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(54) **Title:** CELL SURFACE CONJUGATES AND RELATED CELL COMPOSITIONS AND METHODS

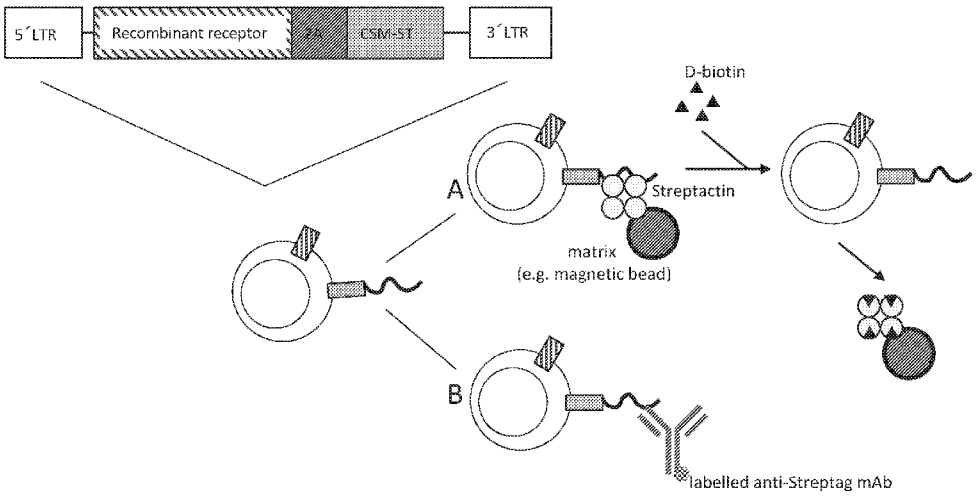

FIG. 1

(57) **Abstract:** Provided herein are cell surface conjugates containing a cell surface molecule and at least one agent, such as at least one affinity tag, and engineered cells expressing such cell surface conjugates. In some embodiments, the cell surface molecule does not contain an intracellular signaling domain or is not capable of mediating intracellular signaling. In some embodiments, the cells engineered to contain the cell surface conjugate, such as T cells, further contain a genetically engineered recombinant receptor that specifically binds to antigens, such as a chimeric antigen receptor (CAR). Also provided are methods of detecting, identifying, selecting or targeting cells expressing the cell surface conjugtes, such as in connection with methods of manufacturing engineered cells or in connection with administration of such cells to subjects, including methods of adoptive cell therapy.

WO 2018/134691 A2

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CELL SURFACE CONJUGATES AND RELATED CELL COMPOSITIONS AND METHODS

Cross-Reference to Related Applications

[0001] This application claims priority from U.S. provisional application No. 62/448,936, filed January 20, 2017, entitled "CELL SURFACE CONJUGATES AND RELATED CELL COMPOSITIONS AND METHODS," the contents of which are incorporated by reference in their entirety.

Incorporation by Reference of Sequence Listing

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled 735042002640SeqList.TXT, created January 19, 2018 which is 209,044 bytes in size. The information in the electronic format of the Sequence Listing is incorporated by reference in its entirety.

Field

[0003] The present disclosure relates in some aspects to cell surface conjugates containing a cell surface molecule and at least one agent, such as at least one affinity tag, and engineered cells expressing such cell surface conjugates. In some embodiments, the cell surface molecule does not contain an intracellular signaling domain or is not capable of mediating intracellular signaling. In some embodiments, the cells engineered to contain the cell surface conjugate, such as T cells, further contain a genetically engineered recombinant receptor, such as a chimeric antigen receptor, that specifically binds to antigen. The present disclosure also provides methods of detecting, identifying, selecting or targeting cells expressing the cell surface conjugtes, such as in connection with methods of manufacturing engineered cells or in connection with administration of such cells to subjects, including methods of adoptive cell therapy.

Background

[0004] Various strategies are available for treatment of diseases or conditions such as cancers or tumors, including the administration of cell therapies. Further, strategies are available for engineering immune cells to express genetically engineered recombinant receptors,

such as chimeric antigen receptors (CARs), and administering compositions containing such cells to subjects. Improved strategies are needed to increase efficacy of the treatments, for example, by improving the engineered compositions and/or improving the ability to monitor or modulate the engineered compositions in connection with such therapies upon administration to subjects. Provided are compositions, cells, and methods that meet such needs.

Summary

[0005] Provided herein is a cell surface conjugate containing a cell surface moleculethat lacks a functional intracellular signaling domain and/or is not capable of mediating intracellular signaling; and at least one agent linked to the cell surface molecule, the agent being capable of binding streptavidin or a streptavidin mutein. In some embodiments, the agent exhibits a binding affinity for streptavidin or a streptavidin, a streptavidin analog or mutein with an equilibrium dissociation constant (K_D) of from or from about 10^{-4} M to or to about 10^{-10} M..

[0006] Also provided is a cell surface conjugate containing a cell surface molecule that lacks a functional intracellular signaling domain and/or is not capable of mediating intracellular signaling; and at least one agent linked to the cell surface molecule and being capable of reversibly binding to a reagent and/or capable of being competed for binding to the reagent in the presence of a competition substance, wherein the agent is a peptide of less than 50 amino acids in length. In some embodiments, the agent exhibits a binding affinity for the reagent with an equilibrium dissociation constant (K_D) of from or from about 10^{-4} M to or to about 10^{-10} M. In some of any such embodiments, the reagent is streptavidin or a streptavidin, a streptavidin analog or mutein.

[0007] In some embodiments, the cell surface molecule comprises a transmembrane domain and/or is capable of being expressed on the surface of the cell. In some embodiments, the cell surface molecule is modified compared to a reference cell surface molecule, optionally wherein the reference cell surface molecule is a cell surface receptor comprising an intracellular signaling domain. In some embodiments, the modified cell surface molecule exhibits altered cellular internalization, enzymatic activity and/or ligand binding, compared to the reference cell surface molecule.

[0008] Also provided are cell surface conjugates, containing (a) a cell surface molecule that is modified compared to a reference cell surface molecule, wherein the modified cell surface molecule exhibits altered cellular internalization, enzymatic activity and/or ligand binding,

compared to the reference cell surface molecule; and (b) at least one agent linked to the cell surface molecule, the agent being capable of binding a streptavidin, a streptavidin analog or a streptavidin mutein.

[0009] In some embodiments, the cell surface molecule lacks a functional intracellular signaling domain and/or is not capable of mediating intracellular signaling.Also provided are cell surface conjugates, containing (a) a cell surface molecule comprising a prostate-specific membrane antigen (PSMA) or a modified cell surface molecule thereof; and (b) at least one agent linked to the cell surface molecule, the agent being capable of binding a streptavidin, a streptavidin analog or a streptavidin mutein. In some embodiments, the modified cell surface molecule lacks a functional intracellular signaling domain and/or is not capable of mediating intracellular signaling; and/or the modified cell surface molecule is modified compared to a reference cell surface molecule, wherein the modified cell surface molecule exhibits altered cellular internalization, enzymatic activity and/or ligand binding, compared to the reference cell surface molecule. In some embodiments, the cell surface molecule comprises a transmembrane domain and/or is capable of being expressed on the surface of the cell. In some embodiments, the agent exhibits a binding affinity for a streptavidin, a streptavidin analog or a streptavidin mutein with an equilibrium dissociation constant (K_D) of from or from about 10^{-4} M to or to about 10^{-10} M.

[0010] Provided herein is a cell surface conjugate containing a cell surface moleculethat lacks a functional intracellular signaling domain and/or is not capable of mediating intracellular signaling; and at least one agent linked to the cell surface molecule, the agent having a binding affinity for a reagent with an equilibrium dissociation constant (K_D) of more than 10^{-7} M or an equilibrium association constant (K_A) of less than 10^7 M^{-1}. In some instances, the reagent is streptavidin or a streptavidin analog or mutein.

[0011] In any of the provided embodiments, the cell surface molecule is a cell surface protein.

[0012] In some of any such embodiments, the binding of the agent to the reagent is reversible and/or is capable of being competed for binding to the reagent in the presence of a competition substance. In some aspects, the competition substance exhibits a higher binding affinity for the reagent than the binding affinity of the agent for the reagent. In some embodiments, the the competition substance exhibits a binding affinity for the reagent with an equilibrium dissociation constant (K_D) of between or about between 10^{-10} M and 10^{-14} M; and/or

the agent exhibits a binding affinity for the reagent with an equilibrium dissociation constant (K_D) of more than 10^{-10} M.

[0013] In some of any such embodiments, the binding of the agent to the streptavidin or a streptavidin, streptavidin analog or mutein is reversible and/or capable of being competed for binding to the reagent in the presence of biotin, a biotin analog or a biologically active fragment thereof.

[0014] In some of any such embodiments, the at least one agent is linked directly to the cell surface molecule. In some of any such embodiments, the at least one agent is linked indirectly to the cell surface molecule via at least one linker.

[0015] In some aspects, the cell surface conjugate containing the cell surface molecule and the at least one agent (e.g. a peptide, such as a streptavidin-binding peptide) is a fusion protein.

[0016] In some of any such embodiments, the at least one agent includes from or from about 1 to 4 or 1 to 2 agents. In some of any such embodiments, the at least one agent is only one agent. In some of any such embodiments, the agent is linked to an extracellular portion or region of the cell surface molecule, optionally wherein the extracellular portion or region is at the N-terminus or C-terminus of the cell surface molecule. In some of any such embodiments, the agent is linked at the N-terminus of the cell surface molecule. In some of any such embodiments, the agent is linked at the C-terminus of the cell surface molecule.

[0017] Also provided is a cell surface conjugate containing a cell surface molecule, such as a cell surface protein, linked at its N-terminus to an agent, the agent being capable of binding a reagent that is or contains streptavidin or a streptavidin mutein.

[0018] Also provided is a cell surface conjugate containing a cell surface molecule, such as a cell surface protein, linked at its N-terminus to an agent capable of reversibly binding to a reagent, wherein the agent is a peptide of less than 50 amino acids in length.

