g., formed) by a heavy chain residue at a position corresponding to Kabat position. In embodiments, the central cavity is lined (e.g., formed) by a heavy chain residue at a position corresponding to Kabat position Pro40. In embodiments, the central cavity is lined (e.g., formed) by a heavy chain residue at a position corresponding to Kabat position Thr91. In embodiments, the central cavity is lined (e.g., formed) by a heavy chain residue at a position corresponding to Kabat position Ala92. In embodiments, the central cavity is lined (e.g., formed) by a heavy chain residue at a position corresponding to Kabat position Ile93. In embodiments, the central cavity is lined (e.g., formed) by a heavy chain residue at a position corresponding to Kabat position Tyr95. In embodiments, the central cavity is lined (e.g., formed) by a heavy chain residue at a position corresponding to Kabat position Gln112. In

embodiments, the central cavity is lined (e.g., formed) by a heavy chain residue at a position corresponding to Kabat position Leu115. In embodiments, the central cavity is lined (e.g., formed) by a heavy chain residue at a position corresponding to Kabat position Glu155. In embodiments, the central cavity is lined (e.g., formed) by a heavy chain residue at a position corresponding to Kabat position Pro156. In embodiments, the central cavity is lined (e.g., formed) by a heavy chain residue at a position corresponding to Kabat position Pro174. In embodiments, the central cavity is lined (e.g., formed) by a heavy chain residue at a position corresponding to Kabat position Ala175. In embodiments, the central cavity is lined (e.g., formed) by a heavy chain residue at a position corresponding to Kabat position Tyr183.

[0141] The central cavity provided herein includes a peptide binding site (also referred to herein as a meditope binding site) including framework region amino acid (FR) residues. In embodiments, the peptide binding site does not include CDR residues of the heavy chain or the light chain. In embodiments, the peptide binding site includes FR residues of the heavy chain or the light chain. In embodiments, the peptide binding site includes FR residues of the heavy chain and the light chain. In embodiments, the peptide binding site includes a residue at a position corresponding to Kabat position 83, a residue at a position corresponding to Kabat position 30 or a residue at a position corresponding to Kabat position 52. In embodiments, the peptide binding site includes a residue at a position corresponding to Kabat position 40, a residue at a position corresponding to Kabat position 41, a residue at a position corresponding to Kabat position 30, a residue at a position corresponding to Kabat position 52, a residue at a position corresponding to Kabat position 83, or a residue at a position corresponding to Kabat position 85. In embodiments, the peptide binding site includes a residue at a position corresponding to Kabat position 40. In embodiments, the peptide binding site includes a residue at a position corresponding to Kabat position 41. In embodiments, the peptide binding site includes a residue at a position corresponding to Kabat position 30. In embodiments, the peptide binding site includes a residue at a position corresponding to Kabat position 52. In embodiments, the peptide binding site includes a residue at a position corresponding to Kabat position 83. In embodiments, the peptide binding site includes a residue at a position corresponding to Kabat position 85. In embodiments, residues forming a peptide binding site are described in published US application US20120301400 A1, which is hereby incorporate by reference in its entirety and for all purposes.

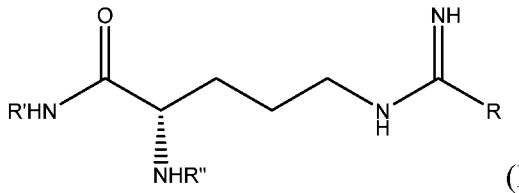
[0142] In embodiments, the central cavity is lined by amino acid residues capable of binding a compound including a peptidyl moiety. Thus, in embodiments, the peptide binding site provided

herein is capable of binding a compound including a peptidyl moiety. In embodiments, the peptide binding site is capable of binding the peptidyl moiety. In embodiments, the peptide binding site provided herein is bound to a compound including a peptidyl moiety. In embodiments, the peptide binding site is bound to the peptidyl moiety. In embodiments, the peptidyl moiety is a moiety as described in published US application US20120301400 A1 and Avery et al. 2015 (Scientific Reports 5:7817) which are hereby incorporated by reference in their entirety and for all purposes.

[0143] In embodiments, the compound that binds to the peptide binding site is a peptide or includes a peptidyl moiety. In embodiments, the compound is a substituted peptide. In embodiments, the peptide is between 5 and 16 amino acids in length. In embodiments, the compound includes a substituted peptidyl moiety. In embodiments, the peptidyl moiety is between 5 and 16 amino acids in length. The peptide or peptidyl moiety provided herein may also be referred to as a "meditope." In embodiments, the peptide or peptidyl moiety has the formula:

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10-X11-X12 (I).

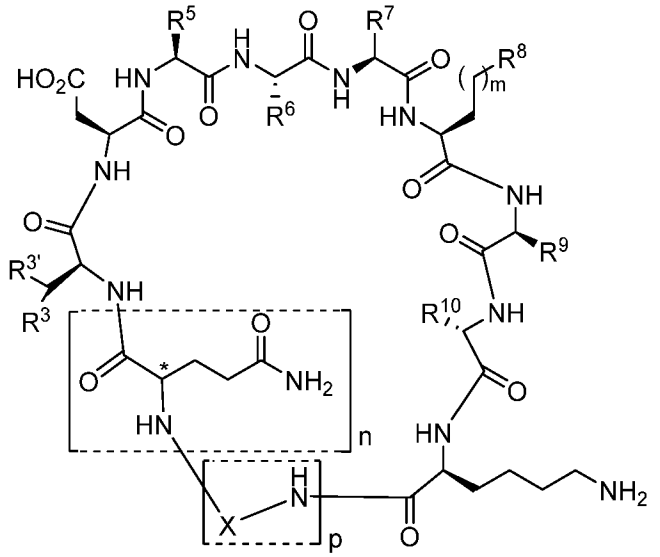
Where the sequence of Formula (I) is a peptidyl moiety, a person having ordinary skill in the art will immediately understand that the peptidyl moiety is attached to the remainder of the compound at one or more attachments points. In formula (I), X1 is Cys, Gly, β-alanine, 2,3-diaminopropionic acid, β-azidoalanine, or null; X2 is Gln or null; X3 is Phe, Tyr, β-β'-diphenyl-Ala, His, Asp, 2-bromo-L-phenylalanine, 3-bromo-L-phenylalanine, 4-bromo-L-phenylalanine, Asn, Gln, a modified Phe, a hydratable carbonyl-containing residue or a boronic acid-containing residue; X4 is Asp or Asn; X5 is Leu; β-β'-diphenyl-Ala, Phe, a non-natural analog of phenylalanine, tryptophan, tyrosine, a hydratable carbonyl-containing residue or a boronic acid-containing residue; X6 is Ser or Cys; X7 is Thr, Ser or Cys; X8 is Arg, a modified (substituted) Arg, a hydratable carbonyl or a boronic acid-containing residue; X9 is Arg or Ala; X10 is Leu, Gln, Glu, β-β'-diphenyl-Ala, Phe, a non-natural analog of phenylalanine, tryptophan, tyrosine, a hydratable carbonyl-containing residue or a boronic acid-containing residue; X11is Lys; and X12 is Cys, Gly, 7-aminoheptanoic acid, β-alanine, diaminopropionic acid, propargylglycine, isoaspartic acid or null; wherein the modified Phe is a Phe with one or more halogen incorporated into the phenyl ring and wherein the modified Arg has a structure of the formula:



(IA). In formula (IA), R, R' and R" are independently substituted or unsubstituted alkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or NHR"' and R"' is substituted or unsubstituted alkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl.

[0144] In embodiments, the peptide is a cyclic peptide. In embodiments, the peptidyl moiety is a cyclic peptidyl moiety. In embodiments, the peptide or peptidyl moiety includes a disulfide bridge, a thioether bridge, a lactam linkage, cycloaddition. In embodiments, the cyclic portion of the cyclic peptide or cyclic peptidyl moiety is formed through binding between X1 and X12, X1 and X11, X3 and X11, X4 and X11, or X2 and X12. In embodiments, the non-natural amino acid is β-β'-diphenyl-Ala, branched alkyl, substituted or unsubstituted aryl or substituted or unsubstituted heteroaryl. In embodiments, each of the one or more halogen is an ortho-, meta-, or para-bromo phenyl substituent.

[0145] In embodiments, the peptide or peptidyl moiety has the formula:



(II).

[0146] In formula (II), R^3 is hydrogen, R^{3A}-substituted or unsubstituted aryl, wherein R^{3A} is hydrogen, halogen or C_{1-4} unsubstituted alkyl. $R^{3'}$ is hydrogen, $R^{3A'}$-substituted or unsubstituted aryl, wherein $R^{3A'}$ is hydrogen, halogen or C_{1-4} unsubstituted alkyl. R^5 is R^{5A}-substituted or unsubstituted C_{1-8} (e.g., C_{1-4}) alkyl. R^{5A} is oxo, acetal, ketal, $-B(OH)_2$, boronic ester, phosphonate ester, ortho ester, $-CO_2C_{1-4}$ alkyl, -CH=CH-CHO, $-CH=CH-C(O)R^{5A'}$, $-CH=CH-CO_2R^{5A'}$, $-CO_2H$, $-CONH_2$, or $R^{5A''}$-substituted or unsubstituted aryl, $R^{5A''}$-substituted

or unsubstituted heteroaryl (e.g., naphthyl, imidazole, indole), wherein $R^{5A'}$ is substituted or unsubstituted C_{1-4} alkyl and $R^{5A''}$ is -OH, fluoro, chloro, bromo or iodo. R^6 is $–L^{6'}OH$ or $–L^{6'}SH$, wherein $L^{6'}$ is substituted or unsubstituted C_{1-4} alkylene. R^7 is $–L^{7'}OH$ or $–L^{7'}SH$, wherein $L^{7'}$ is substituted or unsubstituted C_{1-4} alkyl. The symbol m is 0, 1, 2, 3, 4, or 5.

[0147] In formula (II), R^8 is -OH, $-NR^aR^b$, $-N(R^c)C(O)R^e$, or $-N(R^c)C(=NR^d)R^e$. R^a is H. R^b is H or C_{1-8} alkyl optionally substituted with one or more substituents selected from the group consisting of oxo, acetal, and ketal, $-B(OH)_2$, -SH, boronic ester, phosphonate ester, ortho ester, -CH=CH-CHO, $-CH=CH-C(O)C_{1-4}$ alkyl, $-CH=CH-CO_2C_{1-4}$ alkyl, $–CO_2H$, or $-CO_2C_{1-4}$ alkyl group. R^c is H, C_{1-8} alkyl, C_{3-8} cycloalkyl, branched alkyl, or aryl. R^d is H or a C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{3-8} cycloalkyl, branched alkyl, or aryl group, each optionally substituted with one or more substituents selected from the group consisting of $-N_3$, $-NH_2$, -OH, -SH, halogen, oxo, acetal, ketal, $-B(OH)_2$, boronic ester, phosphonate ester, ortho ester, -CH=CH-CHO, $-CH=CH-C(O)C_{1-4}$alkyl, $-CH=CH-CO_2C_{1-4}$alkyl, $–CO_2H$, and $-CO_2C_{1-4}$ alkyl group. R^e is H, $-NHR^d$; or a C_{1-12} alkyl, C_{3-8} cycloalkyl, C_{2-12} alkenyl, C_{2-8} alkynyl, or aryl group, each optionally substituted with one or more substituents selected from the group consisting of $-N_3$, $-NH_2$, -OH, -SH, oxo, C_{2-4} acetal, C_{2-4} ketal, $-B(OH)_2$, boronic ester, phosphonate ester, ortho ester, -CH=CH-CHO, $-CH=CH-C(O)C_{1-4}$ alkyl, $-CH=CH-CO_2C_{1-4}$ alkyl, and $-CO_2C_{1-4}$ alkyl group.

[0148] In formula (II), R^9 is substituted or unsubstituted C_{1-4} alkyl. R^{10} is R^{10A}-substituted or unsubstituted C_{1-8} alkyl, wherein R^{10A} is oxo, acetal, ketal, $-B(OH)_2$, boronic ester, phosphonate ester, ortho ester, -CH=CH-CHO, $-CH=CH-C(O)C_{1-4}$ alkyl, $-CH=CH-CO_2C_{1-4}$ alkyl, $-CO_2C_{1-4}$ alkyl, $-CO_2H$, $–CONH_2$, R^{10B}-substituted or unsubstituted phenyl, R^{10B}-substituted or unsubstituted naphthyl, R^{10B}-substituted or unsubstituted imidazolyl, or R^{10B}-substituted or unsubstituted indolyl, wherein R^{10B} is –OH or halogen. The symbol n is 0 or 1. The symbol p is 0 or 1.

[0149] In formula (II), X is R^X-substituted or unsubstituted C_{1-8} alkylene, R^x-substituted or unsubstituted C_{2-8} alkenylene, R^x is oxo, -C(O), $-NH_2$, -NHC(O) or $-NHC(O)R^y$, wherein one carbon of the alkenylene is optionally replaced with -C(O)NH, a 5-membered heteroarylene, or -S-S, and R^y is $–C_{1-4}$ alkyl, $–CH(R^z)C(O)$ or $–CH(R^z)CO_2H$, wherein R^z is –H or $R^{z'}$-substituted or unsubstituted $–C_{1-4}$ alkyl, wherein $R^{z'}$ is –OH, -SH, or $-NH_2$. Formula (I) or (II) includes all appropriate pharmaceutically acceptable salts. More information regarding the concepts of peptide binding sites (meditope binding sites) and peptides (meditopes) can be found in international application serial no. PCT/US2011/055656, PCT/US2015/053880,

PCT/US2012/032938 and US application serial no. US 14/453,586, which are hereby incorporated in their entirety and for all purposes.

[0150] The compounds provided herein may include a therapeutic agent, a diagnostic agent or a detectable agent (also referred to herein as a detectable agent) attached to the peptidyl moiety. In embodiments, the compound is conjugated to a therapeutic agent, a diagnostic agent, or a detectable agent. In embodiments, the peptidyl moiety (e.g., the peptide of formula (I) or (II)) is conjugated to a therapeutic agent, a diagnostic agent or a detectable agent. In embodiments, the antibody region is conjugated to a therapeutic agent, a diagnostic agent, or a detectable agent.

[0151] The therapeutic agent, diagnostic agent or detectable agent may be attached through a chemical linker to the compound (e.g. to the peptidyl moiety) and/or the antibody region provided herein including embodiments thereof. In embodiments, the chemical linker is a covalent linker, a non-covalent linker, a peptide linker (a linker including a peptide moiety), a cleavable peptide linker, a substituted or unsubstituted alkylene, substituted or unsubstituted heteroalkylene, substituted or unsubstituted cycloalkylene, substituted or unsubstituted heterocycloalkylene, substituted or unsubstituted arylene or substituted or unsubstituted heteroarylene or any combination thereof.

[0152] A chemical linker as provided herein may include a plurality of chemical moieties, wherein each of the plurality of moieties is chemically different. In embodiments, the therapeutic agent, diagnostic agent or detectable agent is attached to the compound through a non-covalent or covalent linker. In embodiments, the therapeutic agent, diagnostic agent or detectable agent is attached to the peptidyl moiety through a non-covalent or covalent linker. In embodiments, the therapeutic agent, diagnostic agent or detectable agent is attached to the antibody region through a non-covalent or covalent linker. Typically, the linker may be a covalent linker as described herein and formed through conjugate (e.g. "click") chemistry. The linker may further be a cleavable peptide linker as described herein. Where the therapeutic, diagnostic or detectable agent forms part (e.g., through covalent attachment) of the compound, the peptidyl moiety and/or the antibody region provided herein, including embodiments thereof, the therapeutic, diagnostic or detectable agent may be referred to as a "therapeutic moiety", "diagnostic moiety", or "detectable moiety", respectively. In embodiments, the peptide moiety (meditope) contains a reactive amine functionality (e.g., Lysl l), which is used for conjugation of the meditope (peptidyl moiety), e.g., to a scaffold or linker or to a functional moiety, such as a diagnostic, e.g., imaging, agent or therapeutic moiety as described herein. In embodiments, thiol functionalities are introduced in any suitable position on the meditope (peptidyl moiety) and are

selectively modified using reagents containing imagining agents, other proteins and peptides, metal chelators, siRNAs, nanoparticles, and cytotoxic drugs. Coupling of therapeutic or diagnostic moieties to the peptidyl moiety provided herein can be performed using peptide chemistry methodology well known in the art and described, for example in WO 2013055404 A1, which is hereby incorporated by reference for all purposes and its entirety.

[0153] Therapeutic moieties as provided herein may include, without limitation, peptides, proteins, nucleic acids, nucleic acid analogs, small molecules, antibodies, enzymes, prodrugs, cytotoxic agents (e.g. toxins) including, but not limited to ricin, doxorubicin, daunorubicin, taxol, ethidium bromide, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, dihydroxy anthracin dione, actinomycin D, diphteria toxin, Pseudomonas exotoxin (PE) A, PE40, abrin, and glucocorticoid. In embodiments, the therapeutic moiety is an anti-cancer agent or chemotherapeutic agent as described herein. In embodiments, the therapeutic moiety is a nucleic acid moiety, a peptide moiety or a small molecule drug moiety. In embodiments, the therapeutic moiety is a nucleic acid moiety. In embodiments, the therapeutic moiety is an antibody moiety. In embodiments, the therapeutic moiety is a peptide moiety. In embodiments, the therapeutic moiety is a small molecule drug moiety. In embodiments, the therapeutic moiety is a nuclease. In embodiments, the therapeutic moiety is an immunostimulator. In embodiments, the therapeutic moiety is a toxin. In embodiments, the therapeutic moiety is a nuclease.

[0154] The compound, peptidyl moiety or antibody region provided herein may include an imaging or detectable moiety. In embodiments, the detectable moiety is connected to the compound through a covalent linker. In embodiments, the detectable moiety is connected to the antibody region through a covalent linker. In embodiments, detectable moiety is connected to peptidyl moiety through a covalent linker. An "imaging or detectable moiety" as provided herein is a monovalent compound detectable by spectroscopic, photochemical, biochemical, immunochemical, chemical, or other physical means. In embodiments, the imaging moiety is covalently attached to the compound. In embodiments, the imaging moiety is covalently attached to the antibody region. In embodiments, the imaging moiety is covalently attached to the peptidyl moiety. Exemplary imaging moieties include without limitation ^{32}P, radionuclides, positron-emitting isotopes, fluorescent dyes, fluorophores, antibodies, bioluminescent molecules, chemoluminescent molecules, photoactive molecules, metals, electron-dense reagents, enzymes (e.g., as commonly used in an ELISA), magnetic contrast agents, quantum dots, nanoparticles, biotin, digoxigenin, haptens and proteins or other entities which can be made detectable, e.g., by incorporating a radiolabel into a peptide or antibody specifically reactive with a target peptide.

Any method known in the art for conjugating an antibody to the moiety may be employed, e.g., using methods described in Hermanson, Bioconjugate Techniques 1996, Academic Press, Inc., San Diego. Exemplary fluorophores include fluorescein, rhodamine, GFP, coumarin, FITC, AlExa fluor, Cy3, Cy5, BODIPY, and cyanine dyes. Exemplary radionuclides include Fluorine-18, Gallium-68, and Copper-64. Exemplary magnetic contrast agents include gadolinium, iron oxide and iron platinum, and manganese. In embodiments, the imaging moiety is a bioluminescent molecule. In embodiments, the imaging moiety is a photoactive molecule. In embodiments, the imaging moiety is a metal. In embodiments, the imaging moiety is a nanoparticle.

[0155] A transmembrane domain as provided herein refers to a polypeptide forming part of a biological membrane. The transmembrane domain provided herein is capable of spanning a biological membrane (e.g., a cellular membrane) from one side of the membrane through to the other side of the membrane. In embodiments, the transmembrane domain spans from the intracellular side to the extracellular side of a cellular membrane. Transmembrane domains may include non-polar, hydrophobic residues, which anchor the proteins provided herein including embodiments thereof in a biological membrane (e.g., cellular membrane of a T cell). Any transmembrane domain capable of anchoring the proteins provided herein including embodiments thereof are contemplated. In embodiments, the transmembrane domain is L-selectin. The term "L-selectin" as provided herein includes any of the recombinant or naturally-occurring forms of the L-selectin protein, also known as CD62L, or variants or homologs thereof that maintain L-selectin activity (e.g. within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to L-selectin). In embodiments, the variants or homologs have at least 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence (e.g. a 50, 100, 150 or 200 continuous amino acid portion) compared to a naturally occurring L-selectin polypeptide. In embodiments, L-selectin is the protein as identified by the NCBI sequence reference GI:262206315, homolog or functional fragment thereof. Non-limiting examples of transmembrane domains include, the transmembrane domains of CD8, CD4 or CD3-zeta.

[0156] In embodiments, the transmembrane domain is a CD28 transmembrane domain. The term "CD28 transmembrane domain" as provided herein includes any of the recombinant or naturally-occurring forms of the transmembrane domain of CD28, or variants or homologs thereof that maintain CD28 transmembrane domain activity (e.g. within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to the CD28 transmembrane domain). In

some aspects, the variants or homologs have at least 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence (e.g. a 50, 100, 150 or 200 continuous amino acid portion) compared to a naturally occurring CD28 transmembrane domain polypeptide. In embodiments, the CD28 transmembrane domain is the protein as identified by SEQ ID NO:22, SEQ ID NO:2, homolog or functional fragment thereof. In embodiments, CD28 is the protein as identified by the NCBI sequence reference GI:340545506, homolog or functional fragment thereof.

[0157] In embodiments, the transmembrane domain is the protein identified by SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, homolog or functional fragment thereof.

[0158] In embodiments, the isolated nucleic acid provided herein includes an intracellular T-cell signaling sequence encoding an intracellular T-cell signaling domain. In embodiments, the intracellular T-cell signaling domain is a CD3 ζ intracellular T-cell signaling domain. An "intracellular T-cell signaling domain" as provided herein includes amino acid sequences capable of providing primary signaling in response to binding of an antigen to the antibody region provided herein including embodiments thereof. In embodiments, the signaling of the intracellular T-cell signaling domain results in activation of the T cell expressing the same. In embodiments, the signaling of the intracellular T-cell signaling domain results in proliferation (cell division) of the T cell expressing the same. In embodiments, the signaling of the intracellular T-cell signaling domain results expression by said T cell of proteins known in the art to characteristic of activated T cell (e.g., CTLA-4, PD-1, CD28, CD69). In embodiments, the intracellular T-cell signaling domain includes the signaling domain of the zeta chain of the human CD3 complex. In embodiments, the intracellular T-cell signaling domain is a CD3 ζ intracellular T-cell signaling domain. In embodiments, the intracellular T-cell signaling domain is SEQ ID NO:11.

[0159] In embodiments, the isolated nucleic acid provided herein includes an intracellular co-stimulatory signaling sequence encoding an intracellular co-stimulatory signaling domain. An "intracellular co-stimulatory signaling domain" as provided herein includes amino acid sequences capable of providing co-stimulatory signaling in response to binding of an antigen to the antibody region provided herein including embodiments thereof. In embodiments, the signaling of the co-stimulatory signaling domain results in production of cytokines and proliferation of the T cell expressing the same. In embodiments, the intracellular co-stimulatory signaling domain is a CD28 intracellular co-stimulatory signaling domain, a 4-1BB intracellular

co-stimulatory signaling domain, a ICOS intracellular co-stimulatory signaling domain, or an OX-40 intracellular co-stimulatory signaling domain. In embodiments, the intracellular co-stimulatory signaling domain includes a CD28 intracellular co-stimulatory signaling domain, a 4-1BB intracellular co-stimulatory signaling domain, a ICOS intracellular co-stimulatory signaling domain, an OX-40 intracellular co-stimulatory signaling domain or any combination thereof. Exemplary intracellular co-stimulatory signaling domains including sequences and accession numbers are listed in Table 2. In embodiments, the intracellular co-stimulatory signaling domain includes the protein identified by SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15 or SEQ ID NO:16. In embodiments, the intracellular co-stimulatory signaling domain is SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15 or SEQ ID NO:16.

[0160] In embodiments, the isolated nucleic acid provided herein includes a linker sequence encoding a linker domain. In embodiments, the linker domain is between the transmembrane domain and the intracellular T-cell signaling domain. In embodiments, the linker domain is between the intracellular T-cell signaling domain and the intracellular co-stimulatory signaling domain. In embodiments, the linker domain includes the sequence GGCGG or GGG.

[0161] In embodiments, the isolated nucleic acid provided herein includes a spacer sequence encoding a spacer region. In embodiments, the spacer region is between the transmembrane domain and the antibody region. A "spacer region" as provided herein is a polypeptide connecting the antibody region with the transmembrane domain. In embodiments, the spacer region connects the heavy chain constant region with the transmembrane domain. In embodiments, the binding affinity of the antibody region to an antigen is increased compared to the absence of the spacer region. In embodiments, the steric hindrance between an antibody region and an antigen is decreased in the presence of the spacer region.

[0162] In embodiments, the spacer region includes an Fc region. Examples of spacer regions contemplated for the compositions and methods provided herein include without limitation, immunoglobulin molecules or fragments thereof (e.g., IgG1, IgG2, IgG3, IgG4) and immunoglobulin molecules or fragments thereof (e.g., IgG1, IgG2, IgG3, IgG4) including mutations affecting Fc receptor binding. In embodiments, the spacer region is a fragment of an IgG (e.g., IgG4), wherein said fragment includes a deletion of the CH2 domain. The spacer region may be a peptide linker. In embodiments, the spacer region is a serine-glycine linker. In embodiments, the spacer region has the sequence GGSG. In embodiments, the spacer region has the sequence GSGSGSGS. In embodiments, the spacer region is at least 4 amino acids in length.

In embodiments, the spacer region is about 4 amino acids in length. In embodiments, the spacer region is between 4 and 250 amino acids in length. The spacer region may include residues capable of extending the half-life in vivo (e.g., plasma) of the proteins provided herein. In embodiments, the spacer region is 10 amino acids in length. In embodiments, the spacer region is 229 amino acids in length. In embodiments, the spacer region is GGGSSGGGSG. The spacer region may be "pasylated." The term "pasylated" or "pasylation" is used in its customary sense and refers to an amino acid sequences, which due to their high content in proline, alanine and serine form highly soluble biological polymers. Thus, in embodiments, the spacer region includes about 200 proline, alanine and serine residues combined. In embodiments, the spacer region includes from about 10 to about 200 proline, alanine and serine residues combined. In embodiments, the spacer region includes hydrophilic residues. In embodiments, the recombinant protein does not include a spacer region. In embodiments, the nucleic acid does not include a spacer sequence encoding a spacer region. In embodiments, the nucleic acid does not include a spacer sequence encoding a spacer region as described in WO 2015105522 A1.

[0163] In embodiments, the nucleic acid includes (i) a heavy chain sequence encoding a heavy chain domain of the protein, the heavy chain domain includes a variable heavy chain domain and the transmembrane domain; and (ii) a light chain sequence encoding a light chain domain of the protein, the light chain domain includes a variable light chain domain, wherein the variable heavy chain domain and the variable light chain domain together form at least a portion of the antibody region.

[0164] In embodiments, the isolated nucleic acid encodes from the 5' end to 3' end: a light chain sequence, a heavy chain sequence, a transmembrane sequence and an intracellular co-stimulatory signaling sequence. In embodiments, the isolated nucleic acid encodes from the 5' end to 3' end: a heavy chain sequence, a transmembrane sequence, an intracellular co-stimulatory signaling sequence and a light chain sequence. In embodiments, the isolated nucleic acid encodes from the 5' end to 3' end: a light chain sequence, a self-cleaving peptidyl linker sequence, a heavy chain sequence, a spacer sequence, a transmembrane sequence, an intracellular co-stimulatory signaling sequence and an intracellular T-cell signaling sequence. In embodiments, the isolated nucleic acid encodes from the 5' end to 3' end: a heavy chain sequence, a spacer sequence, a transmembrane sequence, an intracellular co-stimulatory signaling sequence, an intracellular T-cell signaling sequence, a self-cleaving peptidyl linker sequence and a light chain sequence.

[0165] A "light chain sequence" as provided herein refers to the nucleic acid sequence encoding for a light chain domain provided herein. A light chain domain provided herein may include a light chain variable (VL) region and/or a light chain constant region (CL). A "heavy chain sequence" as provided herein refers to the nucleic acid sequence encoding for a heavy chain domain provided herein. A heavy chain domain provided herein may include heavy chain variable (VH) region and/or a heavy chain constant region (CH). A "transmembrane sequence" as provided herein refers to the nucleic acid sequence encoding for a transmembrane domain provided herein. An "intracellular T-cell signaling sequence" as provided herein refers to the nucleic acid sequence encoding for a intracellular T-cell signaling domain provided herein. An "intracellular co-stimulatory signaling sequence" as provided herein refers to the nucleic acid sequence encoding for a intracellular co-stimulatory signaling domain provided herein.

[0166] In embodiments, the isolated nucleic acid includes a self-cleaving peptidyl sequence encoding a self-cleaving peptidyl domain between the heavy chain sequence and the light chain sequence. In embodiments, the self-cleaving peptidyl linker sequence is a T2A sequence. In embodiments, the self-cleaving peptidyl linker sequence is a T2A sequence or a 2A sequence. In embodiments, the self-cleaving peptidyl linker sequence is a foot-and-mouth disease virus sequence. In embodiments, the self-cleaving peptidyl linker sequence is PVKQLLNFDLLKLAGDVESNPGP (SEQ ID NO:83). In embodiments, the self-cleaving peptidyl linker sequence is an equine rhinitis A virus sequence. In embodiments, the self-cleaving peptidyl linker sequence is QCTNYALLKLAGDVESNPGP (SEQ ID NO:84). In embodiments, the self-cleaving peptidyl linker sequence is a porcine teschovirus 1 sequence. In embodiments, the self-cleaving peptidyl linker sequence is ATNFSLLKQAGDVEENPGP (SEQ ID NO:85). In embodiments, the self-cleaving peptidyl linker sequence is Thosea asigna virus sequence. In embodiments, the self-cleaving peptidyl linker sequence is EGRGSLLTCGDVESNPGP (SEQ ID NO:86). In embodiments, the light chain sequence is 3′ to the heavy chain sequence. In embodiments, the light chain sequence is 5′ to the heavy chain sequence.

[0167] In embodiments, the antibody region is a cetuximab meditope enabled domain, trastuzumab meditope enabled domain, pertuzumab meditope enabled domain, M5A meditope enabled domain or rituximab meditope enabled domain. In embodiments, the antibody region is a humanized cetuximab meditope enabled domain. In embodiments, the antibody region is a humanized rituximab meditope enabled domain.

[0168] In another aspect, an isolated nucleic acid is provided. The isolated nucleic acid encodes a protein including a first portion including an antibody heavy chain variable domain and a second portion including an antibody light chain variable domain and an antibody light chain constant domain, wherein the first portion further includes a transmembrane domain. In embodiments, the protein is the protein identified by SEQ ID NO:17. In embodiments, the protein is the protein identified by SEQ ID NO:28. In embodiments, the protein is the protein identified by SEQ ID NO:98. In embodiments, the protein is the protein identified by SEQ ID NO:110.

[0169] In embodiments, the protein includes from the N-terminus to the C-terminus: a signaling peptide of SEQ ID NO:18, a heavy chain domain of SEQ ID NO:19, a hinge region of SEQ ID NO:20, a spacer region of SEQ ID NO:21, a transmembrane domain of SEQ ID NO:22, an intracellular co-stimulatory signaling domain of SEQ ID NO:23, a linker domain of SEQ ID NO:24, an intracellular T-cell signaling domain of SEQ ID NO:25, a first self-cleaving peptidyl linker domain of SEQ ID NO:26, a marker peptide of SEQ ID NO:29, a second self-cleaving peptidyl linker domain of SEQ ID NO:30, a signaling peptide of SEQ ID NO:18 and a light chain domain of SEQ ID NO:27. In embodiments, the protein includes from the N-terminus to the C-terminus: a signaling peptide of SEQ ID NO:18, a heavy chain domain of SEQ ID NO:19, a hinge region of SEQ ID NO:20, a spacer region of SEQ ID NO:21, a transmembrane domain of SEQ ID NO:22, an intracellular co-stimulatory signaling domain of SEQ ID NO:23, a linker domain of SEQ ID NO:24, an intracellular T-cell signaling domain of SEQ ID NO:25, a first self-cleaving peptidyl linker domain of SEQ ID NO:26, a marker peptide of SEQ ID NO:29, a second self-cleaving peptidyl linker domain of SEQ ID NO:30, a signaling peptide of SEQ ID NO:18 or a light chain domain of SEQ ID NO:27. In embodiments, the protein includes from the N-terminus to the C-terminus: a signaling peptide of SEQ ID NO:18, a heavy chain domain of SEQ ID NO:19, a hinge region of SEQ ID NO:20, a spacer region of SEQ ID NO:21, a transmembrane domain of SEQ ID NO:22, an intracellular co-stimulatory signaling domain of SEQ ID NO:23, a linker domain of SEQ ID NO:24, an intracellular T-cell signaling domain of SEQ ID NO:25, a self-cleaving peptidyl linker domain of SEQ ID NO:26, a signaling peptide of SEQ ID NO:18 and a light chain domain of SEQ ID NO:27. In embodiments, the protein includes from the N-terminus to the C-terminus: a signaling peptide of SEQ ID NO:18, a heavy chain domain of SEQ ID NO:19, a hinge region of SEQ ID NO:20, a spacer region of SEQ ID NO:21, a transmembrane domain of SEQ ID NO:22, an intracellular co-stimulatory signaling domain of SEQ ID NO:23, a linker domain of SEQ ID NO:24, an intracellular T-cell signaling

domain of SEQ ID NO:25, a self-cleaving peptidyl linker domain of SEQ ID NO:26, a signaling peptide of SEQ ID NO:18 or a light chain domain of SEQ ID NO:27.

[0170] The term "signaling peptide" as referred to herein is used according to its ordinary meaning in the art and refers to a peptide having a length of about 5-30 amino acids. A signaling peptide is present at the N-terminus of newly synthesized proteins that form part of the secretory pathway. Proteins of the secretory pathway include, but are not limited to proteins that reside either inside certain organelles (the endoplasmic reticulum, Golgi or endosomes), are secreted from the cell, or are inserted into a cellular membrane. In embodiments, the signaling peptide forms part of the transmembrane domain of a protein.

[0171] The term "heavy chain domain" as referred to herein is used according to its ordinary meaning in the art and refers to a polypeptide including a heavy chain variable (VH) region and a heavy chain constant region (CH). The term "light chain domain" as referred to herein is used according to its ordinary meaning in the art and refers to a polypeptide including a light chain variable (VL) region and a light chain constant region (CL).

[0172] In embodiments, the protein includes from the N-terminus to the C-terminus: a signaling peptide of SEQ ID NO:87, a light chain domain of SEQ ID NO:88, a self-cleaving peptidyl linker domain of SEQ ID NO:89, a heavy chain domain of SEQ ID NO:90, a hinge region of SEQ ID NO:91, a first spacer region of SEQ ID NO:92, a second spacer region of SEQ ID NO:93, a transmembrane domain of SEQ ID NO:94, an intracellular co-stimulatory signaling domain of SEQ ID NO:95, a linker domain of SEQ ID NO:96, and an intracellular T-cell signaling domain of SEQ ID NO:97. In embodiments, the protein includes from the N-terminus to the C-terminus: a signaling peptide of SEQ ID NO:87, a light chain domain of SEQ ID NO:88, a self-cleaving peptidyl linker domain of SEQ ID NO:89, a heavy chain domain of SEQ ID NO:90, a hinge region of SEQ ID NO:91, a first spacer region of SEQ ID NO:92, a second spacer region of SEQ ID NO:93, a transmembrane domain of SEQ ID NO:94, an intracellular co-stimulatory signaling domain of SEQ ID NO:95, a linker domain of SEQ ID NO:96, or an intracellular T-cell signaling domain of SEQ ID NO:97.

[0173] In embodiments, the protein includes from the N-terminus to the C-terminus: a signaling peptide of SEQ ID NO:99, a heavy chain domain of SEQ ID NO:100, a hinge region of SEQ ID NO:101, a first spacer region of SEQ ID NO:102, a second spacer region of SEQ ID NO:103, a transmembrane domain of SEQ ID NO:104, an intracellular co-stimulatory signaling domain of SEQ ID NO:105, a linker domain of SEQ ID NO:106, and an intracellular T-cell

signaling domain of SEQ ID NO:107, a self-cleaving peptidyl linker domain of SEQ ID NO:108, and a light chain domain of SEQ ID NO:109. In embodiments, the protein includes from the N-terminus to the C-terminus: a signaling peptide of SEQ ID NO:99, a heavy chain domain of SEQ ID NO:100, a hinge region of SEQ ID NO:101, a first spacer region of SEQ ID NO:102, a second spacer region of SEQ ID NO:103, a transmembrane domain of SEQ ID NO:104, an intracellular co-stimulatory signaling domain of SEQ ID NO:105, a linker domain of SEQ ID NO:106, and an intracellular T-cell signaling domain of SEQ ID NO:107, a self-cleaving peptidyl linker domain of SEQ ID NO:108, or a light chain domain of SEQ ID NO:109.

[0174] In embodiments, the first portion further includes an intracellular T-cell signaling domain. In embodiments, the intracellular T-cell signaling domain is SEQ ID NO:11. In embodiments, the intracellular T-cell signaling domain is a CD3 ζ intracellular T-cell signaling domain. In embodiments, the first portion includes an intracellular co-stimulatory signaling domain. In embodiments, the intracellular co-stimulatory signaling domain is a CD28 intracellular co-stimulatory signaling domain, a 4-1BB intracellular co-stimulatory signaling domain, a ICOS intracellular co-stimulatory signaling domain, or an OX-40 intracellular co-stimulatory signaling domain. In embodiments, the intracellular co-stimulatory signaling domain is SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15 or SEQ ID NO:16.

[0175] In embodiments, the first portion includes a linker domain. In embodiments, the linker domain is between the transmembrane domain and the intracellular T-cell signaling domain. In embodiments, the linker domain is between the intracellular T-cell signaling domain and the intracellular co-stimulatory signaling domain. In embodiments, the linker domain comprises the sequence GGCGG or GGG.

[0176] In embodiments, the first portion includes a CD3 ζ intracellular T-cell signaling domain and intracellular co-stimulatory signaling domain. In embodiments, the first portion includes from the amino terminus to the carboxy terminus: the heavy chain variable domain, a heavy chain constant domain, the transmembrane domain, the CD3 ζ intracellular T-cell signaling domain and an intracellular co-stimulatory signaling domain.

[0177] In embodiments, the isolated nucleic acid molecule provided herein includes a spacer region positioned between the heavy chain variable domain and the transmembrane domain. In embodiments, the spacer region includes a hinge region. In embodiments, the hinge region is a CD8 hinge region. In embodiments, the hinge region is a CD28 hinge region. A "spacer region" as provided herein is a polypeptide connecting the antibody heavy chain variable domain with

the transmembrane domain. Where the first portion of the protein provided herein including embodiments thereof, includes a heavy chain constant domain, the heavy chain constant domain connects the heavy chain variable domain with the spacer region and the spacer region connects the heavy chain constant domain with the transmembrane domain. Thus in embodiments, the spacer region connects the heavy chain variable domain with the transmembrane domain. In embodiments, the spacer region connects the heavy chain constant domain with the transmembrane domain.

[0178] In embodiments, the antibody heavy chain variable domain and the antibody light chain variable domain are humanized. In embodiments, the first portion includes a heavy chain constant domain. In embodiments, the isolated nucleic acid includes a self-cleaving peptidyl sequence between the first portion and the second portion. In embodiments, the self-cleaving peptidyl encoding sequence is a T2A encoding sequence or a 2A encoding sequence. In embodiments, the self-cleaving peptidyl encoding sequence is a T2A encoding sequence or 2A encoding sequence. In embodiments, the nucleic acid sequence encoding the second portion is 3′ to the nucleic acid sequence encoding the first portion.

[0179] In embodiments, the protein or antibody region provided herein including embodiments thereof competes for antigen binding with, specifically binds to the same antigen or epitope as, and/or contains one, more, or all CDRs (or CDRs comprising at least at or about 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 % identity to the CDRs), e.g., including a heavy chain CDR 1, 2, and/or 3 and/or a light chain CDR1, 2, and/or 3, of one or more known antibodies, including any commercially available antibody, such as abagovomab, abciximab, adalimumab, adecatumumab, alemtuzumab, altumomab, altumomab pentetate, anatumomab, anatumomab mafenatox, arcitumomab, atlizumab, basiliximab, bectumomab, ectumomab, belimumab, benralizumab, bevacizumab, brentuximab, canakinumab, capromab, capromab pendetide, catumaxomab, certolizumab, clivatuzumab tetraxetan, daclizumab, denosumab, eculizumab, edrecolomab, efalizumab, etaracizumab, ertumaxomab, fanolesomab, Fbta05, fontolizumab, gemtuzumab, girentuximab, golimumab, ibritumomab, igovomab, infliximab, ipilimumab, labetuzumab, mepolizumab, muromonab, muromonab-CD3, natalizumab, necitumumab, nimotuzumab, ofatumumab, omalizumab, oregovomab, palivizumab, panitumumab, ranibizumab, rituximab, satumomab, sulesomab, ibritumomab, ibritumomab tiuxetan, tocilizumab, tositumomab, trastuzumab, Trbs07, ustekinumab, visilizumab, votumumab, zalutumumab, and/or brodalumab; and/or anrukinzumab, bapineuzumab, dalotuzumab, demcizumab, ganitumab, inotuzumab, mavrilimumab, moxetumomab pasudotox, rilotumumab,

sifalimumab, tanezumab, tralokinumab, tremelimumab, urelumab, the antibody produced by the hybridoma 10B5 (see Edelson & Unanue, *Curr Opin Immunol*, 2000 Aug;12(4):425-31), B6H12.2 (abcam) or other anti-CD47 antibody (see Chao et al., *Cell*, 142, 699–713, September 3, 2010).

[0180] In embodiments, the protein or antibody region specifically binds to an antigen selected from the group consisting of: CA-125, glycoprotein (GP) IIb/IIIa receptor, TNF-alpha, CD52, TAG-72, Carcinoembryonic antigen (CEA), interleukin-6 receptor (IL-6R), IL-2, interleukin-2 receptor a-chain (CD25), CD22, B-cell activating factor, interleukin-5 receptor (CD125), VEGF, VEGF-A, CD30, IL-1beta, prostate specific membrane antigen (PSMA), CD3, EpCAM, EGF receptor (EGFR), MUC1, human interleukin-2 receptor, Tac, RANK ligand, a complement protein, e.g., C5, EpCAM, CD11a, e.g., human CD11a, an integrin, e.g., alpha-v beta-3 integrin, vitronectin receptor alpha v beta 3 integrin, HER2, neu, CD3, CD15, CD20 (small and/or large loops), Interferon gamma, CD33, CA-IX, TNF alpha, CTLA-4, carcinoembryonic antigen, IL-5, CD3 epsilon, CAM, Alpha-4-integrin, IgE, e.g., IgE Fc region, an RSV antigen, e.g., F protein of respiratory syncytial virus (RSV), TAG-72, NCA-90 (granulocyte cell antigen), IL-6, GD2, GD3, IL-12, IL-23, IL-17, CTAA16.88, IL13, interleukin-1 beta, beta-amyloid, IGF-1 receptor (IGF-1R), delta-like ligand 4 (DLL4), alpha subunit of granulocyte macrophage colony stimulating factor receptor, hepatocyte growth factor, IFN-alpha, nerve growth factor, IL-13, CD326, Programmed cell death 1 ligand 1 (PD-L1, a.k.a. CD274, B7-H1), CD47, and CD137.

[0181] In embodiments, the protein or antibody region is an anti-CD19 protein, anti-CD20 protein, anti-CD22 protein, anti-CD30 protein, anti-CD33 protein, anti-CD44v6/7/8 protein, anti-CD123 protein, anti-CEA protein, anti-EGP-2 protein, anti-EGP-40 protein, anti-erb-B2 protein, anti-erb-B2,3,4 protein, anti-FBP protein, anti-fetal acetylcholine receptor protein, anti-GD2 protein, anti-GD3 protein, anti-Her2/neu protein, anti-IL-13R-a2 protein, anti-KDR protein, anti k-light chain protein, anti-LeY protein, anti-L1 cell adhesion molecule protein, anti-MAGE-A1 protein, anti-mesothelin protein, anti-murine CMV infected cell protein, anti-MUC2 protein, anti-NKGD2 protein, anti, oncofetal antigen protein, anti-PCSA protein, anti-PSMA protein, anti-TAA (targeted by mAb IfE) protein, anti-EGFR protein, anti-TAG-72 protein or anti-VEGF-72 protein.

[0182] In embodiments, the protein or antibody region has a light chain sequence including P8 , V9 or I9, I10 or L10, Q38, R39, T40, N41 G42, S43, P44, R45, D82, I83, A84, D85, Y86, Y87, G99, A100, G101, T102, K103, L104, E105, R142, S162, V163, T164, E165, Q166, D167, S168, and/or Y173, according to Kabat numbering, and/or has a heavy chain having Q6, P9,

R38, Q39, S40, P41, G42, K43, G44, L45, S84, D86, T87, A88, I89, Y90, Y91, W103, G104, Q105, G106, T107, L108, V111, T110, Y147, E150, P151, V152, T173, F174, P175, A176, V177, Y185, S186, and/or L187, according to Kabat numbering.

[0183] Also provided are complexes including an antibody region or protein bound to one or more compounds including a peptidyl moiety as provided herein. The antibody region or protein may be any of the antibodies described herein including fragments thereof. The one or more compounds including a peptidyl moiety as provided herein may include any one or more of the compounds described herein, such as those described in this section, including monovalent and multivalent compounds, and labeled compounds.

[0184] In another aspect, an expression vector including a nucleic acid provided herein including embodiments thereof is provided. In embodiments, the expression vector is a viral vector. In embodiments, the virus is a lentivirus or onco-retrovirus. In embodiments, the virus is a lentivirus or onco-retrovirus.

[0185] In another aspect, a mammalian cell including the expression vector provided herein including embodiments thereof is provided. In embodiments, the mammalian cell is a natural killer (Nk) cell. In embodiments, the mammalian cell is an induced pluripotent stem cell. In embodiments, the mammalian cell is a hematopoietic stem cell. In embodiments, the mammalian cell includes a first polypeptide and a second polypeptide, the first polypeptide including a heavy chain variable domain, a heavy chain constant domain, a transmembrane domain, a CD3 ζ signaling domain and a co-stimulatory T-cell signaling domain, the second polypeptide including a light chain variable domain and a light chain constant domain.

[0186] In another aspect, a T lymphocyte including the expression vector provided herein including embodiments thereof is provided. In embodiments, the T lymphocyte includes a first polypeptide and a second polypeptide, the first polypeptide including a heavy chain variable domain, a heavy chain constant domain, a transmembrane domain, a CD3 ζ signaling domain and a co-stimulatory T-cell signaling domain, the second polypeptide including a light chain variable domain and a light chain constant domain.

RECOMBINANT PROTEINS

[0187] In another aspect, a recombinant protein is provided. The recombinant protein includes (i) an antibody region including a central cavity formed by a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the central cavity forms a peptide binding site including framework region amino acid residues; and (ii) a transmembrane domain. In

embodiments, the antibody region further includes a heavy chain constant region (CH) and a light chain constant region (CL). In embodiments, the antibody region includes an Fc domain. In embodiments, the antibody region is a humanized antibody region (e.g. a humanized mouse antibody region). In embodiments, the antibody region does not include a scFv antibody region.

[0188] In embodiments, the protein further includes an intracellular T-cell signaling domain as described herein. In embodiments, the intracellular T-cell signaling domain is a CD3 ζ intracellular T-cell signaling domain. In embodiments, the protein further includes an intracellular co-stimulatory signaling domain. In embodiments, the intracellular co-stimulatory signaling domain is a CD28 intracellular co-stimulatory signaling domain, a 4-1BB intracellular co-stimulatory signaling domain, a ICOS intracellular co-stimulatory signaling domain, or an OX-40 intracellular co-stimulatory signaling domain.

[0189] In embodiments, the protein further includes a spacer region. In embodiments, the spacer region is between the transmembrane domain and the antibody region.

[0190] In embodiments, the protein further includes a linker domain. In embodiments, the linker domain is between the transmembrane domain and the intracellular T-cell signaling domain. In embodiments, the linker domain is between the intracellular T-cell signaling domain and the intracellular co-stimulatory signaling domain. In embodiments, the linker domain includes the sequence GGCGG or GGG. In embodiments, the antibody region is a cetuximab meditope enabled domain, trasuzumab meditope enabled domain, pertuzumab meditope enabled domain, M5A meditope enabled domain or rituximab meditope enabled domain. In embodiments, a compound including an peptidyl moiety is bound to the peptide binding site. In embodiments, the compound is a multivalent meditope. A "multivalent meditope" as provided herein is a peptidyl moiety as described herein. Thus, a multivalent meditope is capable of binding the peptide binding site provided herein including embodiments thereof. In embodiments, the multivalent meditope binds to the FR lining the peptide binding site. In embodiments, the multivalent meditope is bound to therapeutic or diagnostic moiety through a chemical linker. In embodiments, the multivalent meditope has the structure of formula (I) or (II). The proteins and compounds may be any of the protein or compounds described herein including embodiments thereof.

[0191] In another aspect, a recombinant protein is provided. The recombinant protein includes a first portion including an antibody heavy chain variable domain and a second portion including an antibody light chain variable domain and an antibody light chain constant domain, wherein

the first portion further includes a transmembrane domain, and wherein the antibody heavy chain variable domain, the antibody light chain variable domain and the antibody light chain constant domain together form an antibody region.

[0192] In another aspect, a mammalian cell including the recombinant protein provided herein including embodiments thereof is provided, wherein the transmembrane domain is within the cell membrane of the mammalian cell. In embodiments, the mammalian cell is a natural killer (Nk) cell. In embodiments, the mammalian cell is an induced pluripotent stem cell. In embodiments, the mammalian cell is a hematopoietic stem cell.

[0193] In another aspect, a T lymphocyte including the recombinant protein provided herein including embodiments thereof is provided, wherein the transmembrane domain is within the cell membrane of the T lymphocyte.

PHARMACEUTICAL COMPOSITIONS

[0194] Pharmaceutical compositions provided by the present invention (e.g., proteins and compounds provided herein) include compositions wherein the active ingredient (e.g. compositions described herein, including embodiments or examples) is contained in a therapeutically effective amount, *i.e.*, in an amount effective to achieve its intended purpose. The actual amount effective for a particular application will depend, *inter alia,* on the condition being treated. When administered in methods to treat a disease, the recombinant proteins described herein will contain an amount of active ingredient effective to achieve the desired result, e.g., modulating the activity of a target molecule, and/or reducing, eliminating, or slowing the progression of disease symptoms. Determination of a therapeutically effective amount of a compound of the invention is well within the capabilities of those skilled in the art, especially in light of the detailed disclosure herein.

[0195] The dosage and frequency (single or multiple doses) administered to a mammal can vary depending upon a variety of factors, for example, whether the mammal suffers from another disease, and its route of administration; size, age, sex, health, body weight, body mass index, and diet of the recipient; nature and extent of symptoms of the disease being treated (e.g. symptoms of cancer and severity of such symptoms), kind of concurrent treatment, complications from the disease being treated or other health-related problems. Other therapeutic regimens or agents can be used in conjunction with the methods and compounds of the invention. Adjustment and manipulation of established dosages (e.g., frequency and duration) are well within the ability of those skilled in the art.

[0196] For any composition (e.g., recombinant protein, nucleic acid) provided herein, the therapeutically effective amount can be initially determined from cell culture assays. Target concentrations will be those concentrations of active compound(s) that are capable of achieving the methods described herein, as measured using the methods described herein or known in the art. As is well known in the art, effective amounts for use in humans can also be determined from animal models. For example, a dose for humans can be formulated to achieve a concentration that has been found to be effective in animals. The dosage in humans can be adjusted by monitoring effectiveness and adjusting the dosage upwards or downwards, as described above. Adjusting the dose to achieve maximal efficacy in humans based on the methods described above and other methods is well within the capabilities of the ordinarily skilled artisan.

[0197] Dosages may be varied depending upon the requirements of the patient and the compound being employed. The dose administered to a patient, in the context of the present invention should be sufficient to affect a beneficial therapeutic response in the patient over time. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects. Determination of the proper dosage for a particular situation is within the skill of the practitioner. Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under circumstances is reached.

[0198] Dosage amounts and intervals can be adjusted individually to provide levels of the administered compound effective for the particular clinical indication being treated. This will provide a therapeutic regimen that is commensurate with the severity of the individual's disease state.

[0199] Utilizing the teachings provided herein, an effective prophylactic or therapeutic treatment regimen can be planned that does not cause substantial toxicity and yet is effective to treat the clinical symptoms demonstrated by the particular patient. This planning should involve the careful choice of active compound by considering factors such as compound potency, relative bioavailability, patient body weight, presence and severity of adverse side effects, preferred

[0200] "Pharmaceutically acceptable excipient" and "pharmaceutically acceptable carrier" refer to a substance that aids the administration of an active agent to and absorption by a subject and can be included in the compositions of the present invention without causing a significant adverse toxicological effect on the patient. Non-limiting examples of pharmaceutically

acceptable excipients include water, NaCl, normal saline solutions, lactated Ringer's, normal sucrose, normal glucose, binders, fillers, disintegrants, lubricants, coatings, sweeteners, flavors, salt solutions (such as Ringer's solution), alcohols, oils, gelatins, carbohydrates such as lactose, amylose or starch, fatty acid esters, hydroxymethycellulose, polyvinyl pyrrolidine, and colors, and the like. Such preparations can be sterilized and, if desired, mixed with auxiliary agents such as lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, and/or aromatic substances and the like that do not deleteriously react with the compounds of the invention. One of skill in the art will recognize that other pharmaceutical excipients are useful in the present invention.

[0201] The term "pharmaceutically acceptable salt" refers to salts derived from a variety of organic and inorganic counter ions well known in the art and include, by way of example only, sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium, and the like; and when the molecule contains a basic functionality, salts of organic or inorganic acids, such as hydrochloride, hydrobromide, tartrate, mesylate, acetate, maleate, oxalate and the like.