[0019] In some of any such embodiments, the agent exhibits a binding affinity for a reagent, e.g. a reagent that is or contains a streptavidin or a streptavidin analog or mutein , with an equilibrium dissociation constant (K_D) of from or from about 10^{-4} to 10^{-10} M.

[0020] Also provided are cell surface conjugates comprising a cell surface molecule linked, at an extracellular portion or region of the cell surface molecule, to an agent, the agent being capable of binding a reagent that is or comprises streptavidin or a streptavidin mutein, optionally wherein the extracellular portion or region is at the N-terminus or C-terminus of the cell surface molecule.

[0021] Also provided are cell surface conjugates comprising a cell surface molecule linked, at an extracellular portion or region of the cell surface molecule, to an agent, the agent being capable of reversibly binding to a reagent, wherein the agent is a peptide of less than 50 amino acids in length optionally wherein the extracellular portion or region is at the N-terminus or C-terminus of the cell surface molecule. In some embodiments, the agent exhibits a binding affinity with an equilibrium dissociation constant (K_D) of from or from about 10^{-4} M to or to about 10^{-10} M.

[0022] Also provided are cell surface conjugates comprising a cell surface molecule linked, at an extracellular portion or region of the cell surface molecule, to an agent, wherein the agent exhibits a binding affinity for a reagent with an equilibrium dissociation constant (K_D) of more than 10^{-7} M or an equilibrium association constant (K_A) of less than 10^7 M^{-1} optionally wherein the extracellular portion or region is at the N-terminus or C-terminus of the cell surface molecule. In some embodiments, the agent is linked at the N-terminus of the cell surface molecule. In some embodiments, the agent is linked at the C-terminus of the cell surface molecule.

[0023] Also provided is a cell surface conjugate containing a cell surface molecule, such as a cell surface protein, linked at its N-terminus to an agent, wherein the agent exhibits a binding affinity for a reagent with an equilibrium dissociation constant (K_D) of more than 10^{-7} M or an equilibrium association constant (K_A) of less than 10^7 M^{-1}. In some of any such embodiments, the reagent is or contains streptavidin or a streptavidin analog or mutein.

[0024] In some of any such embodiments, the binding of the agent to the reagent, e.g. a reagent that is or contains a streptavidin or a streptavidin analog or mutein, is reversible and/or capable of being competed for binding to the reagent in the presence of a competition substance. In some cases, the competition substance exhibits a higher binding affinity for the reagent than the binding affinity of the agent for the reagent. In some examples, the competition substance exhibits a binding affinity for the reagent of between or about between 10^{-10} and 10^{-14}; and/or the agent exhibits a binding affinity for the reagent of less than 10^{-10}. In some of any such embodiments, the reagent is a streptavidin or a streptavidin analog or mutein and the binding of the agent to the streptavidin or a streptavidin analog or mutein is reversible and/or capable of being competed for binding to the reagent in the presence of biotin or a biotin analog.

[0025] In some of any such embodiments, the agent is linked directly to the cell surface molecule, such as to a cell surface protein, including to a modified cell surface molecule as described. In some of any such embodiments, the agent is linked indirectly to the cell surface

molecule via at least one linker. In some of any such embodiments, the cell surface molecule, such as a cell surface protein, is linked to only one agent.

[0026] In some of any such embodiments, the cell surface molecule is not a chimeric antigen receptor (CAR).

[0027] In some of any such embodiments, the cell surface molecule, such as a cell surface protein, lacks a functional intracellular signaling domain and/or is not capable of mediating intracellular signaling. In some of any such embodiments, the cell surface molecule is modified compared to a reference cell surface molecule. In some of any such embodiments, the reference cell surface molecule is a native mammalian cell surface molecule. In some of any such embodiments, the modified cell surface molecule comprises or retains an epitope of the reference cell surface molecule capable of being recognized by an antibody or antigen-binding fragment thereof. In some embodiments, the modified cell surface molecule lacks a functional intracellular signaling domain and/or is not capable of mediating intracellular signaling; and/or the modified cell surface molecule exhibits altered cellular internalization, enzymatic activity and/or ligand binding, compared to the reference cell surface molecule.

[0028] In some of any such embodiments, the cell surface conjugate is a fusion protein.

[0029] In some of any such embodiments, the streptavidin analog or mutein contains the amino acid sequence Val^{44}-Thr^{45}-Ala^{46}-Arg^{47} or Ile^{44}-Gly^{45}-Ala^{46}-Arg^{47} at sequence positions corresponding to positions 44 to 47 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO:1. In some of any such embodiments, the streptavidin analog or mutein contains a) the sequence of amino acids set forth in any of SEQ ID NOS: 3-6, 27 and 28; b) a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS:3-6, 27 and 28 and contains the amino acid sequence corresponding to Val^{44}-Thr^{45}-Ala^{46}-Arg^{47} or Ile^{44}-Gly^{45}-Ala^{46}-Arg^{47} and that reversibly binds to the agent; or c) a functional fragment of a) or b) that reversibly binds to the agent and/or in which binding to the agent is competed in the presence of a competition substance.

[0030] In some of any such embodiments, the streptavidin analog or mutein further contains an amino acid replacement or replacements at a position corresponding to 117, 120 and/or 121 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO:1. In some aspects, the amino acid replacement or replacements are selected from among Glu^{117}, Asp^{117}, Arg^{117}, Ser^{120}, Ala^{120}, Gly^{120}, Trp^{121}, Tyr^{121} or Phe^{121}; or the amino acid

replacement or replacements are selected from one or more of Glu^{117}, Gly^{120} or Tyr^{121}; or the amino acid replacements are selected from Glu^{117}, Gly^{120} or Tyr^{121}.

[0031] In some of any such embodiments, the streptavidin analog or mutein contains a) the sequence of amino acids set forth in SEQ ID NO: 27 or 28; b) a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NOS: 27 or 28 and contains the amino acid sequence corresponding to Val^{44}, Thr^{45}, Ala^{46}, Arg^{47}, Glu^{117}, Gly^{120} and Tyr^{121} and reversibly binds to the agent and/or in which binding to the agent competed in the presence of a competition substance; or c) a functional fragment of a) or b) that reversibly binds to the agent and/or in which binding to the agent is competed in the presence of a competition substance.

[0032] In some of any such embodiments, the agent is an affinity tag. In some of any such embodiments, the agent is or contains a Strep tag, His tag, Flag tag, Xpress tag, Avi tag, Calmodulin tag, Polyglutamate tag, HA tag, Myc tag, Nus tag, S tag, X tag, SBP tag, Softag, V5 tag, CBP, GST, MBP, GFP, Thioredoxin tag, or any combination thereof. In some of any such embodiments, the agent is or comprises one or more streptavidin binding peptide, which optionally is a Strep tag.

[0033] In some of any such embodiments, the streptavidin-binding peptide contains the sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8) or Trp-Arg-His-Pro-Gln-Phe-Gly-Gly (SEQ ID NO:7).

[0034] In some of any such embodiments, the agent contains the sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_3$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 17), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 18) and Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$Gly-Gly-Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 19).

[0035] In some of any such embodiments, the competition substance is or contains biotin, a biotin analog or a biologically active fragment thereof. Among such embodiments are those in which the agent is a streptavidin binding peptide and the reagent is a streptavidin or a streptavidin mutein or analog, including any as described.

[0036] In some of any such embodiments, the reference cell surface molecule is a cell surface protein that is a cell surface receptor, ligand, glycoprotein, cell adhesion molecule, antigen, integrin or cluster of differentiation (CD). In some embodiments, the reference cell surface molecule is a cell surface receptor. In some of any such embodiments, the reference cell surface molecule is selected from EpCAM, VEGFR, integrin (e.g., integrins $\alpha v\beta 3$, $\alpha 4$, $\alpha IIb\beta 3$,

α4β7, α5β1, αvβ3, αv), a member of the TNF receptor superfamily (e.g., TRAIL-R1, TRAIL-R2), a member of the epidermal growth factor receptor family, PDGF Receptor, interferon receptor, folate receptor, GPNMB, ICAM-1, HLA-DR, CEA, CA-125, MUC1, TAG-72, IL-6 receptor, 5T4, GD2, GD3, prostate-specific membrane antigen (PSMA), or clusters of differentiation (e.g., CD2, CD3, CD4, CD5, CD11, CD11a/LFA-1, CD15, CD18/ITGB2, CD19, CD20, CD22, CD23/IgE Receptor, CD25, CD28, CD30, CD33, CD38, CD40, CD41, CD44, CD51, CD52, CD62L, CD74, CD80, CD125, CD147/basigin, CD152/CTLA-4, CD154/CD40L, CD195/CCR5 and CD319/SLAMF7.

[0037] In some of any such embodiments, the reference cell surface molecule, such as reference cell surface protein, is a member of the epidermal growth factor receptor family. In some of any such embodiments, the reference cell surface molecule is an epidermal growth factor receptor (EGFR), an erbB-2 receptor tyrosine-protein kinase (errb2, HER2), an erbB-3 receptor tyrosine-protein kinase, an erbB-4 receptor tyrosine-protein kinase, a hepatocyte growth factor receptor (HGFR/c-MET) or an insulin-like growth factor receptor-1 (IGF-1 R). In some of any such embodiments, the reference cell surface molecule is a prostate-specific membrane antigen (PSMA).

[0038] In some of any such embodiments, the reference cell surface molecule, such as the reference cell surface protein, is human.

[0039] In some of any such embodiments, the modified cell surface molecule lacks a functional intracellular signaling domain and/or is not capable of mediating intracellular signaling. In some of any such embodiments, the modified cell surface molecule is truncated compared to the reference cell surface molecule, such as is truncated to remove or delete all or a portion of an intracellular region, i.e. a portion of the molecule contained inside the cell, of the reference cell surface molecule. In some cases, the intracellular region is a region that contains an intracellular signaling domain or trafficking domain. In some of any such embodiments, the modified cell surface molecule is truncated to lack all or a portion of the intracellular signaling domain or trafficking domain compared to the reference cell surface molecule. In some of any such embodiments, the modified cell surface molecule contains one or more extracellular domains of the reference cell surface molecule. In some embodiments, the modified cell surface molecule exhibits altered cellular internalization, enzymatic activity and/or ligand binding, compared to the reference cell surface molecule.

[0040] In some of any such embodiments, the modified cell surface molecule is capable of binding to a native ligand or substrate of the reference cell surface molecule. In some of any

such embodiments, the modified cell surface molecule is reduced for or does not bind the native ligand or substrate of the reference cell surface molecule. In some of any such embodiments, the modified cell surface molecule contains at least one extracellular domain of the reference cell surface molecule but lacks one or more other extracellular domains recognized by the native ligand or substrate of the reference cell surface molecule. In some aspects, the at least one extracellular domain contains an epitope recognized by an antibody or antigen-binding fragment thereof that specifically binds the reference cell surface molecule. In some of any such embodiments, the antibody or antigen-binding fragment is selected from AMG-102, AMG-479, BIIB022OA-5D5, CP-751,871, IMC-Al2, R1507, 3F8, abagovomab, abciximab, adecatumumab, afutuzumab, alemtuzumab, altumomab pentetate, anatumomab mafenatox, apolizumab, arcitumomab, aselizumab, atlizumab (=tocilizumab), basiliximab, bectumomab, benralizumab, besilesomab, bivatuzumab mertansine, blinatumomab, brentuximab vedotin, cantuzumab mertansine, capromab pendetide, catumaxomab, CC49, cedelizumab, celmoleukin, cetuximab, cixutumumab, clenoliximab, clivatuzumab tetraxetan, CNTO-95, conatumumab, dacetuzumab, daclizumab, daratumumab, detumomab, ecromeximab, ertumaxomab, edrecolomab, efalizumab, elotuzumab, enlimomab pegol, epitumomab cituxetan, epratuzumab, erlizumab, etaracizumab, fanolesomab, faralimomab, farletuzumab, figitumumab, galiximab, gavilimomab, gemtuzumab ozogamicin, glembatumumab vedotin, gomiliximab, ibalizumab, ibritumomab tiuxetan, igovomab, intetumumab, iratumumab, inolimomab, inotuzumab ozogamicin, ipilimumab, keliximab, labetuzumab, lintuzumab, lexatumumab, lucatumumab, lumiliximab, mapatumumab, maslimomab, matuzumab, milatuzumab, minretumomab, mitumomab, muromonab-CD3, naptumomab estafenatox, natalizumab, necitumumab, ocrelizumab, odulimomab, ofatumumab, olaratumab, oportuzumab monatox, oregovomab, otelixizumab, panitumumab, pertuzumab, pemtumomab, priliximab, PRO 140, nimotuzumab, robatumumab, rituximab, rovelizumab, ruplizumab, satumomab pendetide, siplizumab, sontuzumab, tadocizumab, taplitumomab paptox, teneliximab, teplizumab, TGN1412, ticilimumab (=tremelimumab), tigatuzumab, tocilizumab (=atlizumab), toralizumab, tositumomab, trastuzumab, tremelimumab, tucotuzumab, vedolizumab, veltuzumab, visilizumab, vitaxin, volociximab, votumumab, zalutumumab, zanolimumab, ziralimumab, zolimomab aritox, Atezolizumab, bevacizumab (Avastin ®), denosumab, dinutuximab, nivolumab, obinutuzumab, pembrolizumab, pidilizumab (CT-011), ramucirumab, siltuximab, ado-trastuzumab emtansine, CEA-scan Fab fragment, OC125 monoclonal antibody, ab75705, B72.3, MPDL3280A, MSB001078C, MEDI4736, or an antigen binding fragment thereof.

[0041] In some of any such embodiments, the reference cell surface receptor is EGFR and the modified cell surface receptor is a modified EGFR. In some aspects, the modified EGFR contains an epitope specifically recognized by cetuximab or an antigen binding fragment thereof. In some instances, the modified EGFR lacks one or more of an EGFR Domain I, an EGFR Domain II, an EGFR Juxtamembrane Domain, and an EGFR Tyrosine Kinase Domain of the reference EGFR. In some of any such embodiments, the modified EGFR lacks all of the domains EGFR Domain I, an EGFR Domain II, an EGFR Juxtamembrane Domain, and an EGFR Tyrosine Kinase Domain of the reference EGFR of the reference EGFR. In some of any such embodiments, the modified EGFR comprises an extracellular domain that consists of or consists essentially of subdomain III and subdomain IV of the reference EGFR. In some of any such embodiments, the modified EGFR comprises the sequence of amino acids set forth in SEQ ID NOS: 44 or 46 or a sequence of amino acids that exhibits at least at or about 85%, 90%, or 95% sequence identity to SEQ ID NOS: 44 or 46.

[0042] In some of any such embodiments, the reference cell surface receptor is HER2 and the modified cell surface receptor is a modified HER2. In some aspects, the modified HER2 contains an epitope specifically recognized by trastuzumab or an antigen binding fragment thereof. In some of any such embodiments, the modified HER2 lacks one or more of an HER2 Domain I, an HER2 Domain II, an HER2 Domain III of the reference HER2. In some of any such embodiments, the modified HER2 lacks all of the domains HER2 Domain I, HER2 Domain II, and HER2 Domain III of the reference EGFR of the reference HER2. In some of any such embodiments, the modified HER2 comprises an extracellular domain that consists of or consists essentially of Domain IV of the reference HER2. In some of any such embodiments, the modified HER2 contains the sequence of amino acids set forth in SEQ ID NO: 92 or a sequence of amino acids that exhibits at least at or about 85%, 90%, or 95% sequence identity to SEQ ID NO: 92.

[0043] In some embodiments, the reference cell surface molecule is a reference PSMA and the modified cell surface molecule is a modified PSMA. In some embodiments, the reference PSMA is a wild-type PSMA, optionally wild-type human PSMA. In some embodiments, the reference PSMA is a human PSMA and/or comprises the sequence of amino acids set forth in SEQ ID NO: 94 or a sequence of amino acids encoded by the sequence of nucleotides set forth in SEQ ID NO: 96 or 97. In some embodiments, the modified PSMA comprises an extracellular portion and a transmembrane domain of the reference PSMA.

[0044] In some embodiments, the modified PSMA comprises one or more amino acid modifications in the intracellular region compared to the reference PSMA. In some embodiments, the one or more amino acid modification comprises one or more amino acid substitutions, deletions and/or insertions. In some embodiments, the modified PSMA exhibits altered cellular internalization compared to the reference PSMA.

[0045] In some embodiments, the modified PSMA comprises an amino acid substitution corresponding to W2G or does not comprise W2 or does not comprise any residue at position 2, with reference to positions in the sequence of amino acids set forth in SEQ ID NO:94. In some embodiments, the modified PSMA comprises a deletion or truncation of 11 N-terminal amino acids, compared to the reference PSMA.

[0046] In some embodiments, the modified PSMA comprises an epitope capable of being recognized by an antibody or antigen-binding fragment thereof. In some embodiments, the antibody or antigen-binding fragment thereof is selected from among J591, DFO-J591, CYT-356, J415, 3/A12, 3/F11, 3/E7, D2B, 107-1A4, YPSMA-1, YPSMA-2, 3E6, 2G7, 24.4E6, GCP-02, GCP-04, GCP-05, J533, E99, 1G9, 3C6, 4.40, 026, D7-Fc, D7-CH3, 4D4, A5, and antigen-binding fragments thereof.

[0047] In some of any such embodiments, the cell surface conjugate is not immunogenic and/or does not induce an immune response in a subject in which it is administered.

[0048] Also provided is a polynucleotide containing a nucleic acid sequence encoding the cell surface conjugate of any of the embodiments described herein. In some embodiments, the nucleic acid sequence further contains a signal sequence. In some isntances, the signal sequence encodes a signal peptide derived from GMCSFR alpha chain.

[0049] In some of any such embodiments, the nucleic acid sequence is a first nucleic acid sequence and the polynucleotide further contains a second nucleic acid sequence encoding a recombinant receptor. In some cases, the recombinant receptor is or contains a chimeric antigen receptor (CAR). In some of any such embodiments, the first and second nucleic acid sequences are separated by an internal ribosome entry site (IRES), or a nucleotide sequence encoding a self-cleaving peptide or a peptide that causes ribosome skipping, which optionally is T2A or P2A. In some embodiments, the first nucleic acid sequence is upstream of the second nucleic acid sequence. In some embodiments, the first nucleic acid sequence is downstream of the second nucleic acid sequence.

[0050] Provided is a vector containing the polynucleotide of of any of the embodiments desceibed herein. In some embodiments, the vector is a viral vector. In some of any such

embodiments, the vector is a retroviral vector. In some of any such embodiments, the vector is a lentiviral vector or a gammaretroviral vector.

[0051] Also provided is a method of producing an engineered cell including introducing the polynucleotide of any of the embodiments described above or the vector of any of the embodiments described above into a cell. Also provided is an engineered cell produced by the method described herein. In some embodiments, the engineered cell contains the polynucleotide of any of the embodiments described hrein or the vector of any of any of the embodiments described herein.

[0052] In some of any such embodiments, the engineered cell contains the cell surface conjugate of any of the embodiments described above. In some isntances, the engineered cell further contains a recombinant receptor. In some aspects, the recombinant receptor binds to a target antigen that is associated with a disease or disorder. In some cases, the disease or disorder is an infectious disease or disorder, an autoimmune disease, an inflammatory disease, or a tumor or a cancer.