[0202] The term "preparation" is intended to include the formulation of the active compound with encapsulating material as a carrier providing a capsule in which the active component with or without other carriers, is surrounded by a carrier, which is thus in association with it. Similarly, cachets and lozenges are included. Tablets, powders, capsules, pills, cachets, and lozenges can be used as solid dosage forms suitable for oral administration.

[0128] The pharmaceutical preparation is optionally in unit dosage form. In such form the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form. The unit dosage form can be of a frozen dispersion.

METHODS OF TREATMENT

[0203] In another aspect, a method of treating cancer is provided. The method includes administering to a subject in need thereof an effective amount of the mammalian cell provided herein including embodiments thereof, wherein the antibody region is an anti-cancer antibody region.

[0204] In another aspect, a method of treating cancer is provided. The method includes administering to a subject in need thereof an effective amount of the T-lymphocyte provided herein including embodiments thereof, wherein the antibody region is an anti-cancer antibody region. In embodiments, the T-lymphocyte is an autologous T-lymphocyte. In embodiments, the T-lymphocyte is a heterologous T-lymphocyte. In embodiments, the cancer is a solid tumor cancer or hematologic malignancy. In embodiments, the cancer is ovarian cancer, renal cell carcinoma, a B-cell malignancy, leukemia, lymphoma, breast cancer, colorectal cancer, prostate cancer, neuroblastoma, melanoma, medulloblastoma, lung cancer, osteosarcoma, glioblastoma or glioma. In embodiments, the leukemia is acute lymphoid leukemia. In embodiments, the leukemia is chronic lymphocytic leukemia. In embodiments, the leukemia is acute myeloid leukemia. In embodiments, the leukemia is chronic myeloid leukemia.

[0205] In another aspect, a method of reprogramming a T lymphocyte is provided. The method includes contacting a T lymphocyte with the expression vector provided herein including embodiments thereof.

[0206] In another aspect, a method of detecting a cancer is provided. The method includes (i) administering to a cancer patient an effective amount of a T lymphocyte including the recombinant protein provided herein including embodiments thereof and a compound including a peptidyl moiety capable of binding to the peptide binding site, wherein the compound further includes a detectable label, and wherein the antibody region is an anti-cancer antibody region. The method includes (ii) allowing the compound to bind to the peptide binding site thereby forming a recombinant protein-compound complex. And (iii) the recombinant protein-compound complex is detected within the cancer patient thereby detecting the cancer.

[0207] As used herein, "treatment" or "treating," or "palliating" or "ameliorating" are used interchangeably herein. These terms refer to an approach for obtaining beneficial or desired results including but not limited to therapeutic benefit and/or a prophylactic benefit. By therapeutic benefit is meant eradication or amelioration of the underlying disorder being treated. Also, a therapeutic benefit is achieved with the eradication or amelioration of one or more of the physiological symptoms associated with the underlying disorder such that an improvement is observed in the patient, notwithstanding that the patient may still be afflicted with the underlying disorder. For prophylactic benefit, the compositions may be administered to a patient at risk of developing a particular disease, or to a patient reporting one or more of the physiological symptoms of a disease, even though a diagnosis of this disease may not have been made. Treatment includes preventing the disease, that is, causing the clinical symptoms of the disease

not to develop by administration of a protective composition prior to the induction of the disease; suppressing the disease, that is, causing the clinical symptoms of the disease not to develop by administration of a protective composition after the inductive event but prior to the clinical appearance or reappearance of the disease; inhibiting the disease, that is, arresting the development of clinical symptoms by administration of a protective composition after their initial appearance; preventing re-occurring of the disease and/or relieving the disease, that is, causing the regression of clinical symptoms by administration of a protective composition after their initial appearance. For example, certain methods herein treat cancer (e.g. lung cancer, ovarian cancer, osteosarcoma, bladder cancer, cervical cancer, liver cancer, kidney cancer, skin cancer (e.g., Merkel cell carcinoma), testicular cancer, leukemia, lymphoma, head and neck cancer, colorectal cancer, prostate cancer, pancreatic cancer, melanoma, breast cancer, neuroblastoma). For example certain methods herein treat cancer by decreasing or reducing or preventing the occurrence, growth, metastasis, or progression of cancer; or treat cancer by decreasing a symptom of cancer. Symptoms of cancer (e.g. lung cancer, ovarian cancer, osteosarcoma, bladder cancer, cervical cancer, liver cancer, kidney cancer, skin cancer (e.g., Merkel cell carcinoma), testicular cancer, leukemia, lymphoma, head and neck cancer, colorectal cancer, prostate cancer, pancreatic cancer, melanoma, breast cancer, neuroblastoma) would be known or may be determined by a person of ordinary skill in the art.

[0208] As used herein the terms “treatment,” “treat,” or “treating” refers to a method of reducing the effects of one or more symptoms of a disease or condition characterized by expression of the protease or symptom of the disease or condition characterized by expression of the protease. Thus in the disclosed method, treatment can refer to a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% reduction in the severity of an established disease, condition, or symptom of the disease or condition. For example, a method for treating a disease is considered to be a treatment if there is a 10% reduction in one or more symptoms of the disease in a subject as compared to a control. Thus the reduction can be a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or any percent reduction in between 10% and 100% as compared to native or control levels. It is understood that treatment does not necessarily refer to a cure or complete ablation of the disease, condition, or symptoms of the disease or condition. Further, as used herein, references to decreasing, reducing, or inhibiting include a change of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater as compared to a control level and such terms can include but do not necessarily include complete elimination.

[0209] An "effective amount" is an amount sufficient to accomplish a stated purpose (e.g. achieve the effect for which it is administered, treat a disease, reduce enzyme activity, reduce one or more symptoms of a disease or condition). An example of an "effective amount" is an amount sufficient to contribute to the treatment, prevention, or reduction of a symptom or symptoms of a disease, which could also be referred to as a "therapeutically effective amount." A "reduction" of a symptom or symptoms (and grammatical equivalents of this phrase) means decreasing of the severity or frequency of the symptom(s), or elimination of the symptom(s). A "prophylactically effective amount" of a drug is an amount of a drug that, when administered to a subject, will have the intended prophylactic effect, e.g., preventing or delaying the onset (or reoccurrence) of an injury, disease, pathology or condition, or reducing the likelihood of the onset (or reoccurrence) of an injury, disease, pathology, or condition, or their symptoms. The full prophylactic effect does not necessarily occur by administration of one dose, and may occur only after administration of a series of doses. Thus, a prophylactically effective amount may be administered in one or more administrations. An "activity decreasing amount," as used herein, refers to an amount of antagonist required to decrease the activity of an enzyme or protein relative to the absence of the antagonist. A "function disrupting amount," as used herein, refers to the amount of antagonist required to disrupt the function of an enzyme or protein relative to the absence of the antagonist. Guidance can be found in the literature for appropriate dosages for given classes of pharmaceutical products. For example, for the given parameter, an effective amount will show an increase or decrease of at least 5%, 10%, 15%, 20%, 25%, 40%, 50%, 60%, 75%, 80%, 90%, or at least 100%. Efficacy can also be expressed as "-fold" increase or decrease. For example, a therapeutically effective amount can have at least a 1.2-fold, 1.5-fold, 2-fold, 5-fold, or more effect over a control. The exact amounts will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (*see*, *e.g.*, Lieberman, *Pharmaceutical Dosage Forms* (vols. 1-3, 1992); Lloyd, *The Art, Science and Technology of Pharmaceutical Compounding* (1999); Pickar, *Dosage Calculations* (1999); and *Remington: The Science and Practice of Pharmacy*, 20th Edition, 2003, Gennaro, Ed., Lippincott, Williams & Wilkins).

[0210] As used herein, the term "administering" means oral administration, administration as a suppository, topical contact, intravenous, intraperitoneal, intramuscular, intralesional, intrathecal, intranasal or subcutaneous administration, or the implantation of a slow-release device, *e.g.,* a mini-osmotic pump, to a subject. Administration is by any route, including parenteral and transmucosal (*e.g.,* buccal, sublingual, palatal, gingival, nasal, vaginal, rectal, or transdermal). Parenteral administration includes, *e.g.,* intravenous, intramuscular, intra-arteriole, intradermal,

subcutaneous, intraperitoneal, intraventricular, and intracranial. Other modes of delivery include, but are not limited to, the use of liposomal formulations, intravenous infusion, transdermal patches, *etc.* By "co-administer" it is meant that a composition described herein is administered at the same time, just prior to, or just after the administration of one or more additional therapies, for example cancer therapies such as chemotherapy, hormonal therapy, radiotherapy, or immunotherapy. The compounds of the invention can be administered alone or can be coadministered to the patient. Coadministration is meant to include simultaneous or sequential administration of the compounds individually or in combination (more than one compound). Thus, the preparations can also be combined, when desired, with other active substances (e.g. to reduce metabolic degradation). The compositions of the present invention can be delivered by transdermally, by a topical route, formulated as applicator sticks, solutions, suspensions, emulsions, gels, creams, ointments, pastes, jellies, paints, powders, and aerosols.

[0211] The compositions of the present invention may additionally include components to provide sustained release and/or comfort. Such components include high molecular weight, anionic mucomimetic polymers, gelling polysaccharides and finely-divided drug carrier substrates. These components are discussed in greater detail in U.S. Pat. Nos. 4,911,920; 5,403,841; 5,212,162; and 4,861,760. The entire contents of these patents are incorporated herein by reference in their entirety for all purposes. The compositions of the present invention can also be delivered as microspheres for slow release in the body. For example, microspheres can be administered via intradermal injection of drug-containing microspheres, which slowly release subcutaneously (see Rao, *J. Biomater Sci. Polym. Ed.* 7:623-645, 1995; as biodegradable and injectable gel formulations (see, e.g., Gao *Pharm. Res.* 12:857-863, 1995); or, as microspheres for oral administration (see, e.g., Eyles, *J. Pharm. Pharmacol.* 49:669-674, 1997). In embodiments, the formulations of the compositions of the present invention can be delivered by the use of liposomes which fuse with the cellular membrane or are endocytosed, *i.e.,* by employing receptor ligands attached to the liposome, that bind to surface membrane protein receptors of the cell resulting in endocytosis. By using liposomes, particularly where the liposome surface carries receptor ligands specific for target cells, or are otherwise preferentially directed to a specific organ, one can focus the delivery of the compositions of the present invention into the target cells in vivo. (See, *e.g.*, Al-Muhammed, *J. Microencapsul.* 13:293-306, 1996; Chonn, *Curr. Opin. Biotechnol.* 6:698-708, 1995; Ostro, *Am. J. Hosp. Pharm.* 46:1576-1587, 1989). The compositions of the present invention can also be delivered as nanoparticles.

[0136] Utilizing the teachings provided herein, an effective prophylactic or therapeutic treatment regimen can be planned that does not cause substantial toxicity and yet is effective to treat the clinical symptoms demonstrated by the particular patient. This planning should involve the careful choice of active compound by considering factors such as compound potency, relative bioavailability, patient body weight, presence and severity of adverse side effects, preferred mode of administration and the toxicity profile of the selected agent.

[0212] "Anti-cancer agent" is used in accordance with its plain ordinary meaning and refers to a composition (e.g. compound, drug, antagonist, inhibitor, modulator) having antineoplastic properties or the ability to inhibit the growth or proliferation of cells. In embodiments, an anti-cancer agent is a chemotherapeutic. In embodiments, an anti-cancer agent is an agent identified herein having utility in methods of treating cancer. In embodiments, an anti-cancer agent is an agent approved by the FDA or similar regulatory agency of a country other than the USA, for treating cancer.

[0213] The compositions described herein can be used in combination with one another, with other active agents known to be useful in treating a cancer such as anti-cancer agents.

[0214] Examples of anti-cancer agents include, but are not limited to, MEK (e.g. MEK1, MEK2, or MEK1 and MEK2) inhibitors (e.g. XL518, CI-1040, PD035901, selumetinib/ AZD6244, GSK1120212/ trametinib, GDC-0973, ARRY-162, ARRY-300, AZD8330, PD0325901, U0126, PD98059, TAK-733, PD318088, AS703026, BAY 869766), alkylating agents (*e.g.,* cyclophosphamide, ifosfamide, chlorambucil, busulfan, melphalan, mechlorethamine, uramustine, thiotepa, nitrosoureas, nitrogen mustards (e.g., mechloroethamine, cyclophosphamide, chlorambucil, meiphalan), ethylenimine and methylmelamines (e.g., hexamethlymelamine, thiotepa), alkyl sulfonates (e.g., busulfan), nitrosoureas (e.g., carmustine, lomusitne, semustine, streptozocin), triazenes (decarbazine)), anti-metabolites (*e.g.,* 5-azathioprine, leucovorin, capecitabine, fludarabine, gemcitabine, pemetrexed, raltitrexed, folic acid analog (e.g., methotrexate), or pyrimidine analogs (e.g., fluorouracil, floxouridine, Cytarabine), purine analogs (e.g., mercaptopurine, thioguanine, pentostatin), *etc.*), plant alkaloids (*e.g.,* vincristine, vinblastine, vinorelbine, vindesine, podophyllotoxin, paclitaxel, docetaxel, *etc.*), topoisomerase inhibitors (*e.g.,* irinotecan, topotecan, amsacrine, etoposide (VP16), etoposide phosphate, teniposide, *etc.*), antitumor antibiotics (*e.g.,* doxorubicin, adriamycin, daunorubicin, epirubicin, actinomycin, bleomycin, mitomycin, mitoxantrone, plicamycin, *etc.*), platinum-based compounds (*e.g.* cisplatin, oxaloplatin, carboplatin), anthracenedione (e.g., mitoxantrone), substituted urea (e.g., hydroxyurea), methyl hydrazine derivative (e.g.,

procarbazine), adrenocortical suppressant (e.g., mitotane, aminoglutethimide), epipodophyllotoxins (e.g., etoposide), antibiotics (e.g., daunorubicin, doxorubicin, bleomycin), enzymes (e.g., L-asparaginase), inhibitors of mitogen-activated protein kinase signaling (e.g. U0126, PD98059, PD184352, PD0325901, ARRY-142886, SB239063, SP600125, BAY 43-9006, wortmannin, or LY294002, Syk inhibitors, mTOR inhibitors, antibodies (e.g., rituxan), gossyphol, genasense, polyphenol E, Chlorofusin, all trans-retinoic acid (ATRA), bryostatin, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), 5-aza-2'-deoxycytidine, all trans retinoic acid, doxorubicin, vincristine, etoposide, gemcitabine, imatinib (Gleevec.RTM.), geldanamycin, 17-N-Allylamino-17-Demethoxygeldanamycin (17-AAG), flavopiridol, LY294002, bortezomib, trastuzumab, BAY 11-7082, PKC412, PD184352, 20-epi-1, 25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorins; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrodidemnin B; deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; 9-dioxamycin; diphenyl spiromustine; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists;

etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorunicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein

tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylerie conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen-binding protein; sizofuran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; zinostatin stimalamer, Adriamycin, Dactinomycin, Bleomycin, Vinblastine, Cisplatin, acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; aminoglutethimide; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; bropirimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; chlorambucil; cirolemycin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; elsamitrucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride;

estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; fluorocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; iimofosine; interleukin I1 (including recombinant interleukin II, or rlL.sub.2), interferon alfa-2a; interferon alfa-2b; interferon alfa-n1; interferon alfa-n3; interferon beta-1a; interferon gamma-1b; iproplatin; irinotecan hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedepa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazoie; nogalamycin; ormaplatin; oxisuran; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; piposulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; rogletimide; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; tegafur; teloxantrone hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; trestolone acetate; triciribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; zorubicin hydrochloride, agents that arrest cells in the G2-M phases and/or modulate the formation or stability of microtubules, (e.g. Taxol.TM (i.e. paclitaxel), Taxotere.TM, compounds comprising the taxane skeleton, Erbulozole (i.e. R-55104), Dolastatin 10 (i.e. DLS-10 and NSC-376128), Mivobulin isethionate (i.e. as CI-980), Vincristine, NSC-639829, Discodermolide (i.e. as NVP-XX-A-296), ABT-751 (Abbott, i.e. E-7010), Altorhyrtins (e.g. Altorhyrtin A and Altorhyrtin C), Spongistatins (e.g. Spongistatin 1, Spongistatin 2, Spongistatin 3, Spongistatin 4, Spongistatin 5, Spongistatin 6, Spongistatin 7, Spongistatin 8, and Spongistatin 9), Cemadotin hydrochloride (i.e. LU-103793 and NSC-D-669356), Epothilones (e.g. Epothilone A, Epothilone B, Epothilone C (i.e. desoxyepothilone A or dEpoA), Epothilone D (i.e. KOS-862, dEpoB, and desoxyepothilone B), Epothilone E, Epothilone F, Epothilone B N-oxide, Epothilone

A N-oxide, 16-aza-epothilone B, 21-aminoepothilone B (i.e. BMS-310705), 21-hydroxyepothilone D (i.e. Desoxyepothilone F and dEpoF), 26-fluoroepothilone, Auristatin PE (i.e. NSC-654663), Soblidotin (i.e. TZT-1027), LS-4559-P (Pharmacia, i.e. LS-4577), LS-4578 (Pharmacia, i.e. LS-477-P), LS-4477 (Pharmacia), LS-4559 (Pharmacia), RPR-112378 (Aventis), Vincristine sulfate, DZ-3358 (Daiichi), FR-182877 (Fujisawa, i.e. WS-9885B), GS-164 (Takeda), GS-198 (Takeda), KAR-2 (Hungarian Academy of Sciences), BSF-223651 (BASF, i.e. ILX-651 and LU-223651), SAH-49960 (Lilly/Novartis), SDZ-268970 (Lilly/Novartis), AM-97 (Armad/Kyowa Hakko), AM-132 (Armad), AM-138 (Armad/Kyowa Hakko), IDN-5005 (Indena), Cryptophycin 52 (i.e. LY-355703), AC-7739 (Ajinomoto, i.e. AVE-8063A and CS-39.HCl), AC-7700 (Ajinomoto, i.e. AVE-8062, AVE-8062A, CS-39-L-Ser.HCl, and RPR-258062A), Vitilevuamide, Tubulysin A, Canadensol, Centaureidin (i.e. NSC-106969), T-138067 (Tularik, i.e. T-67, TL-138067 and TI-138067), COBRA-1 (Parker Hughes Institute, i.e. DDE-261 and WHI-261), H10 (Kansas State University), H16 (Kansas State University), Oncocidin A1 (i.e. BTO-956 and DIME), DDE-313 (Parker Hughes Institute), Fijianolide B, Laulimalide, SPA-2 (Parker Hughes Institute), SPA-1 (Parker Hughes Institute, i.e. SPIKET-P), 3-IAABU (Cytoskeleton/Mt. Sinai School of Medicine, i.e. MF-569), Narcosine (also known as NSC-5366), Nascapine, D-24851 (Asta Medica), A-105972 (Abbott), Hemiasterlin, 3-BAABU (Cytoskeleton/Mt. Sinai School of Medicine, i.e. MF-191), TMPN (Arizona State University), Vanadocene acetylacetonate, T-138026 (Tularik), Monsatrol, lnanocine (i.e. NSC-698666), 3-IAABE (Cytoskeleton/Mt. Sinai School of Medicine), A-204197 (Abbott), T-607 (Tuiarik, i.e. T-900607), RPR-115781 (Aventis), Eleutherobins (such as Desmethyleleutherobin, Desaetyleleutherobin, lsoeleutherobin A, and Z-Eleutherobin), Caribaeoside, Caribaeolin, Halichondrin B, D-64131 (Asta Medica), D-68144 (Asta Medica), Diazonamide A, A-293620 (Abbott), NPI-2350 (Nereus), Taccalonolide A, TUB-245 (Aventis), A-259754 (Abbott), Diozostatin, (-)-Phenylahistin (i.e. NSCL-96F037), D-68838 (Asta Medica), D-68836 (Asta Medica), Myoseverin B, D-43411 (Zentaris, i.e. D-81862), A-289099 (Abbott), A-318315 (Abbott), HTI-286 (i.e. SPA-110, trifluoroacetate salt) (Wyeth), D-82317 (Zentaris), D-82318 (Zentaris), SC-12983 (NCI), Resverastatin phosphate sodium, BPR-OY-007 (National Health Research Institutes), and SSR-250411 (Sanofi)), steroids (*e.g.,* dexamethasone), finasteride, aromatase inhibitors, gonadotropin-releasing hormone agonists (GnRH) such as goserelin or leuprolide, adrenocorticosteroids (e.g., prednisone), progestins (e.g., hydroxyprogesterone caproate, megestrol acetate, medroxyprogesterone acetate), estrogens (e.g., diethlystilbestrol, ethinyl estradiol), antiestrogen (e.g., tamoxifen), androgens (e.g., testosterone propionate, fluoxymesterone), antiandrogen (e.g., flutamide), immunostimulants (*e.g.,* Bacillus

Calmette-Guérin (BCG), levamisole, interleukin-2, alpha-interferon, *etc.*), monoclonal antibodies (*e.g.,* anti-CD20, anti-HER2, anti-CD52, anti-HLA-DR, and anti-VEGF monoclonal antibodies), immunotoxins (*e.g.,* anti-CD33 monoclonal antibody-calicheamicin conjugate, anti-CD22 monoclonal antibody-pseudomonas exotoxin conjugate, *etc.*), radioimmunotherapy (*e.g.,* anti-CD20 monoclonal antibody conjugated to ^{111}In, ^{90}Y, or ^{131}I, *etc.*), triptolide, homoharringtonine, dactinomycin, doxorubicin, epirubicin, topotecan, itraconazole, vindesine, cerivastatin, vincristine, deoxyadenosine, sertraline, pitavastatin, irinotecan, clofazimine, 5-nonyloxytryptamine, vemurafenib, dabrafenib, erlotinib, gefitinib, EGFR inhibitors, epidermal growth factor receptor (EGFR)-targeted therapy or therapeutic (e.g. gefitinib (Iressa ™), erlotinib (Tarceva ™), cetuximab (Erbitux™), lapatinib (Tykerb™), panitumumab (Vectibix™), vandetanib (Caprelsa™), afatinib/BIBW2992, CI-1033/canertinib, neratinib/HKI-272, CP-724714, TAK-285, AST-1306, ARRY334543, ARRY-380, AG-1478, dacomitinib/PF299804, OSI-420/desmethyl erlotinib, AZD8931, AEE788, pelitinib/EKB-569, CUDC-101, WZ8040, WZ4002, WZ3146, AG-490, XL647, PD153035, BMS-599626), sorafenib, imatinib, sunitinib, dasatinib, or the like. In embodiments, the compositions herein may be used in combination with adjunctive agents that may not be effective alone, but may contribute to the efficacy of the active agent in treating cancer.

[0215] In embodiments, co-administration includes administering one active agent within 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20, or 24 hours of a second active agent. Co-administration includes administering two active agents simultaneously, approximately simultaneously (e.g., within about 1, 5, 10, 15, 20, or 30 minutes of each other), or sequentially in any order. In embodiments, co-administration can be accomplished by co-formulation, i.e., preparing a single pharmaceutical composition including both active agents. In embodiments, the active agents can be formulated separately. In embodiments, the active and/or adjunctive agents may be linked or conjugated to one another.

EXAMPLES

[0216] Applicants have discovered a unique binding site for a cyclic peptide (also referred to herein as a "meditope") within the central cavity of the Fab arm of the therapeutic mAb, cetuximab (1). Applicants demonstrated that this site is unique to cetuximab and absent in human mAbs. Applicants have also shown, biochemically and in cell culture and in animal xenograft studies, that occupancy of this site does not affect antigen binding. Moreover, Applicants demonstrated that this site can be grafted onto human mAbs ("meditope-enabling"), indicating that this peptide binding site may for example be used as a beacon for targeting

imaging agents or as a "hitch" to tether new functionality to mAbs. Through extensive engineering, Applicants have improved the affinity of the meditope-Fab interaction by over 40,000-fold with an estimated half-life that exceeds six days at room temperature. Applicants further demonstrated that the fusion of the meditope to protein L, a Fab-binding protein, significantly improved the affinity and estimated the half-life of this complex to exceed 80 days. Finally, Applicants verified through SPR studies that conjugation of fluorescent markers, DOTA, GFP and other protein domains to the high affinity meditope and to the meditope-protein L (MPL) fusion do not affect the affinity of the MPL-Fab nor the Fab-antigen interactions. Collectively, these data show that functionality can be "snapped" on to any given meditope-enabled mAb.

[0217] CAR T cell therapy, which has produced durable responses especially in B cell malignancies(2-6), involves the reprogramming of patient T cells with an artificial receptor consisting of an extracellular antigen targeting moiety, a transmembrane domain and intracellular signaling modules, including CD3ζ and costimulatory domains of CD28 and/or CD137 (4-1BB), to activate the T cell and elicit an immune response. The antigen-targeting domain of the CAR generally is a tumor antigen recognizing single chain F variable antibody region (scFv). There is a need in the art for the ability to: 1) characterize the density of the CARs on the transformed cells, 2) to track administered CAR T cells at any point during the therapy and correlate this distribution to therapeutic outcomes, 3) to rapidly functionalize CAR T cells, and 4) to selectively eliminate CAR T cells if necessary. In embodiments, the constructs provided herein are capable of meeting these needs.

[0218] In embodiments, the constructs provided herein are useful in the following areas: (i) Application of super resolution microscopy to characterize CAR expression through direct observation of the receptor distribution on the T cells. Applicants have fused a photo-activatable GFP (paGFP) to a high affinity meditope, and demonstrated that meditope-enabled mAbs bound to cell-derived receptors can be "counted" and their cluster size can be quantified. Such information can be correlated with therapeutic efficacy and used in the clinic for "quality control." (ii) Imaging of meditope-enabled CAR T cells with a DOTA-conjugated, high affinity meditope *in situ*. Applicants have demonstrated that high affinity, conjugated meditopes do not affect antigen binding. Thus, meCAR T cells can be pre-labeled with ^{64}Cu-DOTA-conjugated, high affinity meditopes and their migration can be traced. Alternatively, meCAR T cells can be administered, allowed to localize and proliferate, and then subsequently imaged. Pre-targeted, mAb-based imaging methods as proposed have been demonstrated to produce high quality PET

images using engineered antibodies (9-11). (iii) Novel orthogonal functionality that can be rapidly added to the meCAR T cell. Specifically, meditopes may be conjugated to biologics that recognize a second tumor-associated ligand, potent cytotoxins, immune modulators including cytokines, and tumor-activated prodrugs. These meditopes may be directly attached to the meCAR T cells before administration or subsequently added after the meCAR T cells are established.

EXAMPLE 1

[0219] Generation, characterization and identification of meditope-enabled CAR constructs for immunotherapy. Different combinations of meditope-enabled Fab- and mAb-based CAR (meCAR) constructs that target HER2 positive tumors are generated, packaged each into a lentivirus, and transduced T-cells to generate meditope-enabled HER2+-CAR (meHER2+-CAR) T cells. The expression levels of each meCAR are characterized as well as its affinity for soluble extracellular HER2 with and without a DOTA-conjugated meditope. Finally, the tumor cell killing ability of each construct is quantified in the presence and absence of a DOTA-conjugated meditope *in vitro*.