[0053] In some of any such embodiments, the target antigen is a tumor antigen. In some embodiments, the target antigen is the target antigen is selected from the group consisting of αvβ6 integrin (avb6 integrin), B cell maturation antigen (BCMA), B7-H3, B7-H6, carbonic anhydrase 9 (CA9, also known as CAIX or G250), a cancer-testis antigen, cancer/testis antigen 1B (CTAG, also known as NY-ESO-1 and LAGE-2), carcinoembryonic antigen (CEA), a cyclin, cyclin A2, C-C Motif Chemokine Ligand 1 (CCL-1), CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD138, CD171, epidermal growth factor protein (EGFR), truncated epidermal growth factor protein (tEGFR), type III epidermal growth factor receptor mutation (EGFR vIII), epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), ephrinB2, ephrine receptor A2 (EPHa2), estrogen receptor, Fc receptor like 5 (FCRL5; also known as Fc receptor homolog 5 or FCRH5), fetal acetylcholine receptor (fetal AchR), a folate binding protein (FBP), folate receptor alpha, ganglioside GD2, O-acetylated GD2 (OGD2), ganglioside GD3, glycoprotein 100 (gp100), G Protein Coupled Receptor 5D (GPCR5D), Her2/neu (receptor tyrosine kinase erb-B2), Her3 (erb-B3), Her4 (erb-B4), erbB dimers, Human high molecular weight-melanoma-associated antigen (HMW-MAA), hepatitis B surface antigen, Human leukocyte antigen A1 (HLA-A1), Human leukocyte antigen A2 (HLA-A2), IL-22 receptor alpha(IL-22Ra), IL-13 receptor alpha 2 (IL-13Ra2), kinase insert domain receptor (kdr), kappa light chain, L1 cell adhesion molecule (L1-CAM), CE7 epitope of L1-CAM, Leucine Rich Repeat Containing 8 Family Member A (LRRC8A), Lewis Y,

Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, mesothelin, c-Met, murine cytomegalovirus (CMV), mucin 1 (MUC1), MUC16, natural killer group 2 member D (NKG2D) ligands, melan A (MART-1), neural cell adhesion molecule (NCAM), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME), progesterone receptor, a prostate specific antigen, prostate stem cell antigen (PSCA), prostate specific membrane antigen (PSMA), Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1), survivin, Trophoblast glycoprotein (TPBG also known as 5T4), tumor-associated glycoprotein 72 (TAG72), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor receptor 2 (VEGFR2), Wilms Tumor 1 (WT-1).

[0054] In some of any such embodiments, the target antigen is selected from the group consisting of ROR1, HER2, L1-CAM, CD19, CD20, CD22, mesothelin, CEA, hepatitis B surface antigen, anti-folate receptor, CD23, CD24, CD30, CD33, CD38, CD44, EGFR, EGP-2, EGP-4, EPHa2, ErbB2, ErbB3, ErbB4, FBP, fetal acethycholine e receptor, GD2, GD3, HMW-MAA, IL-22R-alpha, IL-13R-alpha2, kdr, kappa light chain, Lewis Y, L1-cell adhesion molecule, MAGE-A1, mesothelin, MUC1, MUC16, PSCA, NKG2D Ligands, NY-ESO-1, MART-1, gp100, oncofetal antigen, TAG72, VEGF-R2, carcinoembryonic antigen (CEA), prostate specific antigen, PSMA, estrogen receptor, progesterone receptor, ephrinB2, CD123, CS-1, c-Met, GD-2, MAGE A3, CE7, Wilms Tumor 1 (WT-1), and cyclin A1 (CCNA1).

[0055] In some of any such embodiments, the recombinant receptor is a functional non-TCR antigen receptor or a transgenic TCR. In some of any such embodiments, the recombinant receptor is a chimeric antigen receptor (CAR). In some of any such embodiments, the recombinant receptor comprises an extracellular portion comprising an antigen-binding domain. In some exmaples, the antigen-binding domain is or contains an antibody or an antibody fragment.

[0056] In some embodiments, the antibody fragment is a single chain fragment. In some embodiments, the fragment contains antibody variable regions joined by a flexible immunoglobulin linker. In some of any such embodiments, the fragment comprises an scFv. In some of any such embodiments, the recombinant receptor comprises an activating intracellular signaling domain.

[0057] In some embodiments of the engineered cell, the activating intracellular signaling domain is capable of inducing a primary activation signal in a T cell, is a T cell receptor (TCR) component, and/or contains an immunoreceptor tyrosine-based activation motif (ITAM). In

some of any such embodiments, the activating intracellular signaling domain is or contains an intracellular signaling domain of a CD3-zeta (CD3ζ) chain or a signaling portion thereof.

[0058] In some of any such embodiments, the engineered cell further contains a transmembrane domain linking the extracellular portion and the activating intracellular signaling domain.

[0059] In some of any such embodiments, the recombinant receptor contains a costimulatory signaling domain. In some instances, the costimulatory signaling domain contains an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof. In some of any such embodiments, the costimulatory signaling domain contains an intracellular signaling domain of CD28, 4-1BB or ICOS or a signaling portion thereof. In some of any such embodiments,the costimulatory signaling domain is between the transmembrane domain and the activating intracellular signaling domain.

[0060] In some of any such embodiments, the cell is an immune cell. In some cases, the cell is a lymphocyte. In some of any such embodiments, the cell is a T cell or an NK cell. In some examples, the cell is a T cell that is a CD8+ T cell or a CD4+ T cell.

[0061] Also provided is a composition containing the engineered cells of any of the embodiments described above. In some cases, the composition further contains a pharmaceutically acceptable excipient.

[0062] Also provided is a method of treatment including administering the engineered cells of any of the embodiments described above or the composition of any of the embodiments described above to a subject having a disease or disorder. In some embodiments, the disease or disorder is a cancer, a tumor, an autoimmune disease or disorder, or an infectious disease. In some of any such embodiments, the method further involves administering to the subject a binding molecule capable of recognizing the agent of the cell surface conjugate expressed on the engineered cell and detecting cells that express the cell surface conjugate. In some aspects, detection includes in vivo imaging.

[0063] Also provided is a method of identifying a cell expressing a cell surface conjugate, including contacting a composition containing cells that express or are likely to express a cell surface conjugate of any of the embodiments described herein with a binding molecule capable of recognizing the agent of the cell surface conjugate. In some aspects, the method is performed in vitro, ex vivo or in vivo. In some embodiments, n the cell expressing the cell surface molecule is detected via in vivo imaging. In some of any such embodiments, the in vivo imaging method is selected from among magnetic resonance imaging (MRI), single-photon

emission computed tomography (SPECT), computed tomography (CT), computed axial tomography (CAT), electron beam computed tomography (EBCT), high resolution computed tomography(HRCT), hypocycloidal tomography, positron emission tomography (PET), scintigraphy, gamma camera, a β+ detector, a γ detector, fluorescence imaging, low-light imaging, X- rays, and bioluminescence imaging.

[0064] In some of any such embodiments, the binding molecule is conjugated to a moiety that provides a signal or induces a signal that is detectable in vivo. In some examples, the moiety is a radioisotope, bioluminescent compound, chemiluminescent compound, fluorescent compound, metal chelate or enzyme.

[0065] Also provided is a method of identifying cells transduced with a cell surface conjugate, including contacting a composition transduced with a polynucleotide of any of the embodiments described herein or the vector of any of the embodiments described herein encoding the cell surface conjugate with a binding molecule capable of recognizing the agent of the cell surface conjugate; and identifying cells bound to the binding molecule. Also provided is a method of identifying cells transduced with a cell surface conjugate including introducing a polynucleotide of any of the embodiments described herein or the vector of any of the embodiments described herein encoding the cell surface conjugate into a cell; contacting a composition comprising the cell with a binding molecule capable of recognizing the agent of the cell surface conjugate; and identifying cells of the composition bound to the binding molecule.

[0066] Also provided is a method of selecting cells transduced with a cell surface conjugate including contacting a composition transduced with a polynucleotide of any of the embodiments described herein or the vector of any of the embodiments described herein encoding the cell surface conjugate with a binding molecule capable of recognizing the agent of the cell surface conjugate; and isolating cells bound to the binding molecule. Further provided is a method of selecting cells transduced with a cell surface conjugate including introducing a polynucleotide of any of the embodiments described herein or the vector of any of the embodiments described herein encoding the cell surface conjugate into a cell; contacting a composition comprising the cell with a binding molecule capable of recognizing the agent of the cell surface conjugate; and isolating cells of the composition bound to the binding molecule.

[0067] In some of any such embodiments, the binding molecule is conjugated to a detectable moiety or is capable of producing a detectable signal. In some instances, the detectable moiety contains a fluorescent protein.

[0068] In some of any such embodiments, the agent is is a streptavidin binding peptide. In some cases, the streptavidin-binding peptide is or comprises the sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8) or Trp-Arg-His-Pro-Gln-Phe-Gly-Gly (SEQ ID NO:7). In some aspects, the streptavidin binding peptide is or contains the sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_3$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 17), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 18) and Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$Gly-Gly-Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 19).

[0069] In some embodiments, the binding molecule is a reagent capable of reversibly binding to the agent. In some aspects, the reagent is a streptavidin analog or mutein. In some instances, the streptavidin analog or mutein contains the amino acid sequence Val^{44}-Thr^{45}-Ala^{46}-Arg^{47} or Ile^{44}-Gly^{45}-Ala^{46}-Arg^{47} at sequence positions corresponding to positions 44 to 47 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO:1.

[0070] In some of any such embodiments, the streptavidin analog or mutein contains a) the sequence of amino acids set forth in any of SEQ ID NOS: 3-6, 27 and 28; b) a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 3-6, 27 and 28 and contains the amino acid sequence corresponding to Val^{44}-Thr^{45}-Ala^{46}-Arg^{47} or Ile^{44}-Gly^{45}-Ala^{46}-Arg^{47} and that reversibly binds to the agent; or c) a functional fragment of a) or b) that reversibly binds to the agent. In some embodiments, the streptavidin analog or mutein further contains an amino acid replacement or replacements at a position corresponding to 117, 120 and/or 121 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO:1.

[0071] In some of any such embodiments, the amino acid replacement or replacements are selected from among Glu^{117}, Asp^{117}, Arg^{117}, Ser^{120}, Ala^{120}, Gly^{120}, Trp^{121}, Tyr^{121} or Phe^{121}; or the amino acid replacement or replacements are selected from one or more of Glu^{117}, Gly^{120} or Tyr^{121}; or the amino acid replacements are selected from Glu^{117}, Gly^{120} or Tyr^{121}.

[0072] In some of any such embodiments, the streptavidin analog or mutein contains a) the sequence of amino acids set forth in SEQ ID NO: 27 or 28; b) a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NOS:27 or 28 and contains the amino acid sequence corresponding to Val^{44}, Thr^{45}, Ala^{46}, Arg^{47}, Glu^{117}, Gly^{120} and Tyr^{121} and

reversibly binds to the agent; or c) a functional fragment of a) or b) that reversibly binds to the agent.

[0073] In some of any such embodiments, the method further includes disrupting the reversible binding of the binding molecule to the agent. In some aspects, the disruption includes contacting the cells with a composition containing a substance capable of reversing the bond between the binding molecule and agent. In some cases, the substance is a free binding partner and/or is a competition agent. In some embodiments, the substance is or contains biotin, a biotin analog or a biologically active fragment thereof.

[0074] In some of any such embodiments, the binding molecule is an antibody or antigen binding fragment that specifically binds the agent. In some examples, the binding molecule is an anti-StrepTag antibody.

[0075] Also provided herein is a molecule containing a streptavidin or a streptavidin analog or mutein conjugated to a cytotoxic agent. In some aspects, the molecule contains a streptavidin analog or mutein. In some embodiments, the streptavidin or streptavidin analog binds to a streptavidin binding peptide.

[0076] In some examples, the streptavidin-binding peptide is or contains the sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8) or Trp-Arg-His-Pro-Gln-Phe-Gly-Gly (SEQ ID NO:7). In some of any such embodiments, the streptavidin binding peptide is or contains the sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_3$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 17), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 18) and Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$Gly-Gly-Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 19).

[0077] In some of any such embodiments, the streptavidin or streptavidin mutein exhibits a binding affinity for the streptavidin binding peptide with an equilibrium dissociation constant (K_D) of from or from about 10^{-4} to 10^{-10} M. In some of any such embodiments, the streptavidin analog or mutein contains the amino acid sequence Val^{44}-Thr^{45}-Ala^{46}-Arg^{47} or Ile^{44}-Gly^{45}-Ala^{46}-Arg^{47} at sequence positions corresponding to positions 44 to 47 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO:1.

[0078] In some of any such embodiments,the streptavidin analog or mutein contains a) the sequence of amino acids set forth in any of SEQ ID NOS: 3-6, 27 and 28; b) a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 3-6, 27 and 28 and contains the amino acid sequence corresponding to Val^{44}-Thr^{45}-Ala^{46}-Arg^{47} or

Ile^{44}-Gly^{45}-Ala^{46}-Arg^{47} and that reversibly binds to the agent; or c) a functional fragment of a) or b) that binds to the streptavidin binding peptide. In some embodiments, the streptavidin analog or mutein further contains an amino acid replacement or replacements at a position corresponding to 117, 120 and/or 121 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO:1.

[0079] In some of any such embodiments, the amino acid replacement or replacements are selected from among Glu^{117}, Asp^{117}, Arg^{117}, Ser^{120}, Ala^{120}, Gly^{120}, Trp^{121}, Tyr^{121} or Phe^{121}; or the amino acid replacement or replacements are selected from one or more of Glu^{117}, Gly^{120} or Tyr^{121}; or the amino acid replacements are selected from Glu^{117}, Gly^{120} or Tyr^{121}.

[0080] In some of any such embodiments, the streptavidin analog or mutein contains a) the sequence of amino acids set forth in SEQ ID NO: 27 or 28; b) a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NOS:27 or 28 and contains the amino acid sequence corresponding to Val^{44}, Thr^{45}, Ala^{46}, Arg^{47}, Glu^{117}, Gly^{120} and Tyr^{121} and reversibly binds to the agent; or c) a functional fragment of a) or b) that reversibly binds to the streptavidin binding peptide.

[0081] In some of any such embodiments, the cytotoxic agent is a toxin. In some examples, the toxin is a peptide toxin, ricin A chain toxin, Abrin A chain, Diptheria Toxin (DT) A chain, Pseudomonas exotoxin, Shiga Toxin A chain, Gelonin, Momordin, Pokeweed Antiviral Protein, Saporin, Trichosanthin, or Barley Toxin. In some instances, the cell toxin is a phototoxin.

[0082] Also provided is a method of killing cells including administering the molecule of any of the embodiments described herein to a subject previously administered the cells of any of the embodiments described herein or the composition of any of the embodiments described above. In some aspects, the molecule is administered at a time at which the subject is exhibiting a toxic outcome associated with the administered cells or at a time at which the subject is exhibiting a detectable and/or cell-mediated immune response to the administered cells. In some instances, the toxic outcome is associated with neurotoxicity or cytokine release syndrome (CRS).

Brief Description of the Drawings

[0083] **FIG. 1** depicts a schematic of a nucleic acid molecule encoding a recombinant receptor (e.g., CAR) and an exemplary cell surface conjugate as provided (e.g. a cell surface

molecule (CSM), linked to a Strep-tag (ST) (CSM-ST) separated by a 2A ribosomal skip element for expressing two proteins in a cell from the same construct. Also shown are exemplary methods of targeting the agent of the expressed cell surface conjugate for selection of gene-modified cells independent of the expressed recombinant receptor by contacting such cell with (A) a non-antibody reagent (e.g. Strep-Tactin) bound to a solid surface or (B) with an anti-Strep-tag antibody specific for the agent of the cell surface conjugate.

[0084] **FIG. 2** depicts a schematic of a nucleic acid molecule encoding a recombinant receptor (e.g. Tg receptor) and an exemplary cell surface conjugate as provided (e.g. truncated epidermal growth factor receptor (tEGFR) linked to a Strep-tag (ST) (tEGFR-ST) separated by a T2A ribosome switch for expressing two proteins in a cell from the same construct. Also shown are exemplary methods of targeting the agent of the expressed cell surface conjugate for selection of gene-modified cells independent of the expressed recombinant receptor by contacting such cell with (A) a non-antibody reagent (e.g. Strep-Tactin) bound to a solid surface or (B) with an anti-Strep-tag antibody specific for the agent of the cell surface conjugate.

Detailed Description

I. CELL SURFACE CONJUGATES FOR PROCESSING OF GENE MODIFIED CELLS

[0085] Provided herein are cell surface conjugates containing a cell surface molecule, such as a cell surface protein, and at least one agent, such as an affinity tag, e.g. a peptide agent. In some embodiments, the provided cell surface conjugates are engineered or expressed in cells to permit one or more of specific targeting of the cell, isolation or selection of the cell or detection of the cell, such as via a binding molecule specific for the agent of the conjugate, which binding molecule does not bind or recognize the cell surface molecule of the conjugate. In some embodiments, the cell surface molecule is not a recombinant receptor, such as is not an antigen receptor, for example, is not a chimeric antigen receptor (CAR). In some embodiments, the provided cell surface conjugates are co-engineered into cells expressing a recombinant receptor (e.g. a CAR), whereby the cell surface conjugate can be exploited for processing of cells expressing the recombinant receptor, such as in connection with methods for detection, selection, isolation or suicide-based deletion of engineered cells.

[0086] Various strategies are available for producing and administering engineered cells for adoptive therapy. The cells generally are engineered by introducing one or more genetically

engineered nucleic acid or product thereof. Among such products are genetically engineered antigen receptors, including engineered T cell receptors (TCRs) and functional non-TCR antigen receptors, such as chimeric antigen receptors (CARs), including activating, stimulatory, and costimulatory CARs, and combinations thereof. For example, strategies are available for engineering cells, such as T cells, expressing chimeric receptors, such as CARs, and administering compositions containing such engineered cells to subjects. Throughout the process of producing engineered cells, it is beneficial to be able to identify, detect, locate, and/or select transduced cells. After administration of the engineered cells for adoptive therapy, there is also a need to monitor the transduced cells and to provide a mechanism to deplete or reduce the number of transduced cells in a subject.

[0087] Known methods for selecting and isolating cells include use of CAR-specific antibodies to bind cells of interest. For example, the use of a biotinylated goat anti-mouse IgG (Fab')2 (Jackson ImmunoReseach) for detection of CAR-modified T cells is known in the art (Brentjens et al., Sci. Transl. Med. 2013 Mar; 5(177): 177ra38). As the sensitivity of this polyclonal antibody is low, in the setting of low lymphocyte numbers in patient samples, detection of CAR-modified T cells was accomplished only after non-specific expansion of T cells using Dynabeads. This prevents a direct assessment of circulating CAR- modified T cell in vivo after infusion. The use of Protein L for detection of CAR-modified T cells by flow cytometry has also been described (Zheng et al., J. Transl. Med. 2012 Feb; 10:29). This reagent has restricted use in terms of detection and sensitivity in a multi-parameter flow cytometry assay. Its use in other assay formats has not been shown. Another approach utilizes Strep-tag II sequences introduced directly into specific sites in the CAR, whereby binding reagents for Strep-Tag are used to directly assess the CAR (Liu et al. (2016) Nature Biotechnology, 34:430; international patent application Pub. No. WO2015095895). Furthermore, monoclonal antibodies that specifically bind to a CAR polypeptide are also known (see international patent application Pub. No. WO2014190273). While useful in some situations, reagents that bind the CAR directly or indirectly may risk activating the CAR to induce cell signaling and activation of the engineered cells, which is not always desired in connection with isolating or selecting cells during ex vivo production and manufacturing.

[0088] In some aspects, extrinsic marker genes are utilized in connection with engineered cell therapies to permit detection or selection of cells and, in some cases, also to promote cell suicide by ADCC. Exemplary of such a marker gene is truncated epidermal growth factor receptor (EGFRt), which can be co-expressed with a transgene of interest (a CAR or TCR) in

transduced cells (see e.g. U.S. Patent No. 8,802,374). EGFRt contains the epitope recognized by the antibody cetuximab (Erbitux®). For this reason, Erbitux® can be used to identify or select cells that have been engineered with the EGFRt construct, including in cells also co-engineered with another recombinant receptor, such as a chimeric antigen receptor (CAR). Additionally, EGFRt is commonly used as a suicide mechanism in connection with cell therapies. In some aspects, when EGFRt is co-expressed in cells with a transgene of interest (e.g. CAR or TCR), it can be targeted by the cetuximab monoclonal antibody to reduce or deplete the transferred gene-modified cells via ADCC (see U.S. Patent No. 8,802,374 and Liu et al., Nature Biotech. 2016 April; 34(4): 430–434). Importantly, the suicide killing approach using tEGFR requires availability of the antibody epitope.