[0220] CARs are a tool in the reprogramming of the immune system to recognize and destroy cancer cells. CARs are generally composed of an antigen recognition domain (e.g., an scFv), a spacer (e.g., the Fc domain of an IgG or hinge domain of CD8), a transmembrane region and intracellular costimulatory and activation domains (e.g., CD28 and/or CD137 and CD3 ζ chain). In embodiments, an antigen recognition domain composed of a meditope-enabled Fab or mAb provides a unique peptide binding site to rapidly and specifically add new functionality through the peptide without recourse to extensive re-engineering of the CAR itself. As noted, these functionalities may include the ability to image, target additional tumor-associated receptors, modulate immune function and selectively kill the CAR T cell. Provided herein are several expression plasmids for trastuzumab, an anti-HER2 mAb that is in the clinic for HER2 positive tumors and which Applicants have meditope-enabled (1). The order of light and heavy chain expression are altered and the efficacy of different internal ribosome entry sites versus the self-cleaving 2A peptide sequence (15) are tested. The binding of soluble HER2 are quantified as well as meditope for each construct using a variety of binding assays and super resolution microscopy. The *in vitro* functionality of the different CAR constructs are characterized by evaluating *in vitro* HER2-dependent T cell killing, degranulation, cytokine production and proliferation. The effect of meditope occupancy of the meCAR T cells are characterized using these same assays. A canonical HER2-specific CAR based on the scFv of trastuzumab are

generated and characterized, which may serve as a reference point for both expression and functional assays.

[0221] A number of tumors aberrantly express HER2 including breast cancer, sarcomas, and lung cancer. Thus, there have been efforts in developing effective therapeutics, trastuzumab being one. However, 70% of HER2+ cancer patients do not respond to these systemic therapies and in fact may rapidly develop resistance to these agents (16). As such, vaccines to HER2 as well as HER2+ CAR T cells have been developed to go beyond the inhibition of HER2 signaling pathways and elicit a powerful immune response. Given the potency of CAR T cells and the possibility of adverse side effects (17), it is useful to monitor, modulate and potentially destroy HER2+ CAR T cells. Enabling CAR T cell with a meditope binding site addresses these problems.

[0222] **Meditope Interaction and Optimization.** Described herein is a unique peptide binding site within the Fab arm of cetuximab including unique amino acid residues lining the site not found in human antibodies. This site may be grafted onto human mAbs including trastuzumab, a humanized anti-CEA, and other mAbs. Peptide binding does not affect the ability of the meditope-enabled antibodies to bind to their antigens. Due to the position of the binding site being in the central cavity of the Fab, the peptide may be referred to as a "meditope" ("medius" and "topo") (Fig.2A). Meditope-enabled antibodies "memAbs" refer to meditope-enabled Fabs as meFabs (1).

[0223] Multiple of meditope variants have been produced, their affinity measure and crystallographic data accumulated for each. In these studies critical residues were identified, non-natural amino acids as well as D-amino acids were introduced, and different cyclization strategies to significantly improve the binding affinity were used(Fig.2B). Further, point mutations were introduced in the Fab at the meditope-binding interface (version 2) and observed a 100 fold increase in the binding affinity (Fig.2B). Through these modifications, the affinity of meditopes increase from 1.2 μM to 860 pM at 37°C (1000-fold increase). In addition, the termini of the meditope and protein L are, in embodiments, in close proximity when bound to the trastuzumab meFab (Fig.2A) and demonstrated favorable avidity through the fusion of meditope to protein L through a short linker (MPL). The affinity of the MPL construct for the original trastuzumab meFab as measured by Kinexa experiments is K_D = 14 pM, or 87,000-fold over the affinity of the individual components at 25 °C (data not shown). Assuming that each modification acts independently, a 258 million-fold increase in affinity for the combination of a synthetic MPL and the memAb. Fusion of GFP to the MPL construct does not affect memAb

binding and the GFP-MPL binding to memAb does not affect the association or dissociation kinetics or the affinity of HER2 binding (as shown in Avery et al. (37)).

[0224] Alexa Fluor 647-labeled MPL was either co-administered with Alexa Fluor 488-labeled memAb to HER2 overexpressing SKBR3 cells or after the cells were treated with the memAb and extensively washed. In both cases, the labeled MPL colocalized with the memAb and antigen (data not shown). In addition, it was demonstrated by fluorescence microscopy that the fusion of the bulky GFP to the MPL does not affect cell binding (data not shown). Lastly, a photoactivatable GFP was fused to the MPL construct and super-resolution microscopy was used to quantify HER2 receptors on BT474 cells (data not shown).

[0225] **HER2-specific scFv-derived CAR T cells target and kill HER2-positive tumors.** A second-generation HER2-specific CAR was generated composed of an scFv based on the trastuzumab antibody and intracellular signaling domains of CD28 and CD3ζ. A self-inactivating (SIN) lentiviral vector cassette was constructed encoding this HER2-specific scFv CAR (HER2-28ζ), followed by a 2A ribosomal skip sequence and a truncated CD19 (CD19t), an inert cell surface marker devoid of intracellular signaling that allows for specific detection of transduced T cells (**Fig.3A**). The truncated CD19 (CD19t) as provided herein is also referred to as "marker peptide". The terms "marker peptide"or "tCD19" may be used interchangeably throughout. A human central memory T cells (Tcm) was constructed to express the HER2-28ζ CAR and CD19t polypeptides via lentiviral transduction, and expanded *ex vivo* using CD3/CD28 Dynabeads® stimulation and growth in X-Vivo media supplemented with IL-2 and IL-15 as per cGMP-compatible manufacturing platform (**Fig.3B**) (18).

[0226] Using mouse and non-human primate models relevant for human translation, it has been observed that T cells derived from $CD62L^+$ Tcm persist in the blood after adoptive transfer, migrate to memory T cell niches in the lymph nodes and bone marrow, re-acquire phenotypic properties of memory T cells, and respond to antigen challenge *in vivo* (21, 22, 24, 25). Tcm or CD62L+ memory/naïve T cells may be engineered to express meditope-enabled HER2-CARs, taking advantage of the intrinsic long-term persistence of memory T cells, and the cGMP-compatible manufacturing platform which has been used to produce clinical products for two phase I clinical trials (BB-INDs 14645 and 15490) (18).

[0227] HER2-28ζ Tcm exhibit potent HER2-specific cytolytic activity *in vitro* against a panel of target cell lines that display both low (MCF7) and high (BT-474 and SK-BR-3) HER2 expression levels (**Fig.4A-4D**). Additionally, intracranial injection of HER2-28ζ Tcm can

mediate regression of established brain tumors derived from the BT-474 HER2+ breast tumor cell line, and result in long term survival for 100% of the mice (data not shown).

[0228] The synthetic meditope-enabled trastuzumab heavy and the light chains may be sublconed into a lentiviral expression vector (Fig.5). Since each chain must be produced individually, an IRES motif will be incorporated between the light and heavy chain or use of a ribosomal skip sequence such as the T2A or the 2A sequence [15]. Further, a monomeric CAR using a meditope-enabled Fab may be created. Thus, the monomeric meditope-enabled Fab may be crosslinked with a bivalent meditope, allowing to regulation of the activity of the CAR T cell (Fig.5). The transmembrane domain is replaced with monomeric L-selectin (26) and a simple poly glycine-serine linker is used.

[0229] Meditope-enabled CAR T cells. Primary human T cells, for example CD62L+ Tcm cells, will be isolated from the peripheral blood of at least three healthy donors, engineered by lentiviral transduction to express HER2-CARs, and evaluated *in vitro* for specificity and functional activity. Following expansion of meditope-enabled HER2+ CAR Tcm with OKT3/CD28 Dynabead® and cytokine (IL-2 and IL-15) stimulation, the expression level of each construct will be characterized by FACS using anti-CD19 as a marker of cell transduction and anti-Fc for CAR expression, an Alexa fluor 488-labeled, extracellular Her2-Fc construct for antigen binding, and an Alexa fluor 647-conjugated meditope for functional meditope-CAR docking. Positive CAR T cells will be enriched, if necessary, by anti-CD19 magnetic cell selection or FACS. The ability of each construct, meditope-enabled Fab and meditope enabled mAb, to target and lyse HER2-positive (low and high HER2-expressing tumor lines; **Fig3A**) and -negative breast cancer cell lines will be examined using standard chromium-release assays, and long-term co-culture assays (24-96 hrs) in the presence and absence of a DOTA-conjugated, high affinity meditope. To examine the effector function of different HER2-CAR T cells, HER2-dependent cytokine production will be measured, including secretion of IFNγ and TNFα following co-culture with tumor cells, again in the presence and absence of a DOTA-conjugated, high affinity meditope. Additionally, markers of activation and cytolytic activity will be included, namely CD69, Granzyme-B, and CD107a, as well as markers of cellular exhaustion, including PD-1. Furthermore, the antigen-dependent proliferative capacity of the different meditope-enabled HER2-CAR T cells in the presence and absence of DOTA-conjugated meditope will be measured by flow cytometry dye dilution analysis using CSFE. In each case, the results will be compared to the scFv CAR T cell. Methodologies for performing these *in vitro* functional assays are readily established in our group(6, 27).

[0230] Super resolution microscopy and autocorrelation analysis (28) will be used to investigate the distribution of receptors for each meditope-enabled CAR T cell. This approach will allow to quantitatively determine the size, occupancy, and density of proteins in the clusters. As demonstrated herein, an ultra-high affinity paGFP-MPL construct was produced and super resolution microscopy was utilized to detect single molecules with 15 nm resolution. In addition, a fluorescently labeled, high affinity 15-mer meditope was produced, which is less sterically constrained than the paGFP-MPL for super-resolution imaging. Using these reagents, ~12 individual cells expressing the meditope-enabled Fab or mAb will be analyzed and the efficacy of the meCAR T cell will be correlated with receptor distribution.

EXAMPLE 2

[0231] **Efficacy and *in vivo* imaging of meditope-enabled CAR in animal models.** The efficacy of meditope-enabled CAR T cells on tumor growth inhibition will be evaluated and PET will be used to image meHER2+-CAR T cells pre-treated with ^{64}Cu-labeled, DOTA-conjugated meditopes in NSG mice. NSG mice will be treated with the meHER2-CAR T cells and ^{64}Cu labeled, DOTA-conjugated meditope will be administered at defined time points to assess meditope uptake by the meHER2-CAR T cells *in situ*.

[0232] Pre-targeted imaging separates the slow accumulation of mAbs at the tumor and slow clearance of mAbs from the blood from the relatively short half-life of useful PET metals through a two-step process. First, the patient is administered a conjugated mAb (streptavidin or with a unique binding domain) and then a homing ligand carrying ^{64}Cu. The homing ligand rapidly binds to the tumor associated, modified mAb or is rapidly excreted. Since the ^{64}Cu undergoes less half-lives, the signal is higher. Also, since the tracer is rapidly cleared, the background is reduced. Pre-clinical images using this approach have produced significantly better images than direct conjugation methods (10). Imaging CAR T cell location, expansion and longevity in patients will be tremendously useful in the development and clinical evaluation of this therapy. *In vivo* CAR T cell mouse models for the treatment of solid tumors, including brain tumors (**Fig.6**), are well established in our lab (27, 34).

[0233] A dual orthotropic and metastatic tumor xenograft model will be employed using female NOD-scid IL2Rγnull (NSG) mice and the HER2-amplified breast cancer line BT474 that has been engineered to express both firefly luciferase (ffLuc) for non-invasive Xenogen imaging and a fluorescent reporter EGFP (27). EGFP-ffLuc+ BT474 tumor cells will be implanted concurrently into the mammary fat pad (1×10^6 in a 50 µL mixture of PBS and Matrigel) to model primary disease, and intracranially (1×10^5 in 2 µL PBS) to model metastatic disease.

Once tumors are established (typically 7-14 days), a single dose of 5 x 10^6 each HER2-meCAR Tcm or un-engineered Tcm (mock) or PBS will be infused intravenously. It has been shown that i.v. administered CAR T cell do traffic to the brain and mediate tumor regression (35, 36). Tumor growth/regression will be non-invasively quantified by Xenogen® IVIS optical imaging and caliper measurement, and survival analyzed by Kaplan-Meier. For these studies, T cell infiltration and persistence in tumors will be evaluated by immunohistochemistry using an Alexa Fluor-labeled meditope and CD3 markers, and Tcm persistence will be quantified by flow cytometry using Alexa Fluor-labeled meditopes, CD45, CD4/CD8, and CD62L markers in tumors and lymphoid tissue. Proliferation/apoptosis in tumors (Ki67, TUNEL), CAR T-cell activation and cytolytic function (CD69, Granzyme B, IFNγ) in tumors and in lymphoid tissues will be measured by flow cytometry. *In vivo* efficacy of meCAR T cells will be compared to previously characterized HER2-28ζ scFv CAR T cells. These studies will establish the capacity of meCAR T cells to mediate HER2+ tumor regression, and reveal potential differences in anti-tumor activity and T cell persistence between the meFab or memAb CAR T cells.

[0234] The high-affinity meditope with a C-terminal DOTA will be directly synthesized. The DOTA-meditope will be charged with ^{64}Cu, purified by gel chromatography and mixed with the meCAR T cells. The cells will be administered to animals bearing EGFP-ffLuc+ BT474 tumors. MicroPET imaging will be conducted immediately following the injection and at defined time points thereafter. At day 1 and day 2, animals will be sacrificed and the bio distribution of the meditope and meCAR T cells will be determined for meCAR T cells at primary and metastatic disease sites. Next, pre-targeted imaging methods will be applied. The meCAR T cells will be administered in the same orthotopic and metastatic xenograft model. ^{64}CU-DOTA meditope will be administered at 1 h post meCAR T cell administration and imaged at defined points thereafter (1, 2, 3 and 6 hours). The same imaging schedule will be conducted 1 day, 3 days and 10 days post meCAR T cell administration. Again, animals will be sacrificed and the bio distribution(s) will be determined through radiography.

TABLES

[0235] **Table 1.** Examples of transmembrane domains.

Protein	NCBI Accession No.	Length	Transmembrane Domain Sequence
CD3z	GI:623041	21 aa	LCYLLDGILFIYGVILTALFL (SEQ ID NO:1)
CD28	GI:340545506	27aa	FWVLVVVGGVLACYSLLVTVAFIIFWV (SEQ ID NO:2)
CD4	GI:179143	22aa	MALIVLGGVAGLLLFIGLGIFF (SEQ ID NO:3)
CD8	GI:225007534	21aa	IYIWAPLAGTCGVLLLSLVIT (SEQ ID NO:4)

CD8	GI:225007534	23aa	IYIWAPLAGTCGVLLLSLVITLY (SEQ ID NO:5)
CD8	GI:225007534	24aa	IYIWAPLAGTCGVLLLSLVITLYC (SEQ ID NO:6)
41BB	GI:315259099	27aa	IISFFLALTSTALLFLLFF LTLRFSVV (SEQ ID NO:7)
OX40	GI:315360637	21 aa	VAAILGLGLVLGLLGPLAILL (SEQ ID NO:8)
ICOS	GI:251823951	21aa	FWLPIGCAAFVVVCILGCILI (SEQ ID NO:9)
CD62L	GI:262206314	23aa	PLFIPVAVMVTAFSGLAFIIWLA (SEQ ID NO:10)

[0236] **Table 2.** Examples of signaling domains.

Protein	**NCBI Accession No.**	**Length**	**Endo Signaling**
CD3ζ	GI:623041	113 aa	SEQ ID NO:11: RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL DKRRGRDPEMGGKPQRRKNPQEGLY
CD28	GI:340545506	42aa	SEQ ID NO:12: RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDF AAYRS
CD28gg*	GI:340545506	42aa	SEQ ID NO:13: RSKRSRGGHSDYMNMTPRRPGPTRKHYQPYAPPRD FAAYRS (ref)
41BB	GI:315259099	42 aa	SEQ ID NO:14: KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEE GGCEL
OX40	GI:315360637	42 aa	SEQ ID NO:15: ALYLLRRDQRLPPDAHKPPGGGSFRTPIQEEQADAH STLAKI
ICOS	GI:251823951	38 aa	SEQ ID NO:16: CWLTKKKYSSSVHDPNGEYMFMRAVNTAKKSRLT DVTL

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INFORMAL SEQUENCE LISTING

[0274] SEQ ID NO:1 LCYLLDGILFIYGVILTALFL

[0275] SEQ ID NO:2: FWVLVVVGGVLACYSLLVTVAFIIFWV

[0276] SEQ ID NO:3: MALIVLGGVAGLLLFIGLGIFF

[0277] SEQ ID NO:4: IYIWAPLAGTCGVLLLSLVIT

[0278] SEQ ID NO:5: IYIWAPLAGTCGVLLLSLVITLY

[0279] SEQ ID NO:6: IYIWAPLAGTCGVLLLSLVITLYC

[0280] SEQ ID NO:7: IISFFLALTSTALLFLLFF LTLRFSVV

[0281] SEQ ID NO:8: VAAILGLGLVLGLLGPLAILL

[0282] SEQ ID NO:9: FWLPIGCAAFVVVCILGCILI

[0283] SEQ ID NO:10: PLFIPVAVMVTAFSGLAFIIWLA

[0284] SEQ ID NO:11:
RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQE
GLY

[0285] SEQ ID NO:12: RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS

[0286] SEQ ID NO:13: RSKRSRGGHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS (ref)

[0287] SEQ ID NO:14: KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

[0288] SEQ ID NO:15: ALYLLRRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKI

[0289] SEQ ID NO:16: CWLTKKKYSSSVHDPNGEYMFMRAVNTAKKSRLTDVTL

[0290] **meHer2Fab-IgG4(HL-CH3)-IgG4-CD28tm-CD28gg-Zeta-T2A-meLc:**

SEQ ID NO:17:
MLLLVTSLLLCELPHPAFLLIPEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVR
QSPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAIYYCS
RWGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE
PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
KKVEPKSCESKYGPPCPPCPGGGSSGGGSGGQPREPQVYTLPPSQEEMTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVM
HEALHNHYTQKSLSLSLGKMFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRGGHSD
YMNMTPRRPGPTRKHYQPYAPPRDFAAYRSGGGRVKFSRSADAPAYQQGQNQLYNEL
NLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR
RGKGHDGLYQGLSTATKDTYDALHMQALPPRLEGGGEGRGSLLTCGDVEENPGPRML
LLVTSLLLCELPHPAFLLIPDIQMTQSPILLSASVGDRVTITCRASQDVNTAVAWYQQRT
NGSPRLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDEADYYCQQHYTTPPTFGAG

TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ
ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0291] GMCSFRa signal peptide SEQ ID NO:18: MLLLVTSLLLCELPHPAFLLIP

[0292] Her2Fab (Her2 heavy chain variable and partial constant region) SEQ ID NO:19:
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQSPGKGLEWVARIYPTNGYTR
YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAIYYCSRWGGDGFYAMDYWGQGTLV
TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC

[0293] IgG4(SmP)-Hinge SEQ ID NO:20: ESKYGPPCPPCP

[0294] Spacer (including IgG4-CH3) SEQ ID NO:21:
GGGSSGGGSGGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

[0295] CD28 transmembrane SEQ ID NO:22:
MFWVLVVVGGVLACYSLLVTVAFIIFWV

[0296] CD28cyto (LLmGG) SEQ ID NO:23:
RSKRSRGGHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS

[0297] Linker ((Gly)3) SEQ ID NO:24: GGG

[0298] Intracellular T-cell signaling domain (CD3-Zeta) SEQ ID NO:25:
RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEG
LYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0299] Self-cleaving peptidyl linker (T2A) SEQ ID NO:26:
LEGGGEGRGSLLTCGDVEENPGPR

[0300] Antibody region (meLc (Her2 Light chain variable and constant region with mutation for meditope)) SEQ ID NO:27:
DIQMTQSPILLSASVGDRVTITCRASQDVNTAVAWYQQRTNGSPRLLIYSASFLYSGVPS
RFSGSRSGTDFTLTISSLQPEDEADYYCQQHYTTPPTFGAGTKVEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0301] meHer2Fab-IgG4(HL-CH3)-IgG4-CD28tm-CD28gg-Zeta-T2A-CD19t-2A-meLc

SEQ ID NO:28:

MLLLVTSLLLCELPHPAFLLIPEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVR
QSPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAIYYCS
RWGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE
PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
KKVEPKSCESKYGPPCPPCPGGGSSGGGSGGQPREPQVYTLPPSQEEMTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVM
HEALHNHYTQKSLSLSLGKMFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRGGHSD
YMNMTPRRPGPTRKHYQPYAPPRDFAAYRSGGGRVKFSRSADAPAYQQGQNQLYNEL
NLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR
RGKGHDGLYQGLSTATKDTYDALHMQALPPRLEGGGEGRGSLLTCGDVEENPGPRMPP
PRLLFFLLFLTPMEVRPEEPLVVKVEEGDNAVLQCLKGTSDGPTQQLTWSRESPLKPFLK
LSLGLPGLGIHMRPLAIWLFIFNVSQQMGGFYLCQPGPPSEKAWQPGWTVNVEGSGELF
RWNVSDLGGLGCGLKNRSSEGPSSPSGKLMSPKLYVWAKDRPEIWEGEPPCVPPRDSLN
QSLSQDLTMAPGSTLWLSCGVPPDSVSRGPLSWTHVHPKGPKSLLSLELKDDRPARDM
WVMETGLLLPRATAQDAGKYYCHRGNLTMSFHLEITARPVLWHWLLRTGGWKVSAV
TLAYLIFCLCSLVGILHLQRALVLRRKRGGSTSEGRGSLLTCGDVEENPGPMETDTLLLW
VLLLWVPGSTGDIQMTQSPILLSASVGDRVTITCRASQDVNTAVAWYQQRTNGSPRLLI
YSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDEADYYCQQHYTTPPTFGAGTKVEIKRT
VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0302] Marker peptide (CD19t) SEQ ID NO:29:

MPPPRLLFFLLFLTPMEVRPEEPLVVKVEEGDNAVLQCLKGTSDGPTQQLTWSRESPLKP
FLKLSLGLPGLGIHMRPLAIWLFIFNVSQQMGGFYLCQPGPPSEKAWQPGWTVNVEGSG
ELFRWNVSDLGGLGCGLKNRSSEGPSSPSGKLMSPKLYVWAKDRPEIWEGEPPCVPPRD
SLNQSLSQDLTMAPGSTLWLSCGVPPDSVSRGPLSWTHVHPKGPKSLLSLELKDDRPAR
DMWVMETGLLLPRATAQDAGKYYCHRGNLTMSFHLEITARPVLWHWLLRTGGWKVS
AVTLAYLIFCLCSLVGILHLQRALVLRRKR

[0303] Self-cleaving peptidyl linker (2A) SEQ ID NO:30:

GGSTSEGRGSLLTCGDVEENPGP

[0304] CAMPATH1 (1CE1) MEFAB (I83) light chain SEQ ID NO:31:

DIQMTQSPILLSASVGDRVTITCKASQNIDKYLNWYQQRTNGSPRLLIYNTNNLQTGVPS

RFSGSGSGTDFTFTISSLQPEDIADYYCLQHISRPRTFGQGTKVEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLS
KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0305] CAMPATH1 (1CE1) MEFAB (I83) heavy chain SEQ ID NO:32:

QVQLQESGGGLVRPSQTLSLTCTVSGFTFTDFYMNWVRQSPGRGLEWIGFIRDKAKGYT
TEYNPSVKGRVTMLVDTSKNQFSLRLSSVTAADTAIYYCAREGHTAAPFDYWGQGSLV
TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE

[0306] CAMPATH1 (1CE1) MEFAB (E83) light chain SEQ ID NO:33:

DIQMTQSPILLSASVGDRVTITCKASQNIDKYLNWYQQRTNGSPRLLIYNTNNLQTGVPS
RFSGSGSGTDFTFTISSLQPEDEADYYCLQHISRPRTFGQGTKVEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0307] CAMPATH1 (1CE1) MEFAB (E83) heavy chain SEQ ID NO:34:

QVQLQESGGGLVRPSQTLSLTCTVSGFTFTDFYMNWVRQSPGRGLEWIGFIRDKAKGYT
TEYNPSVKGRVTMLVDTSKNQFSLRLSSVTAADTAIYYCAREGHTAAPFDYWGQGSLV
TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE

[0308] Tissue factor Fab (1jps) MEFAB light chain SEQ ID NO:35

DIQMTQSPILLSASVGDRVTITCRASRDIKSYLNWYQQRTNGSPRLLIYYATSLAEGVPSR
FSGSGSGTDYTLTISSLQPEDIADYYCLQHGESPWTFGQGTKVEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0309] Tissue factor Fab (1jps) MEFAB heavy chain SEQ ID NO:36

EVQLVESGGGLVQPGGSLRLSCAASGFNIKEYYMHWVRQSPGKGLEWVGLIDPEQGNT
IYDPKFQDRATISADNSKNTAYLQMNSLRAEDTAIYYCARDTAAYFDYWGQGTLVTVS
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT

[0310] Tissue factor Fab (1jps) MEFAB (I83) light chain SEQ ID NO:37

DIQMTQSPILLSASVGDRVTITCRASRDIKSYLNWYQQRTNGSPRLLIYYATSLAEGVPSR
FSGSGSGTDYTLTISSLQPEDEADYYCLQHGESPWTFGQGTKVEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0311] Tissue factor Fab (1jps) MEFAB (I83) heavy chain SEQ ID NO:38

EVQLVESGGGLVQPGGSLRLSCAASGFNIKEYYMHWVRQSPGKGLEWVGLIDPEQGNT
IYDPKFQDRATISADNSKNTAYLQMNSLRAEDTAIYYCARDTAAYFDYWGQGTLVTVS
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT

[0312] ANTI-PRP – prion MEFAB (I83) (GI: 19204296) light chain SEQ ID NO:39

EIVLTQSPIIMSASPGEKVTMTCSASSSVSYMHWYQQRTNGSPRRWIYDTSKLASGVPAR
FSGSGSGTSYSLTISSMEAEDIADYFCHQWRSNPYTFGGGTKLEIKRADAAPTVSIFPPSS
EQLTSGGASVVCFLNNFYPKEINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTL
TKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC

[0313] ANTI-PRP – prion MEFAB (I83) (GI: 19204296) heavy chain SEQ ID NO:40

EVQLQQSGGELVKPGSSVKISCKASRNTFTDYNLDWVKQSHGKTLEWIGNVYPNNGVT
GYNQKFRGKATLTVDKSSSTAYMELHSLTSEDSAIYYCALYYYDVSYWGQGTLVTVSS
AKTTPPSVYPLAPGSAAQTSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSD
LYTLSSSVTVPSSTWPSQSVTCNVAHPASSTKVDKKITPR

[0314] ANTI-PRP – prion MEFAB (IE83) (GI: 19204296) light chain SEQ ID NO:41

EIVLTQSPIIMSASPGEKVTMTCSASSSVSYMHWYQQRTNGSPRRWIYDTSKLASGVPAR
FSGSGSGTSYSLTISSMEAEDEADYFCHQWRSNPYTFGGGTKLEIKRADAAPTVSIFPPSS
EQLTSGGASVVCFLNNFYPKEINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTL
TKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC

[0315] ANTI-PRP – prion MEFAB (E83) (GI: 19204296) heavy chain SEQ ID NO:42

EVQLQQSGGELVKPGSSVKISCKASRNTFTDYNLDWVKQSHGKTLEWIGNVYPNNGVT
GYNQKFRGKATLTVDKSSSTAYMELHSLTSEDSAIYYCALYYYDVSYWGQGTLVTVSS
AKTTPPSVYPLAPGSAAQTSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSD
LYTLSSSVTVPSSTWPSQSVTCNVAHPASSTKVDKKITPR