[0089] The above approaches known in the art can have potential problems that interfere with the processing, production, and/or function of the cells. There is a need for cell surface markers that aid the production, monitoring, and post-administration stages involving transduced cell products. For example, methods for efficient selection and isolation of cells positive for the transgene and for monitoring transgene-expressing cells *in vivo* and *ex vivo*, are desired. The provided cell surface conjugates and methods address such needs and/or address one or more problems associated with existing methods and reagents. In some embodiments, the provided conjugates provide one or more advantages compared to existing markers or selections strategies used in connection with engineered cells.

[0090] In some embodiments, the provided conjugates are conjugates containing a cell surface molecule that lacks an intracellular signaling domain and/or is not capable of mediating intracellular signaling having linked or conjugated thereto an agent, such as an affinity tag (e.g. a peptide). In some embodiments, the agent is one that is recognized by a binding molecule. In some embodiments, the agent of the provided cell surface conjugate is a streptavidin binding peptide (e.g. Strep-tag®) for which well-known binding molecules are available. In some aspects, the cell surface conjugate is a fusion protein comprising a cell surface molecule or a modified form thereof and an agent e.g., an affinity tag.

[0091] In some embodiments, the cell surface molecule is a modified cell surface molecule that is altered compared to a reference cell surface molecule. In some embodiments, the reference cell surface molecule is a native mammalian cell surface molecule. In some cases, the cell surface molecule is modified, e.g. truncated or contains one or more amino acid substitutions, deletions and/or insertions, compared to a reference cell surface molecule. In some embodiments, the cell surface molecule contains a truncation, e.g., a truncation to remove

all or a portion of an intracellular signaling domain and/or other intracellular domains or one or more extracellular domains. In some embodiments, the cell surface molecule contains or retains at least one epitope recognized by an antibody or antigen-binding fragment, which, in some cases, additionally permits targeting of the cell surface molecule of the conjugate independent of the agent, for example, to mediate antibody dependent cell cytotoxicity (ADCC) for selective deletion or suicide of engineered cells as a safety switch mechanism. In some embodiments, the cell surface molecule of the conjugate contains a modified epidermal growth factor receptor (EGFR), such as a truncated EGFR (see e.g. U.S. Patent No. 8,802,374). In some embodiments, the cell surface molecule of the conjugate contains a modified prostate-specific membrane antigen (PSMA), such as a modified PSMA, e.g., a truncated PSMA (tPSMA).

[0092] In some embodiments, the agent is one that is recognized by a binding molecule in which binding thereto is reversible and/or in which binding to a binding molecule is able to be competed or disrupted in the presence of a competition substance. In some embodiments, the binding molecule is or comprises a reagent that exhibits a higher binding affinity for the competition substance than for the agent. In some embodiments, the binding molecule is a reagent that is or comprises a streptavidin or a streptavidin analog or mutein and the agent is a streptavidin binding peptide, e.g. Strep-tag®.

[0093] In some embodiments, certain streptavidin mutein molecules (e.g. Strep-Tactin) are able to reversibly bind to certain streptavidin binding peptides (e.g. Strep-tag®) in the presence of a biotin or a biotin analog or mimic that exhibits a higher binding affinity for the streptavidin mutein than the streptavidin mutein exhibits for the streptavidin binding peptide. Thus, in certain aspects, binding between the agent (e.g. streptavidin binding peptide, such as Strep-tag®) of the cell surface conjugate and the binding molecule (e.g. streptavidin mutein, such as Strep-Tactin) can be disrupted by the addition of the competition substance (e.g. biotin or biotin mimic). In some embodiments, such binding reagents, for example streptavidin mutein binding reagents, do not induce suicide-based killing by ADCC. Furthermore, the streptavidin muteins can be formatted as a soluble reagent or associated in a solid phase, such as in a stationary phase, such as is present in a column, e.g. column chromatography or planar chromatography, to facilitate cell selection or isolation.

[0094] In some embodiments, the cell surface conjugate contains (1) a modified cell surface molecule, such as a modified cell surface receptor that lacks an intracellular signaling domain and/or one or more extracellular domain for binding to a cognate ligand and (2) at least one streptavidin binding peptide agent (Strep-tag®). Exemplary cell surface molecules are described

and include, for example, a modified epidermal growth factor receptor . In some embodiments, the cell surface conjugate can be detected via a binding molecule reagent that is or comprises a streptavidin mutein. In alternative examples, specific detection of a streptavidin or binding peptide (e.g. Strep-tag®) can be achieved by a high affinity monoclonal anti-Streptag antibody.

[0095] In some embodiments, the agent is fused to the extracellular (N-terminal or C-terminal) part of the cell surface molecule, such as the modified cell surface molecule. In some embodiments, linkage of the agent only at the exposed N-terminus or C-terminus of the cell surface molecule exposes the agent so that it is easily detectable and/or its detection is not sterically blocked. In some embodiments, the cell surface molecule contains an epitope that is able to be recognized or bound by a binding molecule, such as an antibody or antigen binding fragment or a ligand, for example, to induce or carry our suicide deletion in connection with safety switch methods. Thus, the linkage of the agent at the N-terminal sequence or the C-terminal sequences of the cell surface molecule can retain access of the epitope of the cell surface molecule for recognition by a specific antibody or antigen binding fragment. In some embodiments, the cell surface conjugates retain the safety switch function of the cell surface molecule, e.g. mediated via cetuximab binding to EGFRt on engineered cells.

[0096] In some embodiments, the provided cell surface conjugates include those in which selection or identification of cells can be uncoupled from activation or suicide of cells. In some cases, selection processes that use antibodies against specific antigen receptors, e.g. CARs, may lead to accidental activation of the receptor, e.g. CAR, and inadvertent signaling through the receptor, e.g. CAR. This problem is avoided by the provided cell surface conjugates, which are expressed on the cell surface independently from the antigen receptor. In some embodiments, the provided cell surface conjugates allow the function of the recombinant receptor (e.g. CAR) to remain separate and unaffected by activity involving the cell surface conjugate. In some cases, the detecting of the conjugate as confirmation of transduction will not lead to accidental activation of the CAR and inadvertent signaling through the CAR. Therefore, in some embodiments, the likelihood of off target effects can be reduced.

[0097] Likewise, since the cell surface molecule of the conjugate is not an antigen receptor, e.g. CAR, and is co-expressed on engineered cells independently from the antigen receptor, recognition of engineered cells can be based on expression of the cell surface conjugate and not on a signaling molecule. In some aspects, the provided cell surface conjugates have the advantage that detection or selection of engineered cells via the agent of the provided conjugates

is independent of expression of the antigen receptor, e.g. CAR, and/or how much antigen receptor, e.g. CAR, is expressed.

[0098] The provided cell surface conjugates also provide a generic or universal marker for engineered cells, which does not need to be reconfigured or developed for each cell therapy. Thus, unlike certain prior art methods, the provided conjugates and methods avoid the need to develop individual marker reagents for each transgene and/or avoid development of individual selection reagents specifically targeting certain domains of the receptor (e.g. CAR or TCR). Thus, the provided methods are less time consuming than prior art methods and conserve reagent resources.

[0099] In available prior art approaches, the selection process can, in some cases, result in the loss or damage of cells. In some aspects, methods of selecting cells and selectively targeting cells for suicide using the same binding molecule (e.g. cetuximab in the case of EGFRt) is not ideal. In some cases, methods of selecting or isolating transduced cells via a protein marker (e.g. EGFRt) using a specific antibody (e.g. cetuximab) can result in a loss of cells due to the ADCC suicide-based mechanisms. During ex vivo production and further processing of engineered cells, however, suicide and loss of cells is not desired. Since the provided cell surface conjugates can be recognized by non-antibody reagents (e.g. streptavidin mutein reagents) such problems associated with loss of cells during selection processes in connection with cell manufacturing can be avoided. Further, unlike reagents that bind the recombinant receptor, e.g. CAR, directly or indirectly, that may risk stimulating the CAR to induce cell signaling and stimulation in the engineered cells, the provided embodiments allow the cells to be engineered, selected, isolated, produced, processed or manufactured without stimulating signals through the CAR.

[0100] Further, in some cases, prior art methods employing antibody molecules for cell selection are carried out in a manner in which the binding of the antibody to specific targets is not reversible or is not efficiently or rapidly reversible. In some cases, high affinity antibodies, and in particular antibodies with a K_D of 10^{-9} M or lower, recognizing a cell surface marker or directly recognizing an antigen receptor (e.g. a CAR) result in a slower detachment of the antibody from the cell. In some cases, when such antibodies are used to select antigen receptor (e.g. CAR) engineered cells in connection with production and manufacturing of cells, there is a risk that residual antibody may be retained in a final formulation or manufacturing product if the antibodies remain attached to cells. Administration of such products to subjects may lead to undesirable effects in the subject. Thus, in some aspects, the cell surface conjugates provided

herein contain an agent (e.g. streptavidin binding peptide, such as StrepTag) that exhibits a lower affinity interaction for certain binding molecule reagents (e.g. streptavidin mutein, such as Strep-Tactin) in order to avoid this problem and to decrease the risk that the binding molecule reagent is retained in the drug product. In addition, methods to completely dissociate or disrupt binding of an agent from its binding molecule are desired in connection with manufacturing cell therapies.

[0101] In some embodiments, the provided cell surface conjugates retain the safety switch functionality of the cell surface molecule (e.g. EGFRt or PSMA) of the conjugate by virtue of retaining or preserving an epitope recognized by a specific antibody. For example, the provided cell surface conjugates can be specifically bound, such as non-competitively bound, by a binding molecule (e.g. antibody or an antigen-binding fragment thereof) specific to the cell surface molecule of the conjugate and a binding molecule specific to the agent of the conjugate. In some aspects, targeting the cell surface molecule through ADCC, which depends on the availability of the antibody epitope of the cell surface molecule, provides for increased functionality of the cell surface conjugate in a variety of applications.

[0102] As an alternative to ADCC mediated activity, depletion or reduction of transduced cell products in a subject can be facilitated by targeting the agent of the cell surface conjugate. In some embodiments, the provided agents can be further modified to exhibit safety switch properties, such as by linkage or conjugation of a binding molecule specific to the agent to a toxin or other cytotoxic agent (hereinafter also called "suicide agent"). In some aspects, the provided suicide agents do not depend on ADCC mechanisms of cell suicide, which in some cases can be slow due to the pharmacokinetics of antibodies. In some embodiment, the binding molecule is not an antibody or antigen-binding fragment. In some embodiments, the binding molecule of the suicide agent is a streptavidin or streptavidin mutein, such as any as described, which, in some cases, bind to an agent that is a streptavidin binding agent. In some embodiments, suicide by delivering a toxin conjugated to the reagent (Strep-Tactin®) to the agent (Strep-tag®) can be used. In some aspects, killing mediated by a toxin-conjugated reagent allows faster delivery compared to using an antibody to activate ADCC. Such suicide agents, e.g. streptavidin mutein-toxins, can exhibit a more rapid or quicker specific cell killing effect on the engineered cells compared to antibody-based suicide mechanisms. In some embodiments, the cell killing is initiated about more than or more than or about 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold or more quickly than cell killing initiated with antibody-based mechanisms.

[0103] Also provided are methods and uses of the cell surface conjugate expressed by cells, such as in connection with processing, manufacture or post-administrative monitoring in connection with adoptive therapy. In some embodiments, provided are methods for *in vivo* or *ex vivo* detection of transduced cells expressing the cell surface conjugate. In some embodiments, methods to select, such as isolate or recover, cells that have been successfully transduced to express the cell surface conjugate are provided. In some embodiments, also provided are methods for suicide killing of cells expressing the cell surface conjugate.

[0104] In some embodiments, the provided methods involve co-engineering cell with the cell surface conjugate and a desired recombinant receptor transgene (e.g. CAR or TCR). Also provided are vectors for co-engineering cells with the cell surface conjugate and the recombinant receptor. In some embodiments, provided is a backbone vector construct containing the coding sequence for the cell surface conjugate. In some cases, improved efficiency is achieved by using such a backbone construct in approaches to genetically engineering cells to independently express the cell surface conjugate and the recombinant receptor. In some embodiments, the backbone expression vector containing the cell surface conjugate can be used to insert transgene sequences for unique recombinant receptors (CAR, TCR, etc.) specifically targeting an antigen. In some embodiments, the resulting vector construct includes nucleic acid sequences encoding the recombinant receptor, a sequence encoding a 2A element, e.g., a T2A ribosomal skip element and the sequence encoding the cell surface conjugate, e.g., downstream of the sequence encoding the CAR. Thus, in some aspects, the construct encoding the recombinant receptor (e.g. CAR) and conjugate are separated by a 2A element, e.g., a T2A ribosome switch for expressing two proteins from the same construct. In some embodiments, such provided constructs can be modified to easily encode any recombinant receptor (e.g. CAR).

[0105] Also provided are methods for using cells expressing the cell surface conjugate. Provided are methods for cell isolation and genetic engineering. Provided are nucleic acids, such as constructs, *e.g.*, viral vectors encoding the cell surface conjugate and/or encoding nucleic acids and/or proteins of the cell surface conjugate, and methods for introducing such nucleic acids into the cells, such as by transduction. Also provided are compositions containing the engineered cells, and methods, kits, and devices for administering and monitoring the cells and compositions to subjects, such as for adoptive cell therapy.

II. CELL SURFACE CONJUGATE

[0106] Provided herein is a cell surface conjugate containing a cell surface molecule and at least one agent (e.g. peptide), such as an affinity tag, which agent is able to be specifically recognized by a binding molecule. In some embodiments, the provided conjugates are or include fusion proteins. In some embodiments, the cell surface molecule of the conjugate lacks a functional intracellular signaling domain and/or is not capable of mediating intracellular signaling. In some aspects, the cell surface molecule is a modified cell surface molecule that is altered compared to a reference cell surface molecule, for example, is a truncated cell surface receptor that lacks all or a portion of the intracellular signaling domain of the reference cell surface molecule, and/or contains one or more amino acid substitutions, deletions and/or insertions. In some embodiments, the cell surface molecule of the conjugate exhibits altered cellular internalization, enzymatic activity and/or ligand binding. In some embodiments, the agent (e.g. peptide), such as an affinity tag, is linked to the N-terminal or C-terminal part of the cell surface molecule. In some embodiments, the agent is a streptavidin binding peptide (e.g. Strep-tag®) and the cell surface molecule is a modified EGFR, such as a truncated EGFR. In some embodiments, the agent is a streptavidin binding peptide (e.g. Strep-tag®) and the cell surface molecule is a modified PSMA, such as a truncated PSMA.

[0107] In some embodiments, the provided conjugates contain the following components: cell surface molecule (CSM), linker (L) and agent (A), which are represented by the formula: CSM-$(L)_q$- $(A)_m$, where q is 0 or more and m is at least 1 or is 1. In some embodiments, the variables q and m are selected such that the resulting cell surface conjugate is expressed on cells and can be detected by a binding molecule via the agent, and optionally, recognized by an antibody or antigen-binding fragment specific to an epitope of the cell surface molecule. In some embodiments, m is 1 to 5, such as 1 to 4 or 1 to 3, for example, at least or at least about or about or 1, 2, 3, 4 or 5. In some embodiments, q is 0 to 5, and can depend on the number of linked agents. In some embodiments, several linkers can be joined in order.

[0108] In some aspects, the at least one agent is linked directly to the cell surface molecule. In some aspects, the at least one agent is linked or joined indirectly to the cell surface molecule via at least one linker. In some embodiments, the agent (e.g. a peptide), such as an affinity tag, is linked via its N-terminus or its C-terminus to the cell surface molecule. In some embodiments, the agent is linked at the membrane distal extracellular portion of the cell surface molecule. In some embodiments, the agent is linked at the N-terminus of the cell surface

molecule. In some embodiments, the agent is linked at the C-terminus of the cell surface molecule. In some embodiments, the agent is a peptide tag of less than 50 amino acids in length fused, directly or indirectly via a linker, to the extracellular (N-terminal) or (C-terminal) part of the cell surface molecule. In some embodiments, the agent is linked to an extracellular portion or domain of the cell surface molecule. In some embodiments, q is 0 and m is 1 and the agent is linked directly to the N-terminus of the cell surface molecule. In some embodiments, the provided conjugates are or include fusion proteins, such as a fusion protein containing components including one or more of cell surface molecule(s), linker(s) or agent(s).

[0109] In some aspects, the linker can be a peptide, a polypeptide or a chemical linker, which can be cleavable or non-cleavable. In some aspects, the linker is a peptide, such as a peptide containing a short sequence of amino acids to join to polypeptide sequences (or nucleic acid encoding such an amino acid sequence). In some embodiments, the linker is one that relieves or decreases steric hindrance that may be caused by proximity of the agent to the cell surface molecule and/or to increase or alter one or more properties of the conjugate, such as expression, specificity or immunogenicity. In some embodiments, the linkage or conjugation can be facilitated by recombinant methods. In some embodiments, the linker is a peptide or a polypeptide and the provided conjugates are fusion proteins.

[0110] A fusion protein can include a cell surface molecule that is a cell surface protein, such as any as described, that is linked directly or indirectly to a peptide or polypeptide agent, e.g. affinity tag, such as a streptavidin-binding peptide. A nucleic acid sequence encoding a fusion protein can contain a coding sequence for the cell surface protein and the at least one peptide or polypeptide agent such that the nucleic acid sequence contains a coding sequence for two or more proteins, in some cases 2, 3, 4, 5 or more proteins. In some embodiments, each of the coding sequences are in the same reading frame such that when the fusion protein is transcribed and translated in a host cell, the protein is produced containing the cell surface protein and the at least one peptide or polypeptide agent, e.g. streptavidin-binding peptide. In some aspects, each of the two or more proteins can be adjavent to another protein in the construct or separated by a linker polypeptide, such as a peptide linker, that contains 1, 2, 3 or more, but typically fewer than 20, 15, 10, 9, 8, 7 or 6 amino acids.

[0111] Exemplary peptide linkers include $(Gly\text{-}Ser)_n$ amino acid sequence, which, in some cases, can include some Glu or Lys residues dispersed throughout to increase solubility. The linker length may be tailored to be longer or shorter to ensure access of a binding molecule for the agent and to ensure access of an antibody or antigen-binding fragment (or other binding

molecule) for the cell surface molecule of the conjugate. In some embodiments, the linker is any set forth as GGGSGGGS (SEQ ID NO:59); GGGGS (SEQ ID NO:60); GGGS (SEQ ID NO:61); GGGGSGGGGSGGGGS (SEQ ID NO:62); GSTSGSGKPGSGEGSTKG (SEQ ID NO:55); GGGGSGGGGS (SEQ ID NO:56). In some embodiments, the linker is a cleavable linker. In some embodiments, the cleavable linker comprises a Phe-Leu linker, a Gly-Phe-Leu-Gly linker (SEQ ID NO:99), a Pro-Leu-Gly-Leu-Trp-Ala linker (set forth in SEQ ID NO:98), a Val-Cit linker or a Phe-Lys linker (*see, e.g.*, U.S. patent 6,214,345).