[0316] daclizumab MEFAB (I83) light chain SEQ ID NO:43

DIQMTQSPILLSASVGDRVTITCSASSSISYMHWYQQRTNGSPRLLIYTTSNLASGVPARF
SGSGSGTEFTLTISSLQPDDIADYYCHQRSTYPLTFGQGTKVEVKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLS
KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0317] daclizumab (I83) heavy chain SEQ ID NO:44

QVQLVQSGGEVKKPGSSVKVSCKASGYTFTSYRMHWVRQSPGQGLEWIGYINPSTGYT
EYNQKFKDKATITADESTNTAYMELSSLRSEDTAIYYCARGGGVFDYWGQGTLVTVSS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP

[0318] daclizumab MEFAB (E83) light chain SEQ ID NO:45

DIQMTQSPILLSASVGDRVTITCSASSSISYMHWYQQRTNGSPRLLIYTTSNLASGVPARF
SGSGSGTEFTLTISSLQPDDEADYYCHQRSTYPLTFGQGTKVEVKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLS
KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0319] daclizumab (E83) heavy chain SEQ ID NO:46

QVQLVQSGGEVKKPGSSVKVSCKASGYTFTSYRMHWVRQSPGQGLEWIGYINPSTGYT
EYNQKFKDKATITADESTNTAYMELSSLRSEDTAIYYCARGGGVFDYWGQGTLVTVSS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP

[0320] MORAb-009 Anti-mesothelin monoclonal antibody MEFAB (I83) light chain SEQ ID NO:47

DIELTQSPIIMSASPGEKVTMTCSASSSVSYMHWYQQRTNGSPRRWIYDTSKLASGVPGR
FSGSGSGNSYSLTISSVEAEDIADYYCQQWSKHPLTFGSGTKVEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0321] MORAb-009 Anti-mesothelin monoclonal antibody MEFAB (I83) heavy chain SEQ ID NO:48

EVQLQQSGGELEKPGASVKISCKASGYSFTGYTMNWVKQSHGKSLEWIGLITPYNGASS
YNQKFRGKATLTVDKSSSTAYMDLLSLTSEDSAIYFCARGGYDGRGFDYWGSGTPVTV

SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC

[0322] MORAb-009 Anti-mesothelin monoclonal antibody MEFAB (E83) light chain SEQ ID NO:49

DIELTQSPIIMSASPGEKVTMTCSASSSVSYMHWYQQRTNGSPRRWIYDTSKLASGVPGR
FSGSGSGNSYSLTISSVEAEDEADYYCQQWSKHPLTFGSGTKVEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0323] MORAb-009 Anti-mesothelin monoclonal antibody MEFAB (E83) heavy chain SEQ ID NO:50

EVQLQQSGGELEKPGASVKISCKASGYSFTGYTMNWVKQSHGKSLEWIGLITPYNGASS
YNQKFRGKATLTVDKSSSTAYMDLLSLTSEDSAIYFCARGGYDGRGFDYWGSGTPVTV
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC

[0324] anti-CD20 antibody Ofatumumab MEFAB (I83) light chain SEQ ID NO:51

EIVLTQSPILLSLSPGERATLSCRASQSVSSYLAWYQQRTNGSPRLLIYDASNRATGIPAR
FSGSGSGTDFTLTISSLEPEDIADYYCQQRSNWPITFGQGTRLEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLS
KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0325] anti-CD20 antibody Ofatumumab MEFAB (I83) heavy chain SEQ ID NO:52

EVQLVESGGGLVQPGRSLRLSCAASGFTFNDYAMHWVRQSPGKGLEWVSTISWNSGSI
GYADSVKGRFTISRDNAKKSLYLQMNSLRAEDTAIYYCAKDIQYGNYYYGMDVWGQG
TTVTVSSASTKGPSVFPLAPGSSKSTSGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP

[0326] anti-CD20 antibody Ofatumumab MEFAB (E83) light chain SEQ ID NO:53

EIVLTQSPILLSLSPGERATLSCRASQSVSSYLAWYQQRTNGSPRLLIYDASNRATGIPAR
FSGSGSGTDFTLTISSLEPEDIADYYCQQRSNWPITFGQGTRLEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLS
KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0327] anti-CD20 antibody Ofatumumab MEFAB (E83) heavy chain SEQ ID NO:54

EVQLVESGGGLVQPGRSLRLSCAASGFTFNDYAMHWVRQSPGKGLEWVSTISWNSGSI
GYADSVKGRFTISRDNAKKSLYLQMNSLRAEDTAIYYCAKDIQYGNYYYGMDVWGQG
TTVTVSSASTKGPSVFPLAPGSSKSTSGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP

[0328] **Notch1 Negative Regulatory Region antibody MEFAB (I83) light chain** SEQ ID NO:55

DIQMTQSPILLSASVGDRVTITCRASQDVSTAVAWYQQRTNGSPRLLIYSASFLYSGVPS
RFSGSGSGTDFTLTISSLQPEDIADYYCQQFYTTPSTFGQGTKVEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0329] **Notch1 Negative Regulatory Region antibody MEFAB (I83) heavy chain** SEQ ID NO:56

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWIHWVRQSPGKGLEWVARINPPNRSNQ
YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAIYYCARGSGFRWVMDYWGQGTLVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT

[0330] **Notch1 Negative Regulatory Region antibody MEFAB (E83) light chain** SEQ ID NO:57

DIQMTQSPILLSASVGDRVTITCRASQDVSTAVAWYQQRTNGSPRLLIYSASFLYSGVPS
RFSGSGSGTDFTLTISSLQPEDEADYYCQQFYTTPSTFGQGTKVEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0331] **Notch1 Negative Regulatory Region antibody MEFAB (E83) heavy chain** SEQ ID NO:58

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWIHWVRQSPGKGLEWVARINPPNRSNQ
YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAIYYCARGSGFRWVMDYWGQGTLVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT

[0332] **anti-HER3 Fab RG7116 MEFAB (I83) light chain** SEQ ID NO:59

DIVMTQSPILLAVSLGERATINCKSSQSVLNSGNQKNYLTWYQQRTNGSPRLLIYWAST
RESGVPDRFSGSGSGTDFTLTISSLQAEDIADYYCQSDYSYPYTFGQGTKLEIKRTVAAPS
VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0333] anti-HER3 Fab RG7116 MEFAB (I83) heavy chain SEQ ID NO:60

QVQLVQSGGGVKKPGASVKVSCKASGYTFRSSYISWVRQSPGQGLEWMGWIYAGTGS
PSYNQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAIYYCARHRDYYSNSLTYWGQGTL
VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH

[0334] anti-HER3 Fab RG7116 MEFAB (E83) light chain SEQ ID NO:61

DIVMTQSPILLAVSLGERATINCKSSQSVLNSGNQKNYLTWYQQRTNGSPRLLIYWAST
RESGVPDRFSGSGSGTDFTLTISSLQAEDEADYYCQSDYSYPYTFGQGTKLEIKRTVAAP
SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST
YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0335] anti-HER3 Fab RG7116 MEFAB (E83) heavy chain SEQ ID NO:62

QVQLVQSGGGVKKPGASVKVSCKASGYTFRSSYISWVRQSPGQGLEWMGWIYAGTGS
PSYNQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAIYYCARHRDYYSNSLTYWGQGTL
VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH

[0336] Rituximab MEFAB (I83) light chain SEQ ID NO:63

QIVLSQSPIILSASPGEKVTMTCRASSSVSYIHWFQQRTNGSPRPWIYATSNLASGVPVRF
SGSGSGTSYSLTISRVEAEDIADYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLS
KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0337] Rituximab MEFAB (I83) heavy chain SEQ ID NO:64

QVQLQQPGGGLVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGD
TSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAIYYCARSTYYGGDWYFNVWGAGT
TVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC

[0338] Rituximab MEFAB (E83) light chain SEQ ID NO:65

QIVLSQSPIILSASPGEKVTMTCRASSSVSYIHWFQQRTNGSPRPWIYATSNLASGVPVRF
SGSGSGTSYSLTISRVEAEDEADYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLS
KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0339] Rituximab MEFAB (E83) heavy chain SEQ ID NO:66

QVQLQQPGGGLVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGD
TSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAIYYCARSTYYGGDWYFNVWGAGT
TVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC

[0340] anti-osteopontin antibody 23C3 MEFAB (I83) light chain SEQ ID NO:67

YIQMTQSPILLSVSVGETVTITCRASENIYSFLAWYQQRTNGSPRLLVYAATNLADGVPS
RFSGSGSGTQFSLKINSLQSEDIADYYCQHFWGTPFTFGSGTKLEIKRSDAAPTVSIFPPSA
AQLSSGGGSVVCFLNNFYPKDINVKWKIDGAERGNGVLNSWTSQDSADSTYSMSSTLT
SGGDEYERHNSYTCEATHKTSTSPIVKSFNRGEC

[0341] anti-osteopontin antibody 23C3 MEFAB (I83) heavy chain SEQ ID NO:68

EVQLVESGGGLVQPKGSLKISCAASGFTFNIYAMNWVRQSPGKGLEWVARIRSQSNNY
TTYYADSVKDRFTISRDDSQSMLYLQMNNLKTEDTAIYYCVRQMGDYWGQGTTLTVS
SAVKTPPSVYPLAPGGGAISNSMVTLGCLVNGYFPEPVTVTWNAGSLGSGVHTFPAVLQ
SDLYTLSSSVTVPVSTWPSEAVTCNVAHPASATSVDKAISPV

[0342] anti-osteopontin antibody 23C3 MEFAB (E83) light chain SEQ ID NO:69

YIQMTQSPILLSVSVGETVTITCRASENIYSFLAWYQQRTNGSPRLLVYAATNLADGVPS
RFSGSGSGTQFSLKINSLQSEDIADYYCQHFWGTPFTFGSGTKLEIKRSDAAPTVSIFPPSA
AQLSSGGGSVVCFLNNFYPKDINVKWKIDGAERGNGVLNSWTSQDSADSTYSMSSTLT
SGGDEYERHNSYTCEATHKTSTSPIVKSFNRGEC

[0343] anti-osteopontin antibody 23C3 MEFAB (E83) heavy chain SEQ ID NO:70

EVQLVESGGGLVQPKGSLKISCAASGFTFNIYAMNWVRQSPGKGLEWVARIRSQSNNY
TTYYADSVKDRFTISRDDSQSMLYLQMNNLKTEDTAIYYCVRQMGDYWGQGTTLTVS
SAVKTPPSVYPLAPGGGAISNSMVTLGCLVNGYFPEPVTVTWNAGSLGSGVHTFPAVLQ
SDLYTLSSSVTVPVSTWPSEAVTCNVAHPASATSVDKAISPV

[0344] anti-uPAR Fab ATN-658 MEFAB (I83) light chain SEQ ID NO:71

DVVMTQTPLLLSVTIGQPASISCKSSQSLLDSDGKTYLNWLLQRTNGSPRRLIYLVSKLD
SGVPDRFTGSGSGTDFTLKISRVEAEDIADYYCWQGTHFPLTFGAGTKLELKRADAAPT
VSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYS
MSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC

[0345] anti-uPAR Fab ATN-658 MEFAB (I83) heavy chain SEQ ID NO:72

EVQLQQSGGGLVKTGASVKISCKASGYSFTSYYMHWVKQSHGKSLEWIGEINPYNGGA
SYNQKIKGRATFTVDTSSRTAYMQFNSLTSEDSAIYYCARSIYGHSVLDYWGQGTSVSV
SSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVL
QSDLYTLSSSVTVPSSTWPSQTVTCNVAHPASSTKVDKKIVPRDCGCKPCIC

[0346] anti-uPAR Fab ATN-658 MEFAB (E83) light chain SEQ ID NO:73

DVVMTQTPLLLSVTIGQPASISCKSSQSLLDSDGKTYLNWLLQRTNGSPRRLIYLVSKLD
SGVPDRFTGSGSGTDFTLKISRVEAEDEADYYCWQGTHFPLTFGAGTKLELKRADAAPT
VSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYS
MSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC

[0347] anti-uPAR Fab ATN-658 MEFAB (E83) heavy chain SEQ ID NO:74

EVQLQQSGGGLVKTGASVKISCKASGYSFTSYYMHWVKQSHGKSLEWIGEINPYNGGA
SYNQKIKGRATFTVDTSSRTAYMQFNSLTSEDSAIYYCARSIYGHSVLDYWGQGTSVSV
SSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVL
QSDLYTLSSSVTVPSSTWPSQTVTCNVAHPASSTKVDKKIVPRDCGCKPCIC

[0348] therapeutic influenza A MEFAB (I83) light chain SEQ ID NO:75

IVLTQSPILLSVSPGERATLSCRASQVISHNLAWYQQRTNGSPRLLIYGASTRASGIPARFS
GSGSGTDYTLTITSLQPEDIADYYCQHYSNWPPRLTFGGGTKVEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0349] therapeutic influenza A MEFAB (I83) heavy chain SEQ ID NO:76

EVQLQQSGGGLVKTGASVKISCKASGYSFTSYYMHWVKQSHGKSLEWIGEINPYNGGA
SYNQKIKGRATFTVDTSSRTAYMQFNSLTSEDSAIYYCARSIYGHSVLDYWGQGTSVSV
SSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVL
QSDLYTLSSSVTVPSSTWPSQTVTCNVAHPASSTKVDKKIVPRDCGCKPCIC

[0350] therapeutic influenza A MEFAB (E83) light chain SEQ ID NO:77

IVLTQSPILLSVSPGERATLSCRASQVISHNLAWYQQRTNGSPRLLIYGASTRASGIPARFS
GSGSGTDYTLTITSLQPEDEADYYCQHYSNWPPRLTFGGGTKVEIKRTVAAPSVFIFPPS
DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL
TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0351] therapeutic influenza A MEFAB (E83) heavy chain SEQ ID NO:78

EVQLQQSGGGLVKTGASVKISCKASGYSFTSYYMHWVKQSHGKSLEWIGEINPYNGGA
SYNQKIKGRATFTVDTSSRTAYMQFNSLTSEDSAIYYCARSIYGHSVLDYWGQGTSVSV
SSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVL
QSDLYTLSSSVTVPSSTWPSQTVTCNVAHPASSTKVDKKIVPRDCGCKPCIC

[0352] West Nile Virus Envelope Protein DIII in complex with neutralizing E16 antibody MEFAB (I83) light chain SEQ ID NO:79

DIVMTQSHILMSTSVGDRVSITCKASQDVSTAVAWYQQRTNGSPRLLISWASTRHTGVP
DRFTGSGSGTDYTLTISSVQAEDIADYYCQQHYTTPLTFGAGTKLELKRADAAPTVSIFP
PSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSST
LTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRGEC

[0353] West Nile Virus Envelope Protein DIII in complex with neutralizing E16 antibody MEFAB (I83) heavy chain SEQ ID NO:80

QVQLQQSGGGLMKPGASVQISCKATGYTFSDYWIEWVKQSPGHGLEWIGDILCGTGRT
RYNEKLKAMATFTADTSSNTAFMQLSSLTSEDSAIYYCARSASYGDYADYWGHGTTLT
VSSAKTTPPSVYPLAPGCGDTTGSSVTLGCLVKGYFPESVTVTWNSGSLSSSVHTFPALL
QSGLYTMSSSVTVPSSTWPSQTVTCSVAHPASSTTVDKKLEPS

[0354] West Nile Virus Envelope Protein DIII in complex with neutralizing E16 antibody MEFAB (E83) light chain SEQ ID NO:81

DIVMTQSHILMSTSVGDRVSITCKASQDVSTAVAWYQQRTNGSPRLLISWASTRHTGVP
DRFTGSGSGTDYTLTISSVQAEDEADYYCQQHYTTPLTFGAGTKLELKRADAAPTVSIFP
PSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSST
LTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRGEC

[0355] West Nile Virus Envelope Protein DIII in complex with neutralizing E16 antibody MEFAB (E83) heavy chain SEQ ID NO:82

QVQLQQSGGGLMKPGASVQISCKATGYTFSDYWIEWVKQSPGHGLEWIGDILCGTGRT
RYNEKLKAMATFTADTSSNTAFMQLSSLTSEDSAIYYCARSASYGDYADYWGHGTTLT
VSSAKTTPPSVYPLAPGCGDTTGSSVTLGCLVKGYFPESVTVTWNSGSLSSSVHTFPALL
QSGLYTMSSSVTVPSSTWPSQTVTCSVAHPASSTTVDKKLEPS

[0356] PVKQLLNFDLLKLAGDVESNPGP (SEQ ID NO:83)

[0357] QCTNYALLKLAGDVESNPGP (SEQ ID NO:84)

[0358] ATNFSLLKQAGDVEENPGP (SEQ ID NO:85)

[0359] EGRGSLLTCGDVESNPGP (SEQ ID NO:86)

[0360] **Humanized anti-CD19 meditope enabled heavy chain** SEQ ID NO:111

EVQLVESGGGLVQPGGSLRLSCAASGVSLPDYGVSWVRQSPGKGLEWVAVIWGSETTY
YADSVKGRFTISADTSKNTYLQMNSLRAEDTAIYYCSRHYYYGGSYAMDYWGQGTLV
TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP

[0361] **Humanized anti-CD19 meditope enabled light chain** SEQ ID NO:112

DIQMTQSPILLSASVGDRVTITCRASQDISKYLNWYQQ RTNGSPR
LLIYHTSRLHSGVPSRFSGSRSGTDFTLTISSLQPEDIADYYCQQGNTLPYTFGAGTKVEI
KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE
QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0362] **Humanized anti-CD19 meditope enabled I83E heavy chain** SEQ ID NO:113

EVQLVESGGGLVQPGGSLRLSCAASGVSLPDYGVSWVRQSPGKGLEWVAVIWGSETTY
YADSVKGRFTISADTSKNTYLQMNSLRAEDTAIYYCSRHYYYGGSYAMDYWGQGTLV
TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP

[0363] **Humanized anti-CD19 meditope enabled I83E light chain** SEQ ID NO:114

DIQMTQSPILLSASVGDRVTITCRASQDISKYLNWYQQRTNGSPRLLIYHTSRLHSGVPSR
FSGSRSGTDFTLTISSLQPEDEADYYCQQGNTLPYTFGAGTKVEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0364] Anti-TAG72 (CC49) CC49 memAb I83E enhanced Chothia heavy chain SEQ ID NO:115

EVQLVESGGGLVQPGGSLRLSCAASGYTFTDHAIHWVRQSPGKGLEWVAYFSPGNDDF
KYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAIYYCSRSLNMAYWGQGTLVTVSSA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP

[0365] Anti-TAG72 (CC49) CC49 memAb I83E enhanced Chothia light chain SEQ ID NO:116

DIQMTQSPILLSASVGDRVTITCKSSQSLLYSGNQKNYLAWYQQRTNGSPRLLIYWASA
RESGVPSRFSGSRSGTDFTLTISSLQPEDEADYYCQQYYSYPLTFGAGTKVEIKRTVAAPS
VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0366] Humanized CA19.9 memAb I83E enhanced Chothia heavy chain SEQ ID NO:117

EVQLVESGGGLVQPGGSLRLSCAASGFTFEAYAMHWVRQSPGKGLEWVASINWNSGRI
AYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAIYYCSRDIRRFSTGAEFEYWGQGTL
VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP

[0367] Humanized CA19.9 memAb I83E enhanced Chothia light chain SEQ ID NO:118

DIQMTQSPILLSASVGDRVTITCSGSSSNIGSNFVYWYQQRTNGSPRLLIYRNNQRPSGVP
SRFSGSRSGTDFTLTISSLQPEDEADYYCAAWDDSLG????HYVFGAGTKVEIKRTVAAPS
VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0368] YP218 anti-mesothelin - Humanized YP218 memAb I83E enhanced Chothia heavy chain SEQ ID NO:119

EVQLVESGGGLVQPGGSLRLSCAASGFDLGFYFYACWVRQSPGKGLEWVACIYTAGSG
STYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAIYYCSRSTANTRSTYLNLWGQGT
LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP

[0369] YP218 anti-mesothelin - Humanized YP218 memAb I83E enhanced Chothia light chain SEQ ID NO:120

DIQMTQSPILLSASVGDRVTITCQASQRISSYLSWYQQRTNGSPRLLIYGASTLASGVPSR
FSGSRSGTDFTLTISSLQPEDEADYYCQSYFDSNWHAFGAGTKVEIKRTVAAPSVFIFPPS
DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL
TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

EMBODIMENTS

[0370] Embodiment 1. An isolated nucleic acid encoding a protein comprising: (i) an antibody region comprising a central cavity formed by a heavy chain variable (VH) region, a light chain variable (VL) region, a heavy chain constant region (CH) and a light chain constant region (CL), wherein said central cavity forms a peptide binding site comprising framework region amino acid residues; and (ii) a transmembrane domain.

[0371] Embodiment 2. The isolated nucleic acid of embodiment 1, wherein said antibody region is an antibody fragment.

[0372] Embodiment 3. The isolated nucleic acid of embodiment 1 or 2, wherein said antibody region comprises an Fc domain.

[0373] Embodiment 4. The isolated nucleic acid of one of embodiments 1 to 3, wherein said antibody region is a humanized antibody region.

[0374] Embodiment 5. The isolated nucleic acid of one of embodiment 1 to 4, further comprising an intracellular T-cell signaling sequence encoding an intracellular T-cell signaling domain.

[0375] Embodiment 6. The isolated nucleic acid of embodiment 5, wherein said intracellular T-cell signaling domain is a CD3 ζ intracellular T-cell signaling domain.

[0376] Embodiment 7. The isolated nucleic acid of one of embodiments 1-6 further comprising an intracellular co-stimulatory signaling sequence encoding an intracellular co-stimulatory signaling domain.

[0377] Embodiment 8. The isolated nucleic acid of embodiment 7, wherein said intracellular co-stimulatory signaling domain is a CD28 intracellular co-stimulatory signaling domain, a 4-1BB intracellular co-stimulatory signaling domain, a ICOS intracellular co-stimulatory signaling domain, or an OX-40 intracellular co-stimulatory signaling domain.

[0378] Embodiment 9. The isolated nucleic acid of one of embodiments 1-8 further comprising a spacer sequence encoding a spacer region.

[0379] Embodiment 10. The isolated nucleic acid of embodiment 9, wherein said spacer region is between said transmembrane domain and said antibody region.

[0380] Embodiment 11. The isolated nucleic acid of one of embodiments 1-10 further comprising a linker sequence encoding a linker domain.

[0381] Embodiment 12. The isolated nucleic acid of embodiment 11, wherein said linker domain is between said transmembrane domain and said intracellular T-cell signaling domain.

[0382] Embodiment 13. The isolated nucleic acid of embodiment 11, wherein said linker domain is between said intracellular T-cell signaling domain and said intracellular co-stimulatory signaling domain.

[0383] Embodiment 14. The isolated nucleic acid of embodiment 11, wherein said linker domain comprises the sequence GGCGG or GGG.

[0384] Embodiment 15. The isolated nucleic acid of one of embodiments 1 to 14 comprising: (i) a heavy chain sequence encoding a heavy chain domain of said protein, said heavy chain domain comprising a variable heavy chain domain and said transmembrane domain; and (ii) a light chain sequence encoding a light chain domain of said protein, said light chain domain comprising a variable light chain domain, wherein said variable heavy chain domain and said variable light chain domain together form at least a portion of said antibody region.

[0385] Embodiment 16. The isolated nucleic acid of embodiment 15 comprising a self-cleaving peptidyl sequence between said heavy chain sequence and said light chain sequence.

[0386] Embodiment 17. The isolated nucleic acid of embodiment 16, wherein said self-cleaving peptidyl linker sequence is a T2A sequence or a 2A sequence.

[0387] Embodiment 18. The isolated nucleic acid of one of embodiments 15 to 17, wherein said light chain sequence is 3′ to said heavy chain sequence.

[0388] Embodiment 19. The isolated nucleic acid of one of embodiments 1 to 18, wherein said antibody region is a cetuximab meditope enabled domain, trastuzumab meditope enabled domain, pertuzumab meditope enabled domain, M5A meditope enabled domain or rituximab meditope enabled domain.

[0389] Embodiment 20. An isolated nucleic acid encoding a protein comprising a first portion comprising an antibody heavy chain variable domain and a second portion comprising an

antibody light chain variable domain and an antibody light chain constant domain, wherein the first portion further comprises a transmembrane domain.

[0390] Embodiment 21. The isolated nucleic acid of embodiment 20, wherein said first portion further comprises an intracellular T-cell signaling domain.

[0391] Embodiment 22. The isolated nucleic acid of embodiment 20, wherein said intracellular T-cell signaling domain is a CD3 ζ intracellular T-cell signaling domain.

[0392] Embodiment 23. The isolated nucleic acid of one of embodiments 20-22, wherein said first portion further comprises an intracellular co-stimulatory signaling domain.

[0393] Embodiment 24. The isolated nucleic acid of embodiment 23, wherein said intracellular co-stimulatory signaling domain is a CD28 intracellular co-stimulatory signaling domain, a 4-1BB intracellular co-stimulatory signaling domain, a ICOS intracellular co-stimulatory signaling domain, or an OX-40 intracellular co-stimulatory signaling domain.

[0394] Embodiment 25. The isolated nucleic acid of one of embodiments 20-24 wherein said first portion further comprises a linker domain.

[0395] Embodiment 26. The isolated nucleic acid of embodiment 25, wherein said linker domain is between said transmembrane domain and said intracellular T-cell signaling domain.

[0396] Embodiment 27. The isolated nucleic acid of embodiment 25, wherein said linker domain is between said intracellular T-cell signaling domain and said intracellular co-stimulatory signaling domain.

[0397] Embodiment 28. The isolated nucleic acid of embodiment 25, wherein said linker domain comprises the sequence GGCGG or GGG.

[0398] Embodiment 29. The isolated nucleic acid of embodiment 20, wherein said first portion further comprises a CD3 ζ intracellular T-cell signaling domain and an intracellular co-stimulatory signaling domain.

[0399] Embodiment 30. The isolated nucleic acid molecule of embodiment 23, wherein the first portion comprises from the amino terminus to the carboxy terminus: the heavy chain variable domain, a heavy chain constant domain, the transmembrane domain, the CD3 ζ intracellular T-cell signaling domain and an intracellular co-stimulatory signaling domain.

[0400] Embodiment 31. The isolated nucleic acid molecule of one of embodiments 20-30 further comprising a spacer region positioned between the heavy chain variable domain and the transmembrane domain.

[0401] Embodiment 32. The isolated nucleic acid of embodiment 31, wherein said spacer region further comprises a hinge region.

[0402] Embodiment 33. The isolated nucleic acid of embodiment 20, wherein the antibody heavy chain variable domain and the antibody light chain variable domain are humanized.

[0403] Embodiment 34. The isolated nucleic acid of embodiment 20, wherein said first portion comprises a heavy chain constant domain.

[0404] Embodiment 35. The isolated nucleic acid of embodiment 20 comprising a self-cleaving peptidyl sequence between said first portion and said second portion.

[0405] Embodiment 36. The isolated nucleic acid of embodiment 35, wherein said self-cleaving peptidyl encoding sequence is a T2A encoding sequence or a 2A encoding sequence.

[0406] Embodiment 37. The isolated nucleic acid of one of embodiment 20, wherein the nucleic acid sequence encoding the second portion is 3´ to the nucleic acid sequence encoding the first portion.

[0407] Embodiment 38. The isolated nucleic acid of one of embodiment 1 to 37, wherein said protein is an anti-CD19 protein, anti-CD20 protein, anti-CD22 protein, anti-CD30 protein, anti-CD33 protein, anti-CD44v6/7/8 protein, anti-CD123 protein, anti-CEA protein, anti-EGP-2 protein, anti-EGP-40 protein, anti-erb-B2 protein, anti-erb-B2,3,4 protein, anti-FBP protein, anti-fetal acetylcholine receptor protein, anti-GD2 protein, anti-GD3 protein, anti-Her2/neu protein, anti-IL-13R-a2 protein, anti-KDR protein, anti k-light chain protein, anti-LeY protein, anti-L1 cell adhesion molecule protein, anti-MAGE-A1 protein, anti-mesothelin protein, anti-murine CMV infected cell protein, anti-MUC2 protein, anti-NKGD2 protein, anti, oncofetal antigen protein, anti-PCSA protein, anti-PSMA protein, anti-TAA (targeted by mAb IfE) protein, anti-EGFR protein, anti-TAG-72 protein or anti-VEGF-72 protein.

[0408] Embodiment 39. The isolated nucleic acid of one of embodiments 1 to 38, further comprising a suicide gene sequence.

[0409] Embodiment 40. An expression vector comprising the nucleic acid of one of embodiments 1 to 39.

[0410] Embodiment 41. The expression vector of embodiment 40, wherein said expression vector is a viral vector.

[0411] Embodiment 42. The expression vector of embodiment 41, wherein said virus is a lentivirus or onco-retrovirus.

[0412] Embodiment 43. A T lymphocyte comprising the expression vector of one of embodiments 40 to 42.

[0413] Embodiment 44. A T lymphocyte of embodiment 43, comprising a first polypeptide and a second polypeptide, the first polypeptide comprising a heavy chain variable domain, a heavy chain constant domain, a transmembrane domain, a CD3 ζ signaling domain and a co-stimulatory T-cell signaling domain, the second polypeptide comprising a light chain variable domain and an light chain constant domain.

[0414] Embodiment 45. A recombinant protein comprising: (i) an antibody region comprising a central cavity formed by a heavy chain variable (VH) region and a light chain variable (VL) region, wherein said central cavity forms a peptide binding site comprising framework region amino acid residues; and (ii) a transmembrane domain.

[0415] Embodiment 46. The recombinant protein of embodiment 45, wherein said antibody region further comprises a heavy chain constant region (CH) and a light chain constant region (CL).

[0416] Embodiment 47. The recombinant protein of embodiment 45 or 46, wherein said antibody region comprises an Fc domain.

[0417] Embodiment 48. The recombinant protein of one of embodiments 45 to 47, wherein said antibody region is a humanized antibody region.

[0418] Embodiment 49. The recombinant protein of one of embodiments 45 to 48, wherein said antibody region does not comprise a scFv antibody region.

[0419] Embodiment 50. The recombinant protein of one of embodiments 45 to 49, wherein said protein further comprises an intracellular T-cell signaling domain.

[0420] Embodiment 51. The recombinant protein of embodiment 50, wherein said intracellular T-cell signaling domain is a CD3 ζ intracellular T-cell signaling domain.

[0421] Embodiment 52. The recombinant protein of one of embodiments 45 to 51, further comprising an intracellular co-stimulatory signaling domain.

[0422] Embodiment 53. The recombinant protein of embodiment 52, wherein said intracellular co-stimulatory signaling domain is a CD28 intracellular co-stimulatory signaling domain, a 4-1BB intracellular co-stimulatory signaling domain, a ICOS intracellular co-stimulatory signaling domain, or an OX-40 intracellular co-stimulatory signaling domain.

[0423] Embodiment 54. The recombinant protein of one of embodiments 45 to 53, further comprising a spacer region.

[0424] Embodiment 55. The recombinant protein of embodiment 54, wherein said spacer region is between said transmembrane domain and said antibody region.

[0425] Embodiment 56. The recombinant protein of embodiment one of embodiments 45-55, further comprising a linker domain.

[0426] Embodiment 57. The recombinant protein of embodiment 56, wherein said linker domain is between said transmembrane domain and said intracellular T-cell signaling domain.

[0427] Embodiment 58. The recombinant protein of embodiment 56, wherein said linker domain is between said intracellular T-cell signaling domain and said intracellular co-stimulatory signaling domain.

[0428] Embodiment 59. The recombinant protein of embodiment 57 or 58, wherein said linker domain comprises the sequence GGCGG or GGG.

[0429] Embodiment 60. The recombinant protein of one of embodiments 45 to 59, wherein said antibody region is a cetuximab meditope enabled domain, trasuzumab meditope enabled domain, pertuzumab meditope enabled domain, M5A meditope enabled domain or rituximab meditope enabled domain.

[0430] Embodiment 61. The recombinant protein of one of embodiments 45 to 60, wherein a compound comprising an peptidyl moiety is bound to said peptide binding site.

[0431] Embodiment 62. The recombinant protein of embodiment 61, wherein said compound is a multivalent meditope.

[0432] Embodiment 63. A recombinant protein comprising a first portion comprising an antibody heavy chain variable domain and a second portion comprising an antibody light chain variable domain and an antibody light chain constant domain, wherein the first portion further comprises a transmembrane domain, and wherein said antibody heavy chain variable domain,

said antibody light chain variable domain and said antibody light chain constant domain together form an antibody region.

[0433] Embodiment 64. A T lymphocyte comprising the recombinant protein of one of embodiments 45 to 63, wherein said transmembrane domain is within the cell membrane of said T lymphocyte.

[0434] Embodiment 65. A method of treating cancer, said method comprising administering to a subject in need thereof an effective amount of the T-lymphocyte of embodiment 64, wherein said antibody region is an anti-cancer antibody region.

[0435] Embodiment 66. The method of embodiment 65, wherein said T-lymphocyte is an autologous T-lymphocyte.

[0436] Embodiment 67. The method of embodiment 65, wherein said T-lymphocyte is a heterologous T-lymphocyte.

[0437] Embodiment 68. The method of embodiment 65, wherein said cancer is a solid tumor cancer or hematologic malignancy.

[0438] Embodiment 69. The method of one of embodiments 65 to 68, wherein said cancer is ovarian cancer, renal cell carcinoma, a B-cell malignancy, leukemia, lymphoma, breast cancer, colorectal cancer, prostate cancer, neuroblastoma, melanoma, medulloblastoma, lung cancer, osteosarcoma, glioblastoma or glioma.

[0439] Embodiment 70. A method of reprogramming a T lymphocyte, said method comprising contacting a T lymphocyte with the expression vector of one of embodiments 40 to 42.

[0440] Embodiment 71. A method of detecting a cancer, said method comprising: (i) administering to a cancer patient an effective amount of a T lymphocyte comprising the recombinant protein of one of embodiments 45 to 63 and a compound comprising a peptidyl moiety capable of binding to said peptide binding site, wherein said compound further comprises a detectable label, and wherein said antibody region is an anti-cancer antibody region; (ii) allowing said compound to bind to said peptide binding site thereby forming a recombinant protein-compound complex; and (iii) detecting said recombinant protein-compound complex within said cancer patient thereby detecting said cancer.

[0441] Embodiment 72. A T lymphocyte comprising the isolated nucleic acid of claim 1.

WHAT IS CLAIMED IS:

1. An isolated nucleic acid encoding a protein comprising:

(i) an antibody region comprising a central cavity formed by a heavy chain variable (VH) region, a light chain variable (VL) region, a heavy chain constant region (CH) and a light chain constant region (CL), wherein said central cavity forms a peptide binding site comprising framework region amino acid residues; and

(ii) a transmembrane domain.

2. The isolated nucleic acid of claim 1, wherein said antibody region is an antibody fragment.

3. The isolated nucleic acid of claim 1 or 2, wherein said antibody region comprises an Fc domain.

4. The isolated nucleic acid of one of claims 1 to 3, wherein said antibody region is a humanized antibody region.

5. The isolated nucleic acid of one of claim 1 to 4, further comprising an intracellular T-cell signaling sequence encoding an intracellular T-cell signaling domain.

6. The isolated nucleic acid of claim 5, wherein said intracellular T-cell signaling domain is a CD3 ζ intracellular T-cell signaling domain.

7. The isolated nucleic acid of one of claims 1-6 further comprising an intracellular co-stimulatory signaling sequence encoding an intracellular co-stimulatory signaling domain.

8. The isolated nucleic acid of claim 7, wherein said intracellular co-stimulatory signaling domain is a CD28 intracellular co-stimulatory signaling domain, a 4-1BB intracellular co-stimulatory signaling domain, a ICOS intracellular co-stimulatory signaling domain, or an OX-40 intracellular co-stimulatory signaling domain.

9. The isolated nucleic acid of one of claims 1-8 further comprising a spacer sequence encoding a spacer region.

10. The isolated nucleic acid of claim 9, wherein said spacer region is between said transmembrane domain and said antibody region.

11. The isolated nucleic acid of one of claims 1-10 further comprising a linker sequence encoding a linker domain.

12. The isolated nucleic acid of claim 11, wherein said linker domain is between said transmembrane domain and said intracellular T-cell signaling domain.

13. The isolated nucleic acid of claim 11, wherein said linker domain is between said intracellular T-cell signaling domain and said intracellular co-stimulatory signaling domain.

14. The isolated nucleic acid of claim 11, wherein said linker domain comprises the sequence GGCGG or GGG.

15. The isolated nucleic acid of one of claims 1 to 14 comprising:

(i) a heavy chain sequence encoding a heavy chain domain of said protein, said heavy chain domain comprising a variable heavy chain domain and said transmembrane domain; and

(ii) a light chain sequence encoding a light chain domain of said protein, said light chain domain comprising a variable light chain domain, wherein said variable heavy chain domain and said variable light chain domain together form at least a portion of said antibody region.

16. The isolated nucleic acid of claim 15 comprising a self-cleaving peptidyl sequence between said heavy chain sequence and said light chain sequence.

17. The isolated nucleic acid of claim 16, wherein said self-cleaving peptidyl linker sequence is a T2A sequence or a 2A sequence.

18. The isolated nucleic acid of one of claims 15 to 17, wherein said light chain sequence is 3′ to said heavy chain sequence.

19. The isolated nucleic acid of one of claims 1 to 18, wherein said antibody region is a cetuximab meditope enabled domain, trastuzumab meditope enabled domain, pertuzumab meditope enabled domain, M5A meditope enabled domain or rituximab meditope enabled domain.

20. An isolated nucleic acid encoding a protein comprising a first portion comprising an antibody heavy chain variable domain and a second portion comprising an antibody light chain variable domain and an antibody light chain constant domain, wherein the first portion further comprises a transmembrane domain.

21. The isolated nucleic acid of claim 20, wherein said first portion further comprises an intracellular T-cell signaling domain.

22. The isolated nucleic acid of claim 20, wherein said intracellular T-cell signaling domain is a CD3 ζ intracellular T-cell signaling domain.

23. The isolated nucleic acid of one of claims 20-22, wherein said first portion further comprises an intracellular co-stimulatory signaling domain.

24. The isolated nucleic acid of claim 23, wherein said intracellular co-stimulatory signaling domain is a CD28 intracellular co-stimulatory signaling domain, a 4-1BB intracellular co-stimulatory signaling domain, a ICOS intracellular co-stimulatory signaling domain, or an OX-40 intracellular co-stimulatory signaling domain.

25. The isolated nucleic acid of one of claims 20-24 wherein said first portion further comprises a linker domain.

26. The isolated nucleic acid of claim 25, wherein said linker domain is between said transmembrane domain and said intracellular T-cell signaling domain.

27. The isolated nucleic acid of claim 25, wherein said linker domain is between said intracellular T-cell signaling domain and said intracellular co-stimulatory signaling domain.

28. The isolated nucleic acid of claim 25, wherein said linker domain comprises the sequence GGCGG or GGG.

29. The isolated nucleic acid of claim 20, wherein said first portion further comprises a CD3 ζ intracellular T-cell signaling domain and an intracellular co-stimulatory signaling domain.

30. The isolated nucleic acid molecule of claim 23, wherein the first portion comprises from the amino terminus to the carboxy terminus: the heavy chain variable domain, a heavy chain constant domain, the transmembrane domain, the CD3 ζ intracellular T-cell signaling domain and an intracellular co-stimulatory signaling domain.

31. The isolated nucleic acid molecule of one of claims 20-30 further comprising a spacer region positioned between the heavy chain variable domain and the transmembrane domain.

32. The isolated nucleic acid of claim 31, wherein said spacer region further comprises a hinge region.

33. The isolated nucleic acid of claim 20, wherein the antibody heavy chain variable domain and the antibody light chain variable domain are humanized.

34. The isolated nucleic acid of claim 20, wherein said first portion comprises a heavy chain constant domain.

35. The isolated nucleic acid of claim 20 comprising a self-cleaving peptidyl sequence between said first portion and said second portion.

36. The isolated nucleic acid of claim 35, wherein said self-cleaving peptidyl encoding sequence is a T2A encoding sequence or a 2A encoding sequence.

37. The isolated nucleic acid of one of claim 20, wherein the nucleic acid sequence encoding the second portion is 3′ to the nucleic acid sequence encoding the first portion.

38. The isolated nucleic acid of one of claim 1 to 37, wherein said protein is an anti-CD19 protein, anti-CD20 protein, anti-CD22 protein, anti-CD30 protein, anti-CD33 protein, anti-CD44v6/7/8 protein, anti-CD123 protein, anti-CEA protein, anti-EGP-2 protein, anti-EGP-40 protein, anti-erb-B2 protein, anti-erb-B2,3,4 protein, anti-FBP protein, anti-fetal acetylcholine receptor protein, anti-GD2 protein, anti-GD3 protein, anti-Her2/neu protein, anti-IL-13R-a2 protein, anti-KDR protein, anti k-light chain protein, anti-LeY protein, anti-L1 cell adhesion molecule protein, anti-MAGE-A1 protein, anti-mesothelin protein, anti-murine CMV infected cell protein, anti-MUC2 protein, anti-NKGD2 protein, anti, oncofetal antigen protein,

anti-PCSA protein, anti-PSMA protein, anti-TAA (targeted by mAb IfE) protein, anti-EGFR protein, anti-TAG-72 protein or anti-VEGF-72 protein.

39. The isolated nucleic acid of one of claims 1 to 38, further comprising a suicide gene sequence.

40. An expression vector comprising the nucleic acid of one of claims 1 to 39.

41. The expression vector of claim 40, wherein said expression vector is a viral vector.

42. The expression vector of claim 41, wherein said virus is a lentivirus or onco-retrovirus.

43. A T lymphocyte comprising the expression vector of one of claims 40 to 42.

44. A T lymphocyte of claim 43, comprising a first polypeptide and a second polypeptide, the first polypeptide comprising a heavy chain variable domain, a heavy chain constant domain, a transmembrane domain, a CD3 ζ signaling domain and a co-stimulatory T-cell signaling domain, the second polypeptide comprising a light chain variable domain and an light chain constant domain.

45. A recombinant protein comprising:

(i) an antibody region comprising a central cavity formed by a heavy chain variable (VH) region and a light chain variable (VL) region, wherein said central cavity forms a peptide binding site comprising framework region amino acid residues; and

(ii) a transmembrane domain.

46. The recombinant protein of claim 45, wherein said antibody region further comprises a heavy chain constant region (CH) and a light chain constant region (CL).

47. The recombinant protein of claim 45 or 46, wherein said antibody region comprises an Fc domain.

48. The recombinant protein of one of claims 45 to 47, wherein said antibody region is a humanized antibody region.

49. The recombinant protein of one of claims 45 to 48, wherein said antibody region does not comprise a scFv antibody region.

50. The recombinant protein of one of claims 45 to 49, wherein said protein further comprises an intracellular T-cell signaling domain.

51. The recombinant protein of claim 50, wherein said intracellular T-cell signaling domain is a CD3 ζ intracellular T-cell signaling domain.

52. The recombinant protein of one of claims 45 to 51, further comprising an intracellular co-stimulatory signaling domain.

53. The recombinant protein of claim 52, wherein said intracellular co-stimulatory signaling domain is a CD28 intracellular co-stimulatory signaling domain, a 4-1BB intracellular co-stimulatory signaling domain, a ICOS intracellular co-stimulatory signaling domain, or an OX-40 intracellular co-stimulatory signaling domain.

54. The recombinant protein of one of claims 45 to 53, further comprising a spacer region.

55. The recombinant protein of claim 54, wherein said spacer region is between said transmembrane domain and said antibody region.

56. The recombinant protein of claim one of claims 45-55, further comprising a linker domain.

57. The recombinant protein of claim 56, wherein said linker domain is between said transmembrane domain and said intracellular T-cell signaling domain.

58. The recombinant protein of claim 56, wherein said linker domain is between said intracellular T-cell signaling domain and said intracellular co-stimulatory signaling domain.

59. The recombinant protein of claim 57 or 58, wherein said linker domain comprises the sequence GGCGG or GGG.

60. The recombinant protein of one of claims 45 to 59, wherein said antibody region is a cetuximab meditope enabled domain, trasuzumab meditope enabled domain,

pertuzumab meditope enabled domain, M5A meditope enabled domain or rituximab meditope enabled domain.

61. The recombinant protein of one of claims 45 to 60, wherein a compound comprising an peptidyl moiety is bound to said peptide binding site.

62. The recombinant protein of claim 61, wherein said compound is a multivalent meditope.

63. A recombinant protein comprising a first portion comprising an antibody heavy chain variable domain and a second portion comprising an antibody light chain variable domain and an antibody light chain constant domain, wherein the first portion further comprises a transmembrane domain, and wherein said antibody heavy chain variable domain, said antibody light chain variable domain and said antibody light chain constant domain together form an antibody region.

64. A T lymphocyte comprising the recombinant protein of one of claims 45 to 63, wherein said transmembrane domain is within the cell membrane of said T lymphocyte.

65. A method of treating cancer, said method comprising administering to a subject in need thereof an effective amount of the T-lymphocyte of claim 64, wherein said antibody region is an anti-cancer antibody region.

66. The method of claim 65, wherein said T-lymphocyte is an autologous T-lymphocyte.

67. The method of claim 65, wherein said T-lymphocyte is a heterologous T-lymphocyte.

68. The method of claim 65, wherein said cancer is a solid tumor cancer or hematologic malignancy.

69. The method of one of claims 65 to 68, wherein said cancer is ovarian cancer, renal cell carcinoma, a B-cell malignancy, leukemia, lymphoma, breast cancer, colorectal cancer, prostate cancer, neuroblastoma, melanoma, medulloblastoma, lung cancer, osteosarcoma, glioblastoma or glioma.

70. A method of reprogramming a T lymphocyte, said method comprising contacting a T lymphocyte with the expression vector of one of claims 40 to 42.

71. A method of detecting a cancer, said method comprising:

(i) administering to a cancer patient an effective amount of a T lymphocyte comprising the recombinant protein of one of claims 45 to 63 and a compound comprising a peptidyl moiety capable of binding to said peptide binding site, wherein said compound further comprises a detectable label, and wherein said antibody region is an anti-cancer antibody region;

(ii) allowing said compound to bind to said peptide binding site thereby forming a recombinant protein-compound complex; and

(iii) detecting said recombinant protein-compound complex within said cancer patient thereby detecting said cancer

FIG. 1A

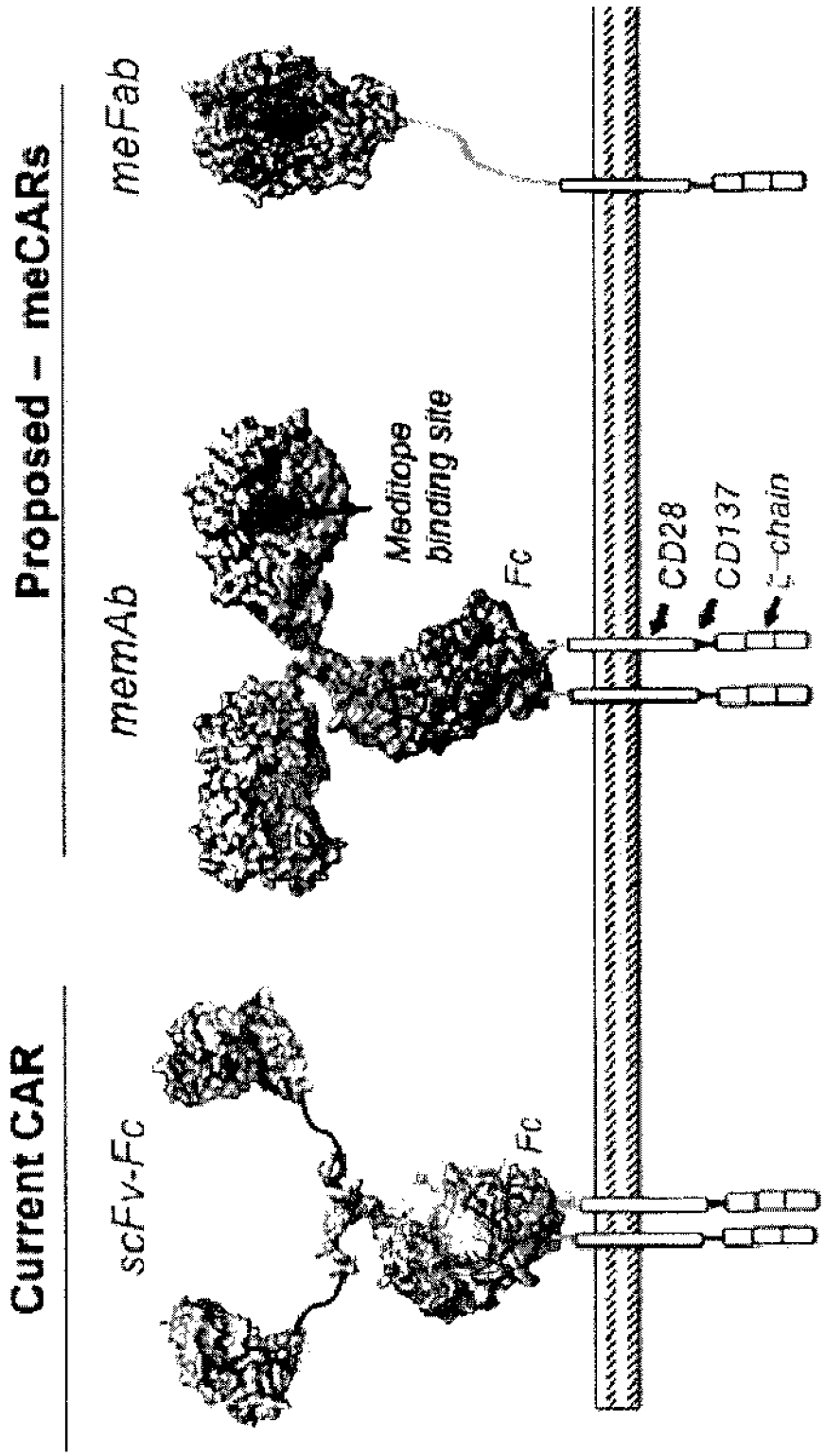

Proposed – meCARs
meFab
memAb
Meditope binding site
Fc
CD28
CD137
ζ-chain
Current CAR
scFv-Fc
Fc

FIG. 1B

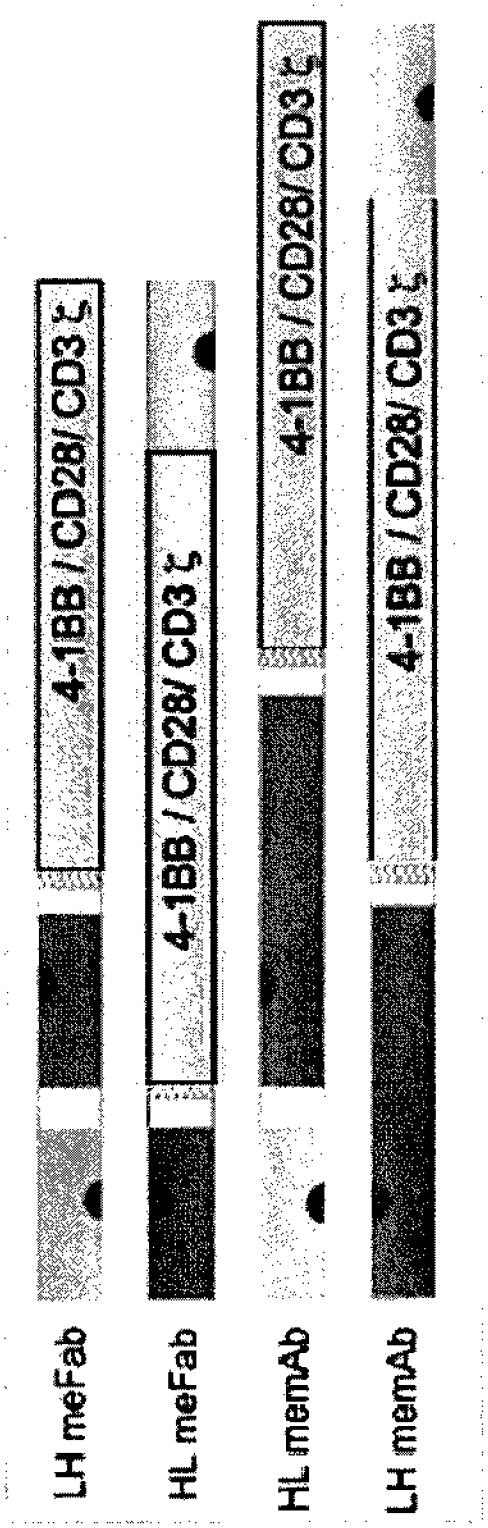

Light Chain
TM
Heavy Chain
Mediloge Site
4-1BB / CD28/ CD3 ζ
scFv
LH meFab
HL meFab
HL memAb
LH memAb

FIG. 2A

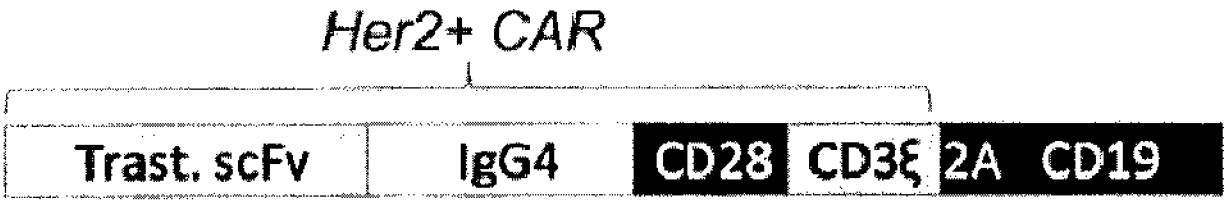

Her2+ CAR
Trast. scFv
IgG4
CD28
CD3ξ
2A
CD19

FIG. 2B

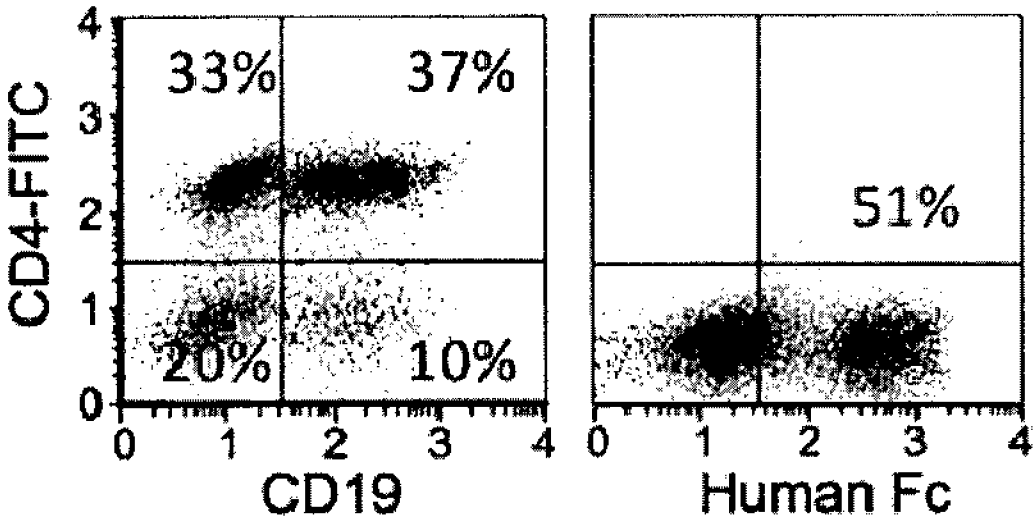

CD4-FITC
33%
37%
20%
10%
CD19
51%
Human Fc
0
1
2
3
4

FIG. 3A

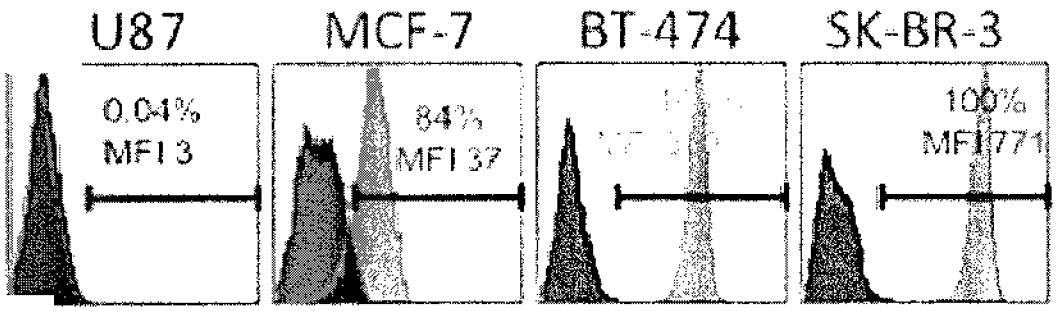

U87
MCF-7
BT-474
SK-BR-3
0.04%
MFI 3
84%
MFI 37
100%

FIG. 3B

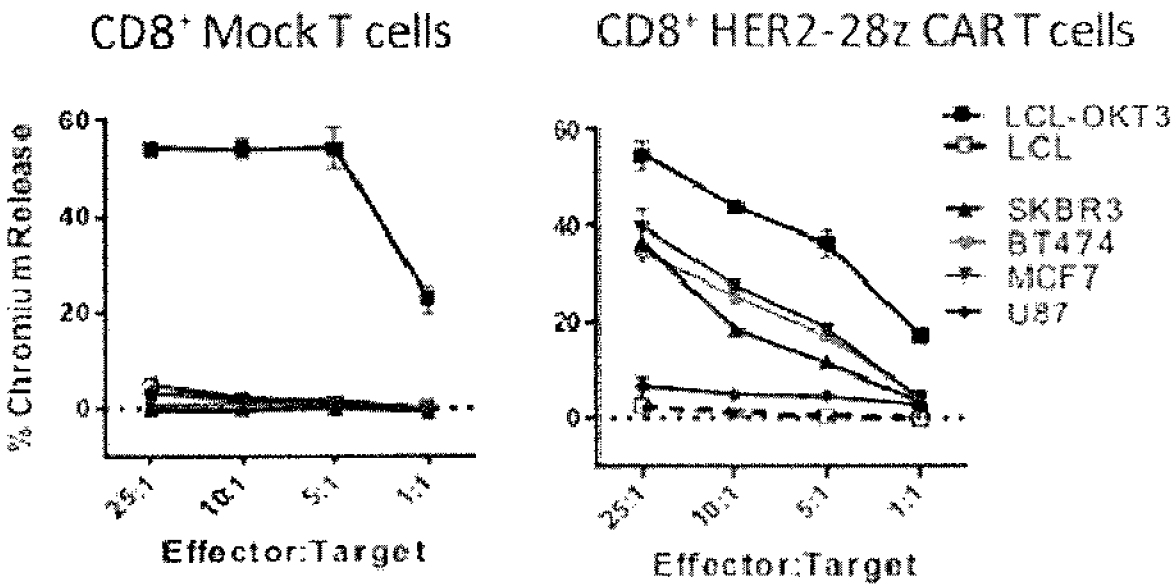

CD8+ Mock T cells
CD8+ HER2-28z CAR T cells
% Chromium Release
60
40
20
0
25:1
10:1
5:1
1:1
Effector:Target
LCL-OKT3
LCL
SKBR3
BT474
MCF7
U87

FIG. 4A

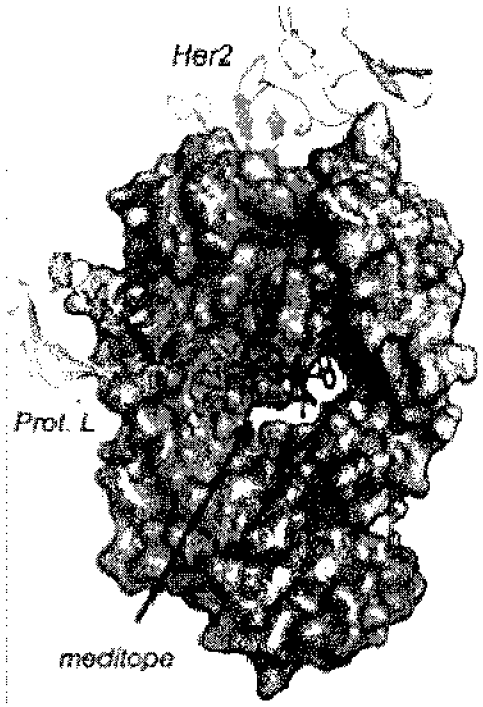

Her2
Prot. L
epitope

FIG. 4B

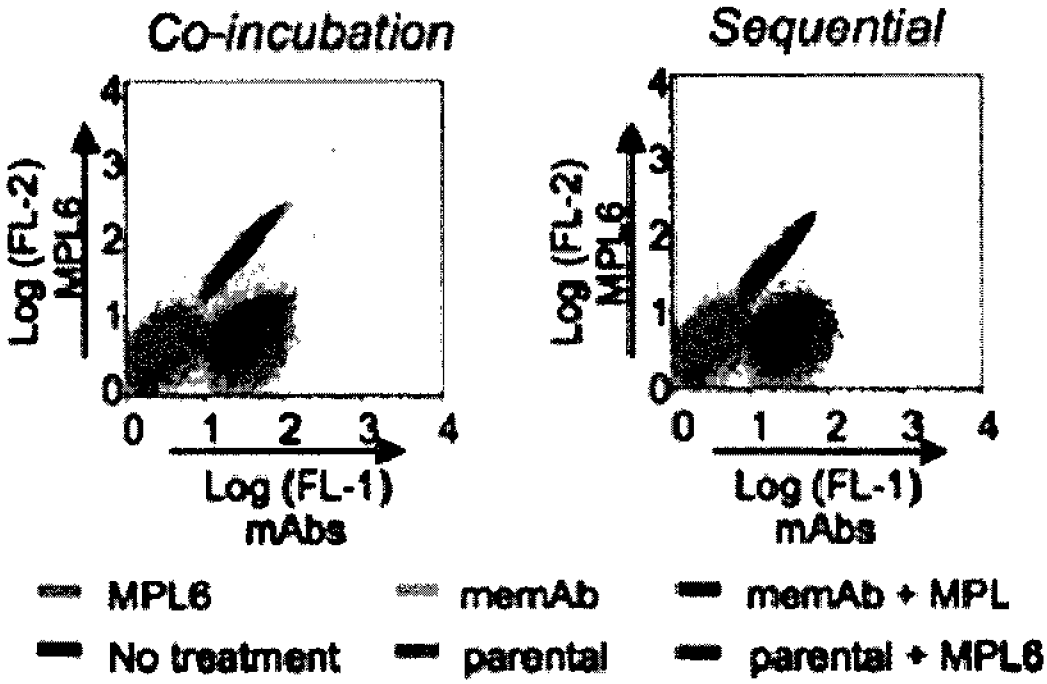

Co-incubation
Sequential
Log (FL-2) MPL6
Log (FL-1) mAbs
0
1
2
3
4
MPL6
No treatment
memAb
parental
memAb + MPL
parental + MPL6

FIG. 4C

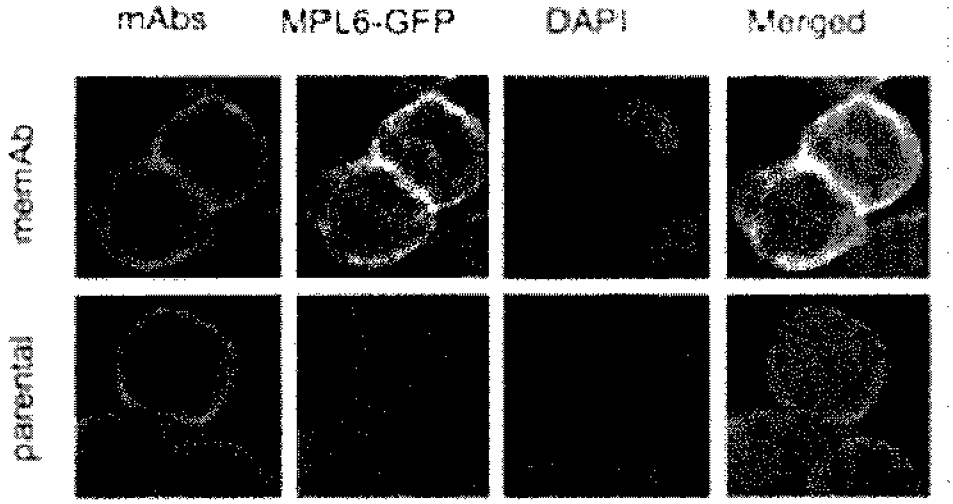


FIG. 4D

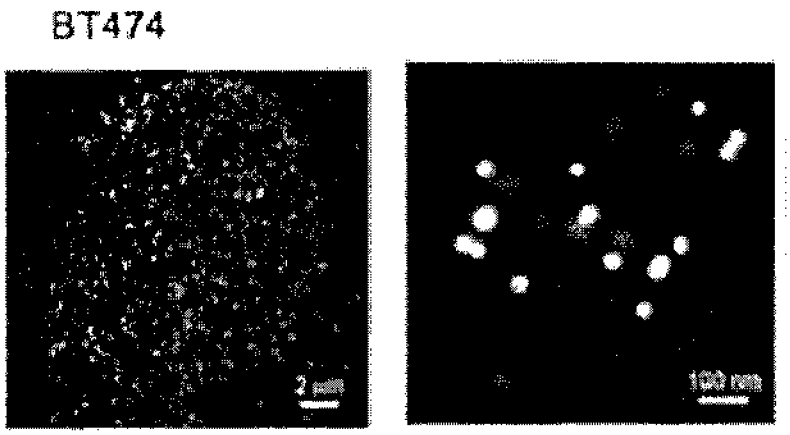


FIG. 5

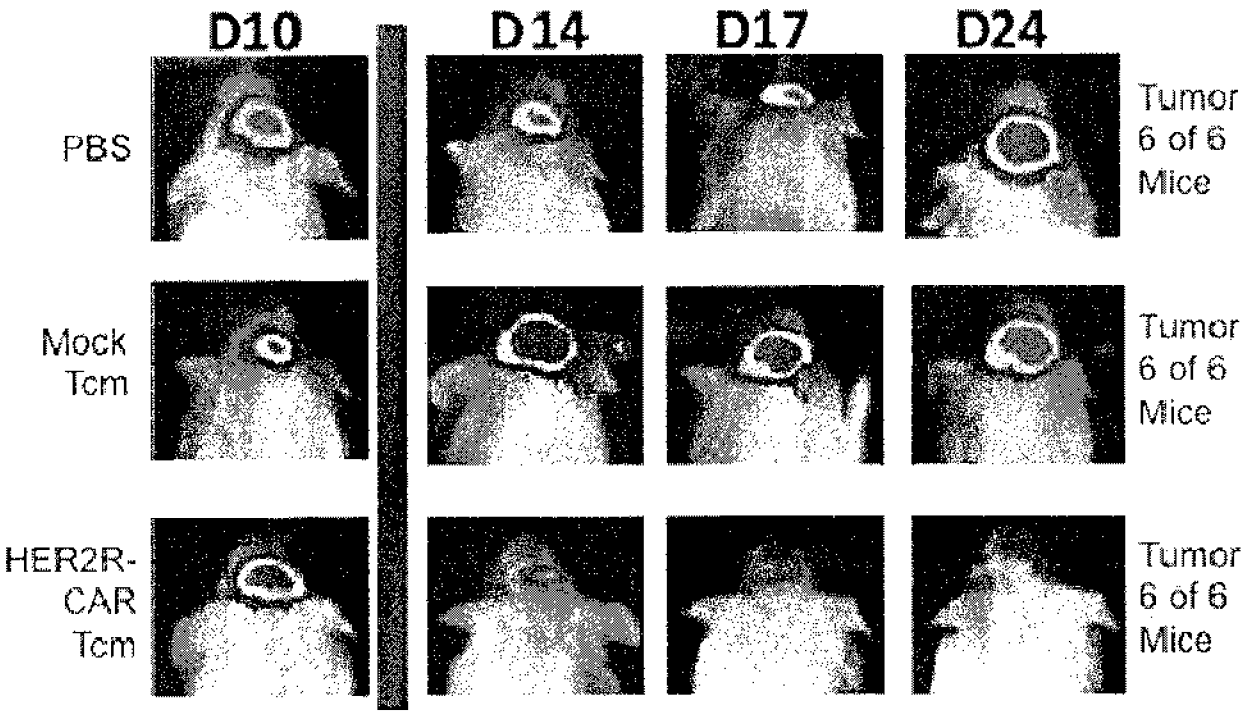

D10
D14
D17
D24
PBS
Mock Tcm
HER2R-CAR Tcm
Tumor 6 of 6 Mice
Tumor 6 of 6 Mice
Tumor 6 of 6 Mice

FIG. 6

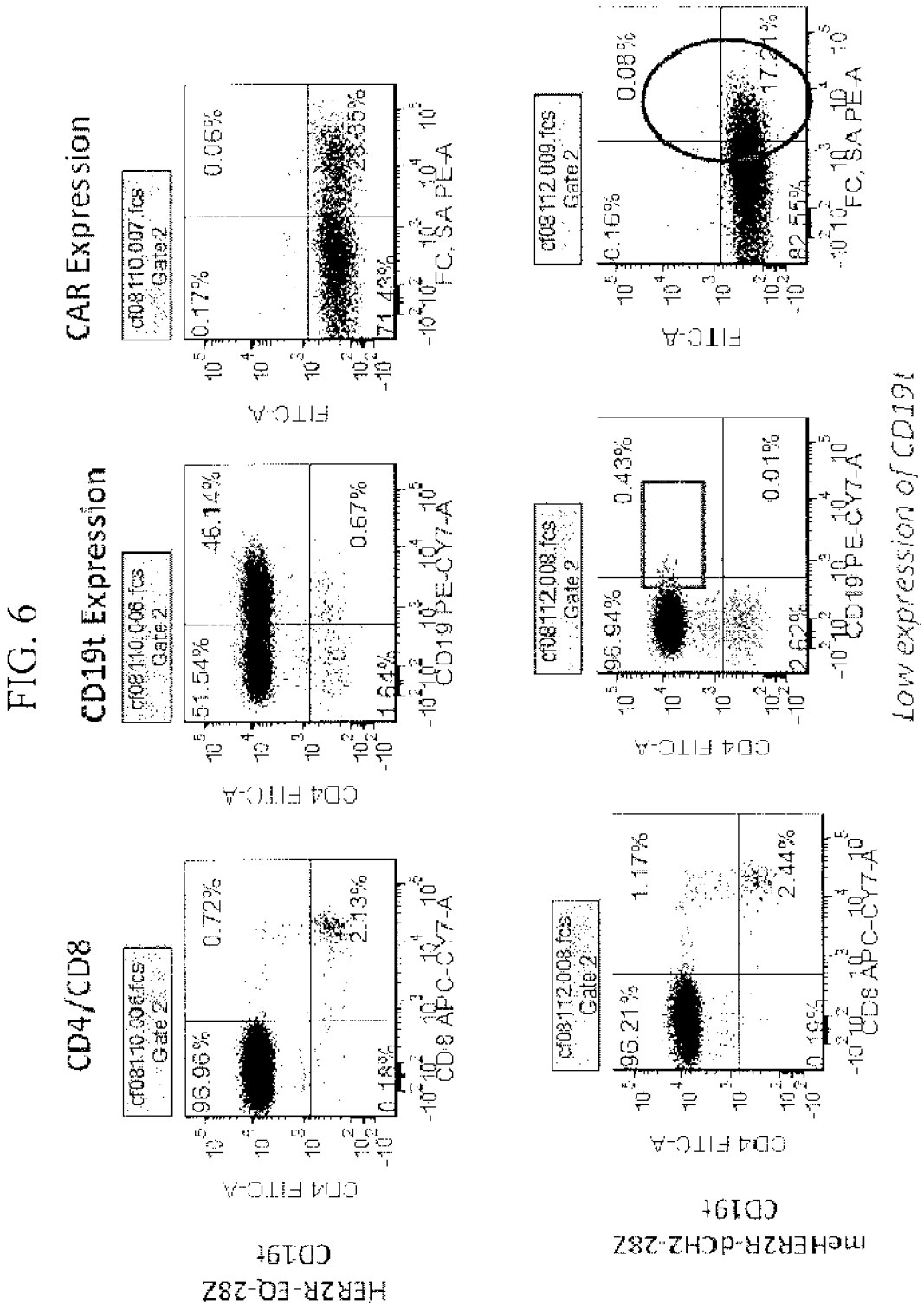

CD4/CD8
CD19t Expression
CAR Expression
HER2R-EQ-28Z
meHER2R-dCH2-28Z
CD19t
low expression of CD19t

FIG. 7

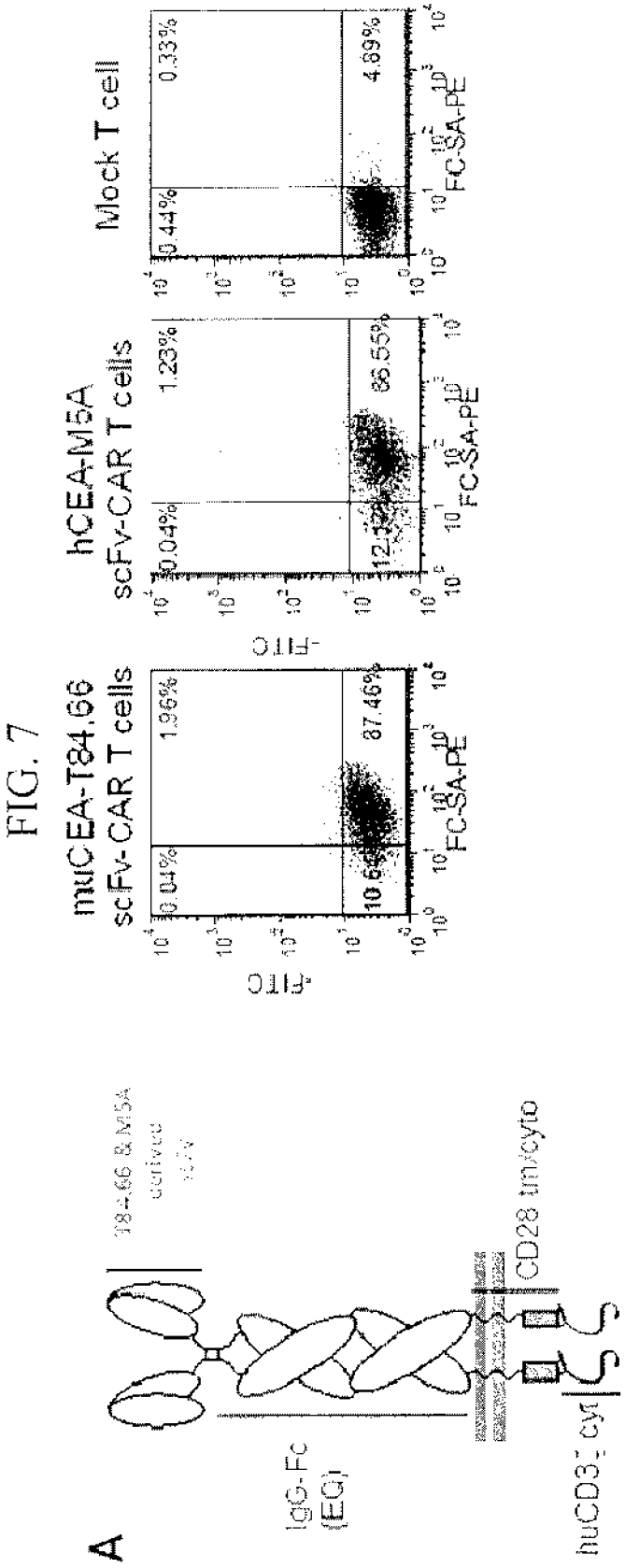

A
IgG-Fc (EQ)
CD28 tm/cyto
huCD3ζ cyt
muCEA-T84.66 scFv-CAR T cells
hCEA-M5A scFv-CAR T cells
Mock T cell
FITC
FC-SA-PE
0.04%
1.96%
87.46%
0.04%
1.23%
86.55%
0.44%
0.33%
4.89%

FIG.8A

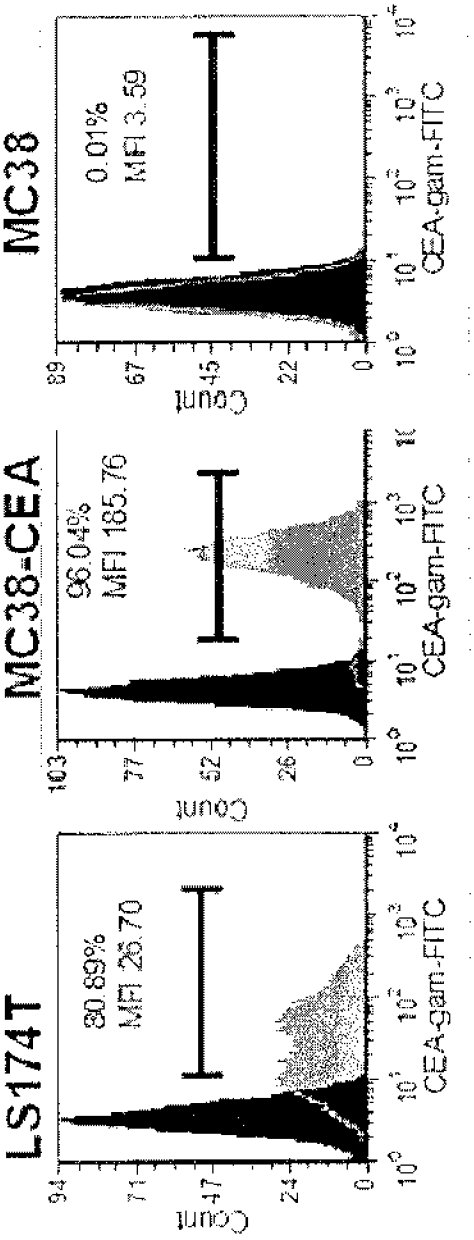

LS174T
80.89%
MFI 26.70
MC38-CEA
96.04%
MFI 185.76
MC38
0.01%
MFI 3.59
Count
CEA-gam-FITC

FIG.8B

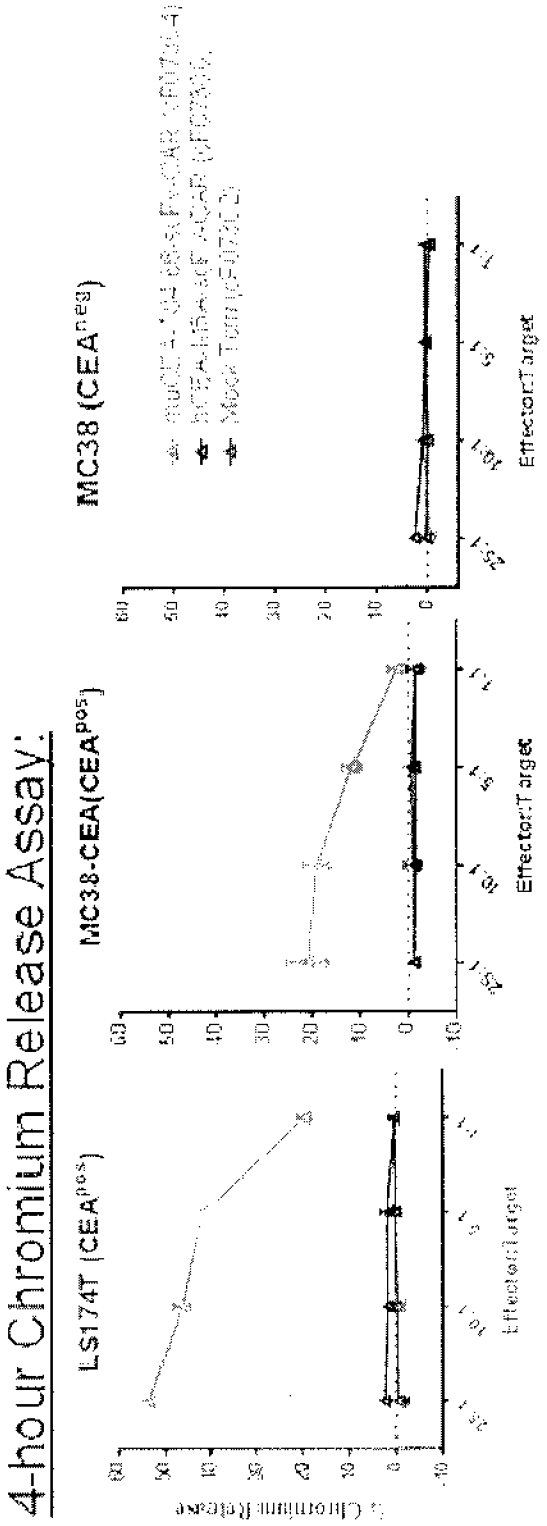

4-hour Chromium Release Assay:
LS174T (CEA^{POS})
MC38-CEA(CEA^{POS})
MC38 (CEA^{neg})
% Chromium Release
Effector:Target

FIG. 9

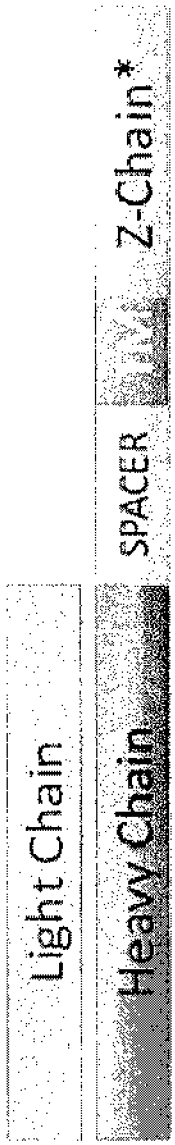

Light Chain
Heavy Chain
SPACER
Z-Chain*

FIG. 10

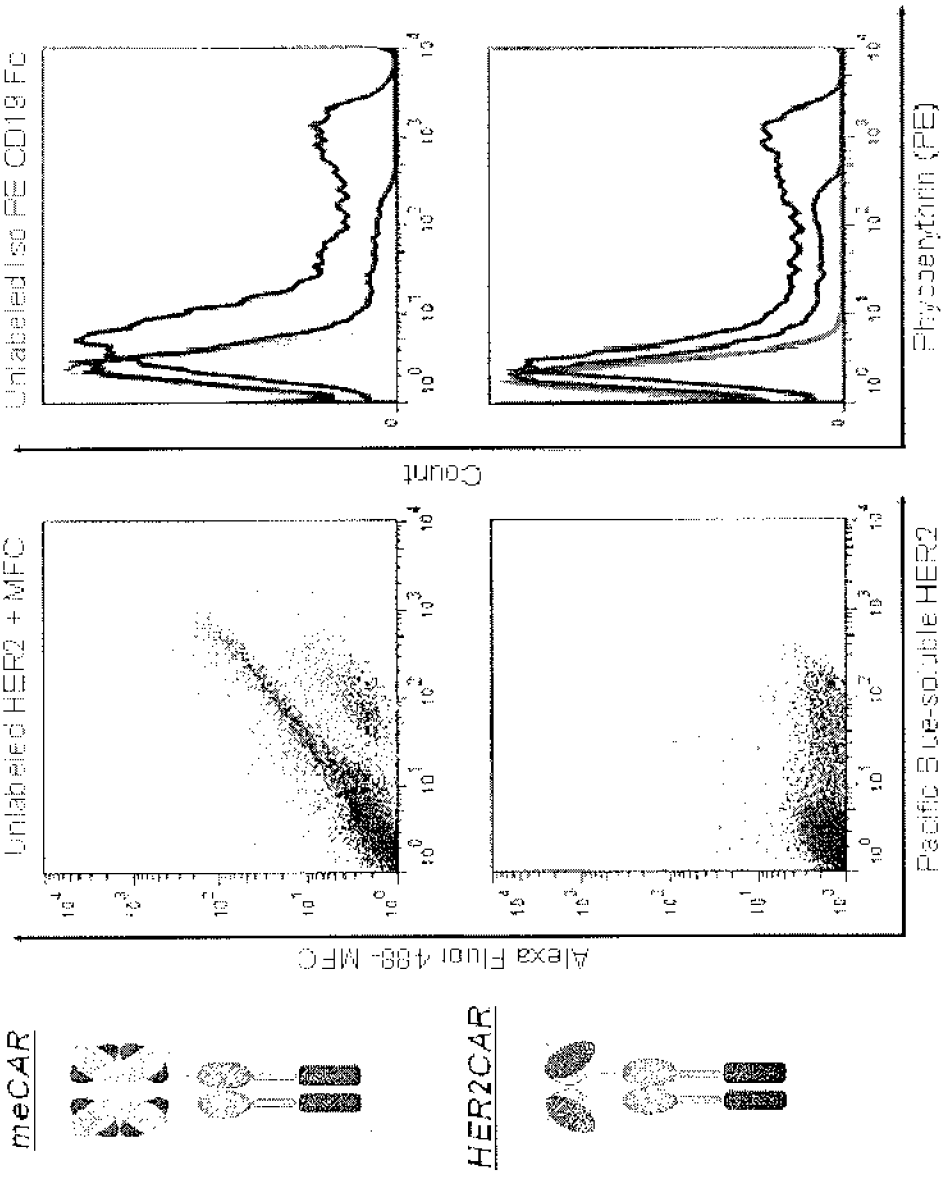

meCAR
HER2CAR
Alexa Fluor 488- MFC
Pacific Blue-soluble HER2
Unlabeled HER2 + MFC
Count
Unlabeled Iso PE CD19 Fc
Phycoerythrin (PE)

FIG. 11A

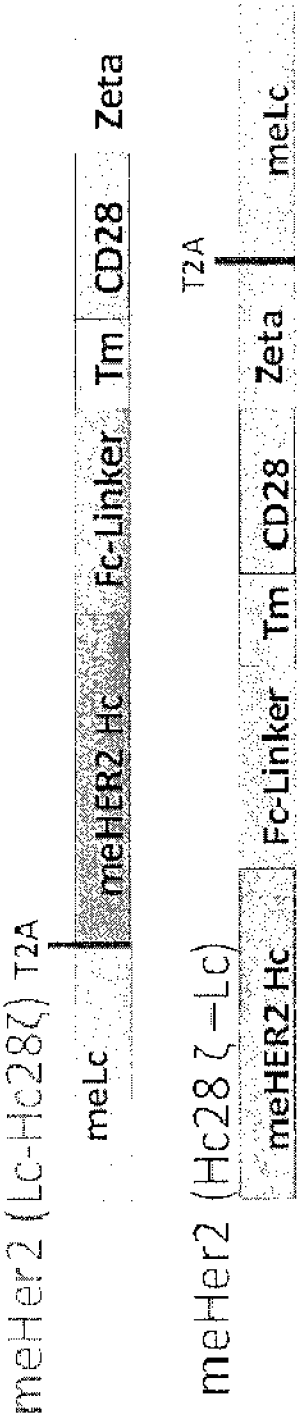
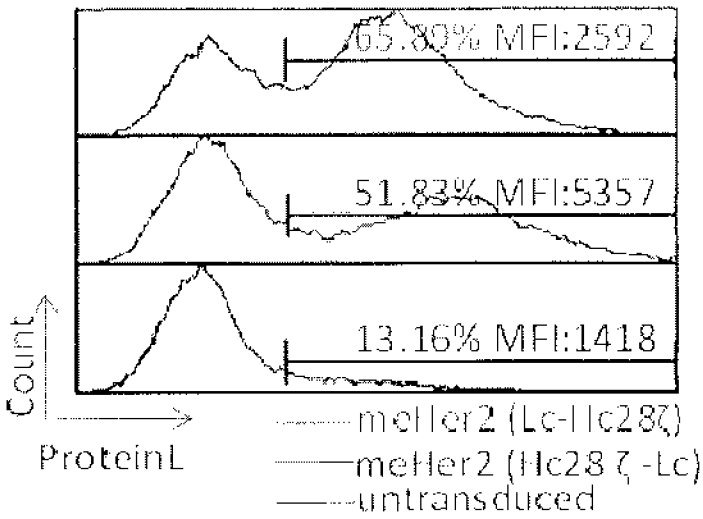

meHer2 (Lc-Hc28ζ) T2A
meLc
meHER2 Hc
Fc-Linker
Tm
CD28
Zeta
meHer2 (Hc28ζ-Lc)
T2A
meHER2 Hc
Fc-Linker
Tm
CD28
Zeta
meLc

FIG. 11B


65.80% MFI:2592
51.83% MFI:5357
13.16% MFI:1418
Count
ProteinL
meHer2 (Lc-Hc28ζ)
meHer2 (Hc28 ζ -Lc)
untransduced

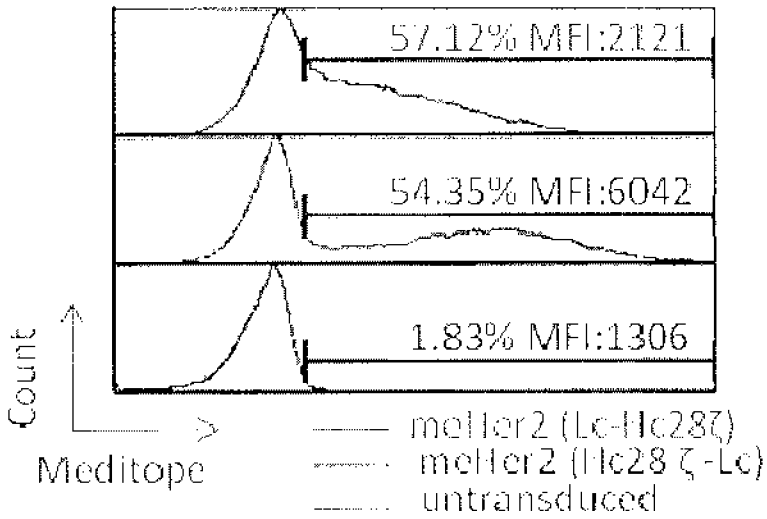

57.12% MFI:2121
54.35% MFI:6042
1.83% MFI:1306
Count
Meditope
meHer2 (Lc-Hc28ζ)
meHer2 (Hc28 ζ -Lc)
untransduced

FIG. 11C

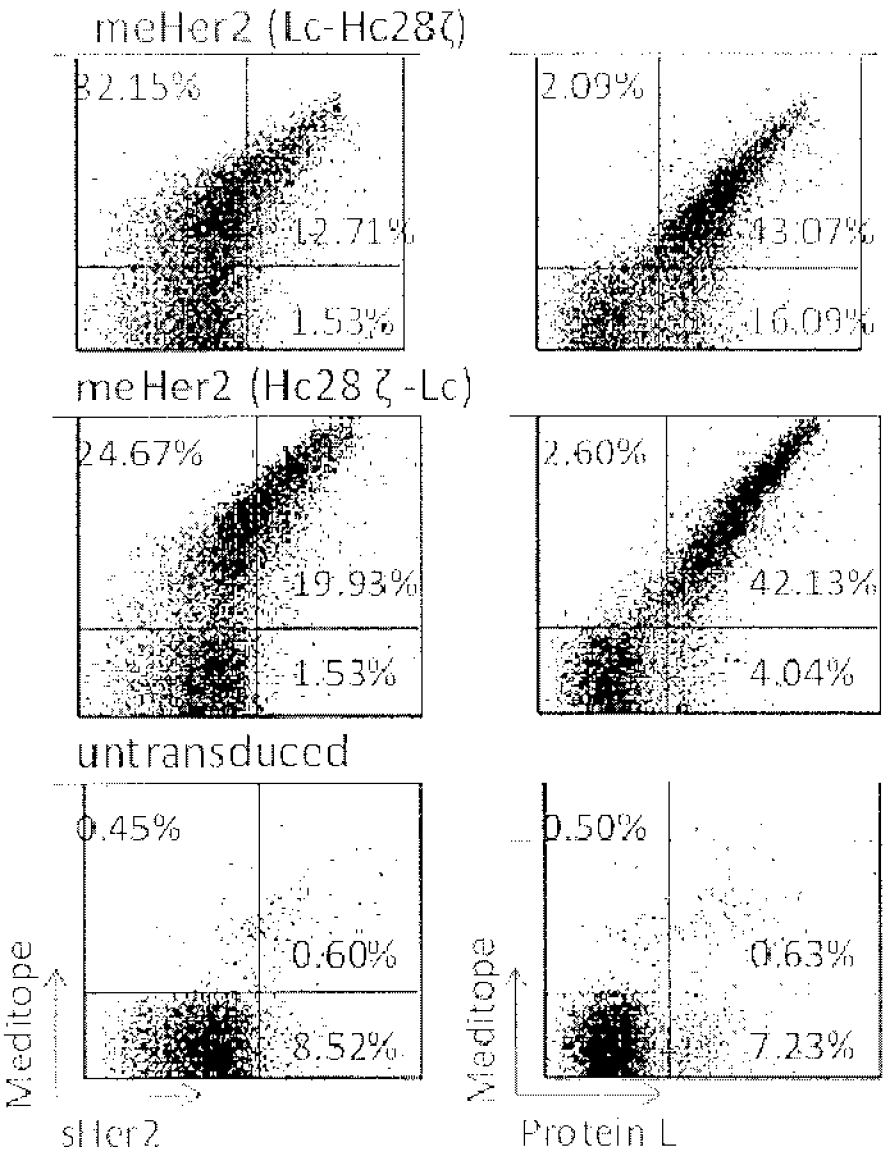

meHer2 (Lc-Hc28ζ)
82.15%
12.71%
1.53%
2.09%
13.07%
16.09%
meHer2 (Hc28ζ-Lc)
24.67%
19.93%
1.53%
2.60%
42.13%
4.04%
untransduced
0.45%
0.60%
8.52%
0.50%
0.63%
7.23%
Meditope
sHer2
Meditope
Protein L

FIG. 12A

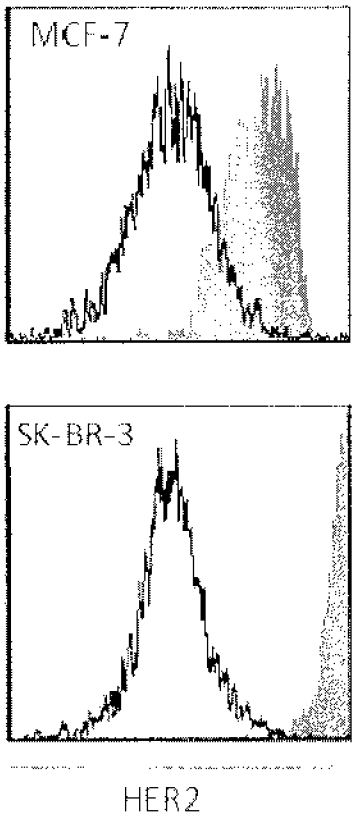


FIG. 12B

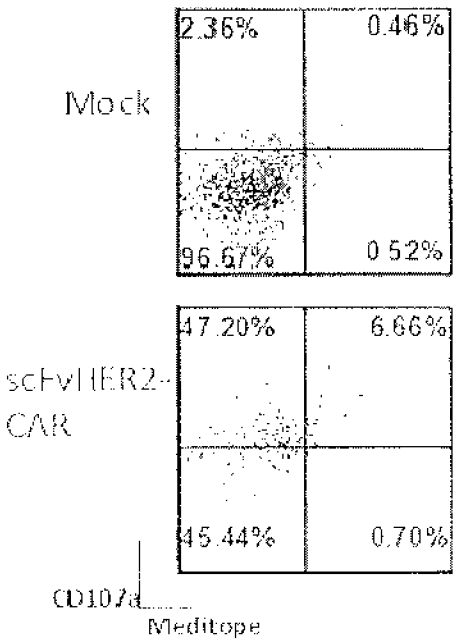


FIG. 12C

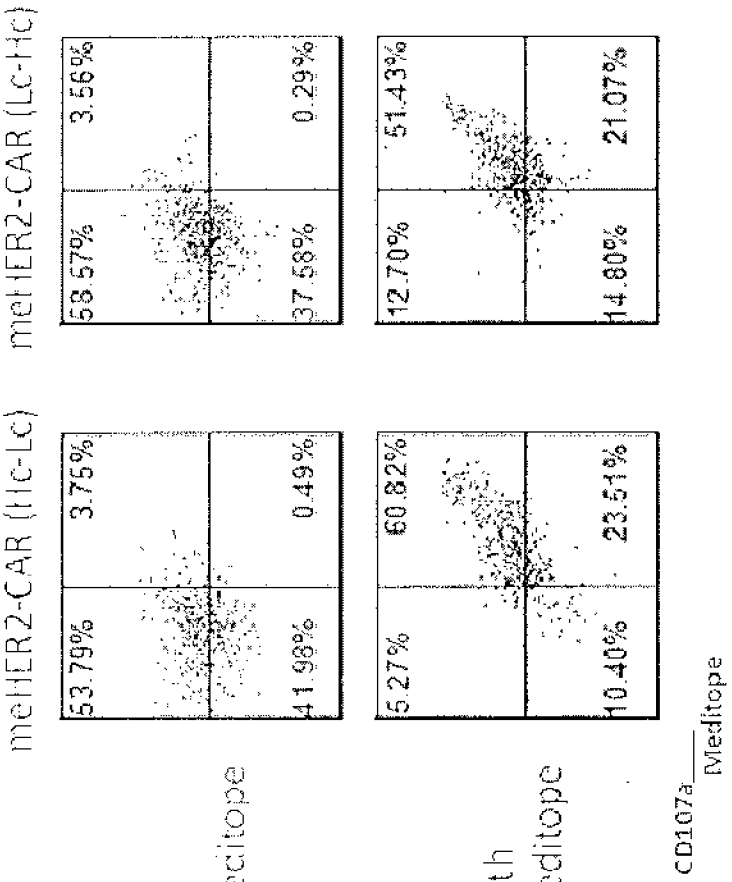

meHER2-CAR (Hc-Lc)
meHER2-CAR (Lc-Hc)
53.79%
3.75%
41.98%
0.49%
58.57%
3.56%
37.58%
0.29%
No Meditope
5.27%
60.82%
10.40%
23.51%
12.70%
51.43%
14.80%
21.07%
With Meditope
CD107a
Meditope

FIG. 12D

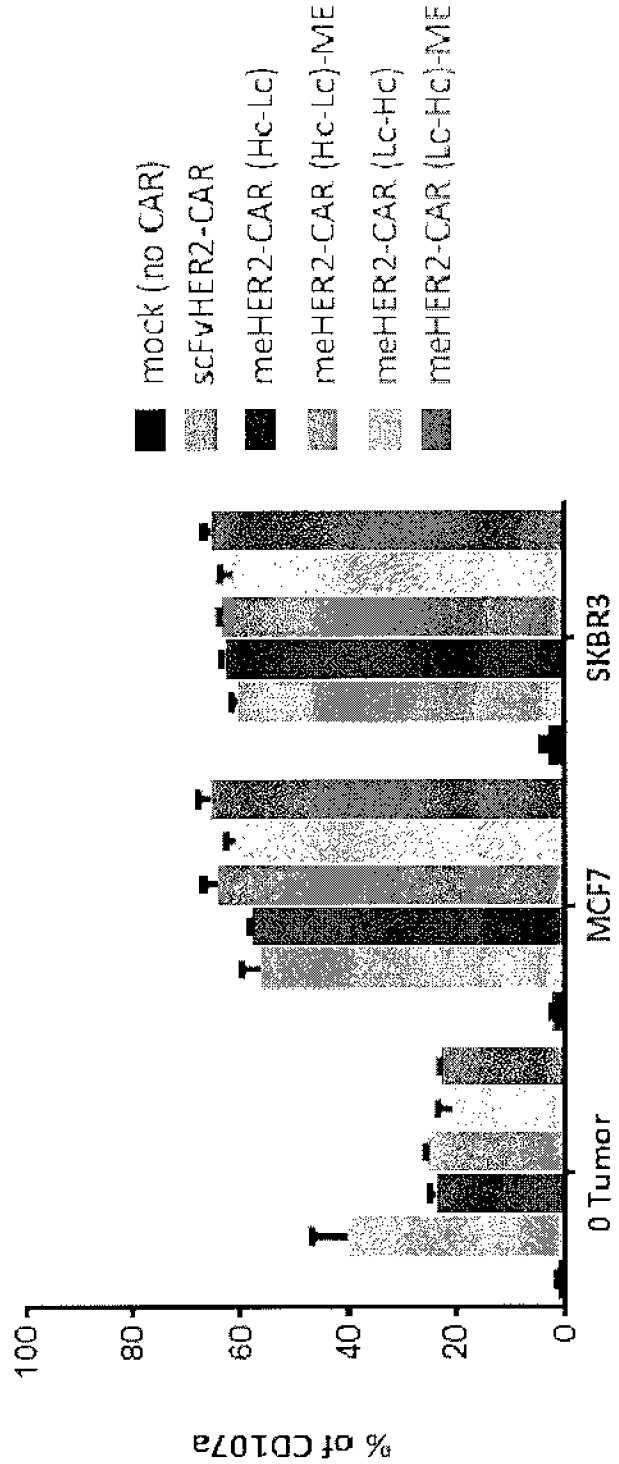

mock (no CAR)
scFvHER2-CAR
meHER2-CAR (Hc-Lc)
meHER2-CAR (Hc-Lc)-ME
meHER2-CAR (Lc-Hc)
meHER2-CAR (Lc-Hc)-ME
% of CD107a
100
80
60
40
20
0
0 Tumor
MCF7
SKBR3

FIG. 13A

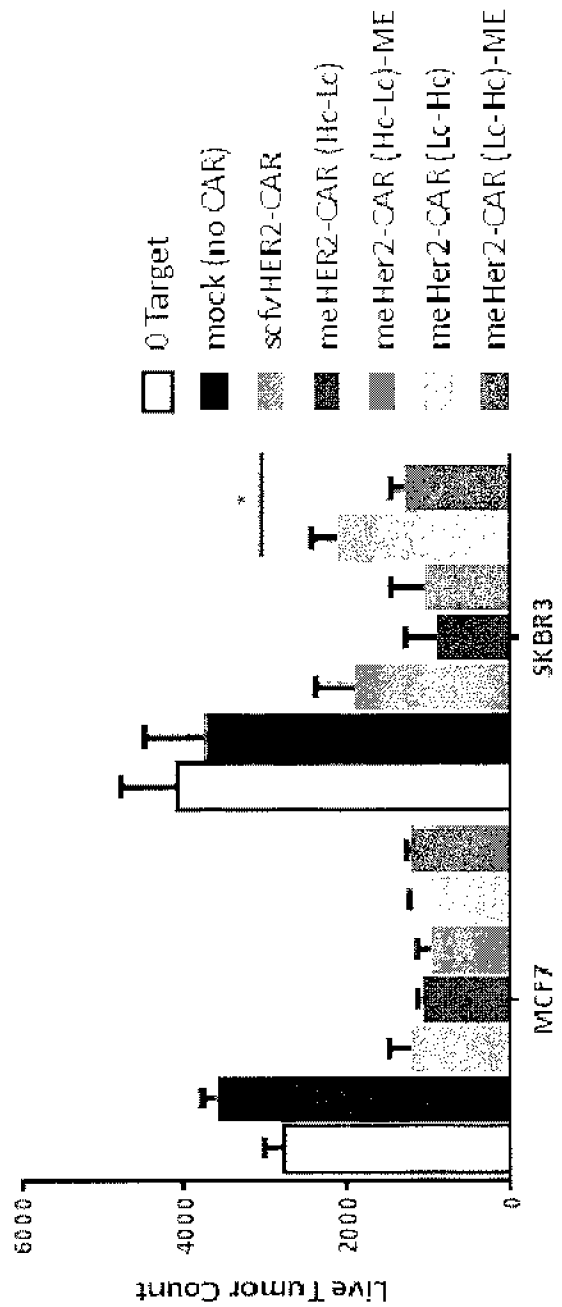
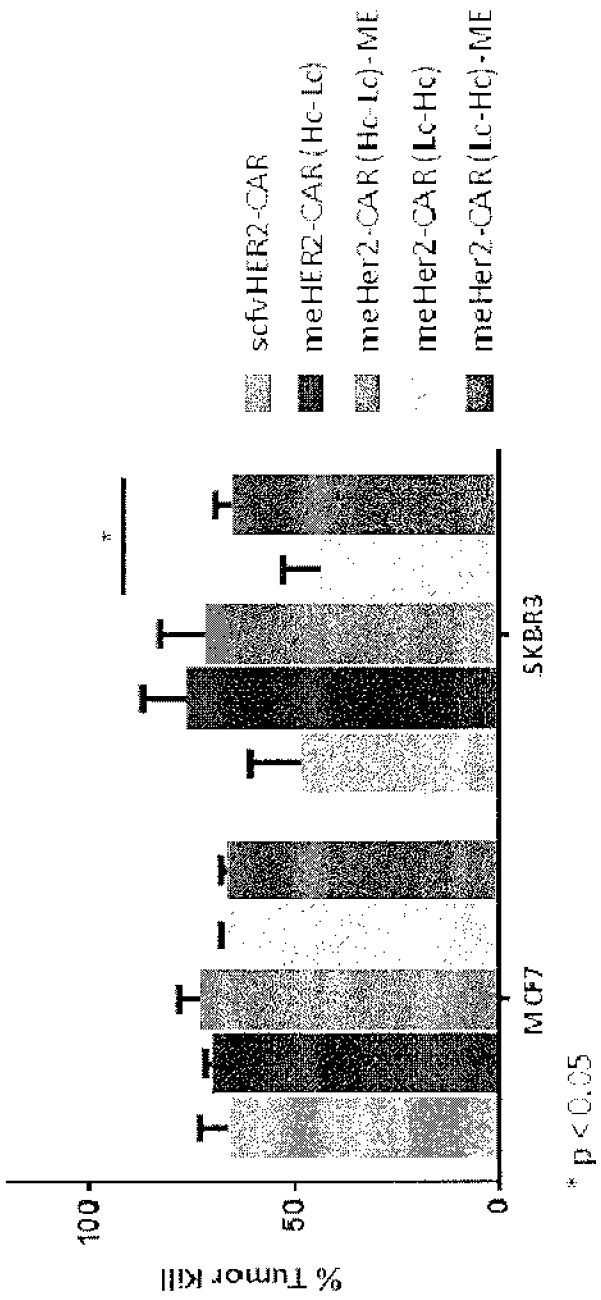

0 Target
mock (no CAR)
scfvHER2-CAR
meHER2-CAR (Hc-Lc)
meHer2-CAR (Hc-Lc)-ME
meHer2-CAR (Lc-Hc)
meHer2-CAR (Lc-Hc)-ME
*
Live Tumor Count
6000
4000
2000
0
MCF7
SKBR3

FIG. 13B



47979178v.1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/32780

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07K 14/435, 16/28; C12N 5/10, 9/00, 15/09, 15/63 (2016.01)
CPC - C07K 16/28, 16/2863; C12N 5/06, 9/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC (8) Classifications: C12P 21/02; C07K 14/435, 16/28; C07H 21/00, 21/04; C12N 5/10, 7/00, 9/00, 15/09, 15/63 (2016.01)
CPC Classifications: C07K 16/28, 16/2863; C12N 5/06, 9/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data), Google Scholar, EBSCO, PubMed; antibody, heavy, light, transmembrane, domain, protein, polypeptide, chain, chimeric, constant, variable, sequence, nucleic, cleave, splice, constant, expression, vector, plasmid

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 2014/180306 A1 (GAO, H et al.) 13 November 2014; paragraphs [0006]-[0009], [0019], [0025]	20-22, 23/20-22, 24/23/20-22, 29, 33-36 ----- 30/23/20-22, 37, 63
Y	US 7,563,441 B2 (GRAUS, Y et al.) 21 July 2009; column 1, line 21; column 3, lines 13- 64; column 4, lines 15-17; column 5, lines 53-55	1-2, 3/1-2, 30/23/20-22, 45-46, 47/45-46, 63
Y	(DONALDSON, JM et al.) Identification and grafting of a unique peptide-binding site in the Fab framework monoclonal antibodies. PNAS. 22 October 2013 Vol. 110, no 43, pages 17456-17461.	1-2, 3/1-2, 30/23/20-22, 45-46, 47/45-46
Y	US 5,359,046 A (CAPON, DJ et al.) 25 October 1994; figures 2D, 2E	37
P,X	EP 2 995 682 A1 (CARSGEN THERAPEUTICS LIMITED) 16 March 2016; entire document	1-2, 3/1-2, 20-22, 23/20-22, 24/23/20-22, 29, 30/23/20-22, 33-37, 45-46, 47/45-46, 63

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 July 2016 (20.07.2016)	31 AUG 2016
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/32780

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/32780

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-19, 25-28, 31-32, 38-44, 48-62, 64-71
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.