[0112] In some embodiments, the cell surface conjugate is non-immunogenic. In some embodiments, the cell surface conjugate does not comprise an immunogenic epitope and/or is not recognized by the immune response or is not able to induce, elicit or initiate a detectable immune response in an animal, e.g. humoral or cell-mediated immune. Cell-mediated immune responses include, for example, T cell responses, such as T cell proliferation, lymphokine secretion, cytotoxic responses, local inflammatory reactions and/or recruitment of additional immune cells. Humoral responses include, for example, activation of B cells leading to production of antibodies against an immunogenic epitope. The ability of cells expressing a cell surface conjugate to induce or elicit an immune response, such as a humoral or cell-mediated immune response, can be assessed following administration of such cells to a subject. In some embodiments, the presence of antibodies that specifically bind to and/or neutralize binding epitopes of the cell surface conjugate can be identified by methods such as ELISpot, intracellular cytokine staining, ELISAs (e.g. for cytokines), or cell-based antibody detection methods, for example, by flow cytometry, on serum from the subject. In some embodiments, a cell-mediated immune response to the cell surface conjugate can be assessed using a cytotoxic T-lymphocyte (CTL) assay for detection of CD8+ T cells that specifically bind to and induce cytotoxicity and/or a mixed lymphocyte reaction, using cells, e.g., irradiated cells, expressing the cell surface conjugate, as stimulator cells.

[0113] In some embodiments, the polynucleotide encoding the cell surface conjugate also contains a signal sequence encoding a signal peptide, such as for targeting the expressed protein to the secretory pathway for insertion of the conjugate into the cell membrane. In some aspects, the signal peptide is about 5-30 amino acids in length and is present at the N-terminus of the encoded conjugate. In some embodiments, the polynucleotide encodes a conjugate containing in order N- to C-terminus: signal peptide, agent (e.g. affinity tag, such as a streptavidin binding peptide) and cell surface molecule (e.g. modified cell surface molecule, such as EGFRt). In some embodiments, the signal peptide is the native signal peptide of the reference cell surface

molecule (e.g. native signal peptide contained in a sequence set forth in any of SEQ ID NOS: 64-69). In some embodiments, the signal peptide is a heterologous or non-native signal peptide, such as the GMCSFR alpha chain signal peptide set forth in SEQ ID NO: 48 which, in some cases, is encoded by nucleotides set forth in SEQ ID NO:47. In some embodiments, the polynucleotide encodes a conjugate containing in order N- to C-terminus: cell surface molecule (e.g. PSMA) or a modified cell surface molecule (e.g., tPSMA) and an agent (e.g. affinity tag, such as a streptavidin binding peptide).

A. Cell Surface Molecule, e.g. Modified Cell Surface Molecule

[0114] In some embodiments, the cell surface molecule of the conjugate contains at least one extracellular domain and a transmembrane domain. In some embodiments, the cell surface molecule is capable of being expressed on the surface of the cell. In some embodiments, the cell surface molecule is a cell surface receptor, ligand, glycoprotein, cell adhesion molecule, antigen, integrin, or cluster of differentiation (CD) or is a modified form thereof. In some embodiments, the cell surface molecule is not a chimeric antigen receptor. In some embodiments, the cell surface molecule is a modified cell surface molecule that is altered compared to a reference cell surface molecule. In some cases, the modified cell surface molecule lacks a functional intracellular signaling domain and/or is not capable of mediating intracellular signaling.

[0115] In some embodiments, the cell surface molecule of the cell surface conjugate contains a modified cell surface molecule that is altered compared to a reference cell surface molecule. In some embodiments, the reference cell surface molecule is a cell surface receptor, ligand, glycoprotein, cell adhesion molecule, antigen, integrin, or cluster of differentiation (CD). In some embodiments, the reference cell surface molecule is a cell surface receptor. In some embodiments, the reference cell surface molecule is a native mammalian cell surface molecule, such as a native mammalian cell surface receptor. In some cases, the cell surface molecule is a native human membrane protein.

[0116] In some embodiments, the reference cell surface molecule can be one that contains an extracellular domain or regions containing one or more epitope(s) recognized by an antibody or an antigen-binding fragment thereof. The antibody or antigen-binding fragment can include polyclonal and monoclonal antibodies, including intact antibodies and functional (antigen-binding) antibody fragments, including fragment antigen binding (Fab) fragments, $F(ab')_2$ fragments, Fab' fragments, Fv fragments, recombinant IgG (rIgG) fragments, variable heavy

chain (V_H) regions capable of specifically binding the antigen, single chain antibody fragments, including single chain variable fragments (scFv), and single domain antibodies (*e.g.*, sdAb, sdFv, nanobody) fragments. Antibodies or antigen-binding fragment thereof can include intact or full-length antibodies, including antibodies of any class or sub-class, including IgG and sub-classes thereof, IgM, IgE, IgA, and IgD, or portion or fragments of a full length antibody. In some aspects, the antibody is an antibody or antigen-binding fragment thereof that is clinically approved. In some aspects, the one or more epitopes can contain contiguous or non-contiguous sequences of a molecule or protein. In some aspects, the one or more epitope(s) is present in the extracellular portion or region of the reference cell surface molecule, such that the reference cell surface molecule can be recognized, identified or detected by the antibody or antigen-binding fragment.

[0117] In some embodiments, the extracellular domain of the reference cell surface molecule, in some cases, also contains a binding domain capable of specifically binding to a binding partner, an antigen, a substrate or a ligand. In such embodiments, among the provided cell surface molecules are modified cell surface molecules in which such a binding domain is modified or altered, e.g. is mutated or deleted, such that the ability of the modified cell surface molecule to bind to its normal cognate binding partner, antigen, substrate or ligand is reduced compared to the binding of the reference cell surface molecule to the binding partner, antigen, substrate or ligand. In some cases, the altered binding is reduced by greater than or greater than about 40%, greater than or greater than about 50%, greater than or greater than about 60%, greater than or greater than about 70%, greater than or greater than about 80%, greater than or greater than about 90% or more.

[0118] In some embodiments, the cell surface molecule is a membrane protein or a membrane-integrated protein. In some embodiments, the cell surface molecule contains a transmembrane domain. In some aspects, the cell surface molecule is a type I, type II, type III or type IV membrane protein. In some aspects, type I proteins have a single transmembrane stretch of hydrophobic residues, with the portion of the polypeptide on the amino (N)-terminal side of the transmembrane domain exposed on the exterior side of the membrane and the carboxy (C)-terminal portion exposed on the cytoplasmic side. In some aspects, type I membrane proteins are subdivided into types Ia (with cleavable signal sequences) and Ib (without cleavable signal sequence). In some aspects, type II membrane proteins span the membrane only once, but they have their amino terminus on the cytoplasmic side of the cell and the carboxy terminus on the exterior. In some aspects, type III membrane proteins have multiple

transmembrane domains in a single polypeptide chain and can be sub-divided into type IIIa proteins (with cleavable signal sequences) and type IIIb (with amino termini exposed on the exterior surface of the membrane, but without cleavable signal sequences). In some aspects, type IV proteins have multiple homologous domains which make up an assembly that spans the membrane multiple times, with the domains present on a single polypeptide chain or one or more different polypeptide chains.

[0119] In some embodiments, the reference cell surface molecule further contains an intracellular (or cytoplasmic) region or domain, i.e., a region of one or more contiguous amino acids present inside the cell and/or in the cytoplasmic side of the cell. In some cases, the intracellular region of a reference cell surface molecule contains an intracellular signaling domain and/or is capable of mediating intracellular signaling by directly or indirectly modulating cellular signal transduction pathways, and/or downstream responses, functions or activities, such as gene and protein expression, changes in subcellular localization of molecules, intracellular trafficking, changes in protein-protein interaction, receptor internalization, cellular differentiation, proliferation and/or survival.

[0120] In some embodiments, the intracellular signaling region or domain, e.g. present in or containing a cytoplasmic tail of the reference cell surface molecule, contains one or more motifs or residues that are capable of being phosphorylated and/or interacting with one or more adaptor proteins in a signal transduction pathway or downstream process in the cell upon a molecular or cellular signal, e.g., when activated or exposed to its cognate antigen or ligand. In some embodiments, the motif is or contains a tyrosine-based motif (e.g. YXXO, where Y is tyrosine, X is any amino acid and O is an amino acid with a bulky hydrophobic group), or a dileucine-based motif (e.g. LL). In some aspects, the intracellular signaling domain of a reference cell surface molecule can be present at or near the C-terminus of type I membrane proteins or at or near the N-terminus of type II membrane proteins. In such embodiments, among the provided cell surface molecules are modified cell surface molecules in which amino acid residues of such an intracellular region or domain is modified or altered, such as mutated, e.g., by one or more substitution, deletion, truncation and/or insertion, such that the ability of the modified cell surface molecule to modulate cellular signal transduction pathways, and/or downstream responses, functions or activities is reduced or prevented. In some cases, the altered signaling and/or downstream responses, functions or activities is reduced by greater than or greater than about 40%, greater than or greater than about 50%, greater than or greater than about 60%, greater than or greater than about 70%, greater than or greater than about 80%, greater than or

greater than about 90% or more compared to such signaling and/or downstream responses, functions or activities of a reference cell surface molecule.

[0121] In some embodiments, the reference cell surface molecule is different from and/or not identical to the antigen, e.g., a cell surface-expressed antigen, targeted by the recombinant receptor, e.g., chimeric antigen receptor (CAR). In some embodiments, the reference cell surface molecule or modified form thereof, is not specifically bound and/or recognized by the ligand- or antigen-binding domain of the recombinant receptor, e.g., chimeric antigen receptor (CAR).

[0122] In some embodiments, the reference cell surface molecule is or includes a cell surface protein and/or a receptor. In some embodiments, the reference cell surface molecule is EpCAM, VEGFR, integrins (e.g., integrins αvβ3, α4, αIIbβ3, α4β7, α5β1, αvβ3, αv), TNF receptor superfamily (e.g., TRAIL-R1, TRAIL-R2), PDGF Receptor, interferon receptor, folate receptor, GPNMB, ICAM-1, HLA-DR, CEA, CA-125, MUC1, TAG-72, IL-6 receptor, 5T4, GD2, GD3, prostate-specific membrane antigen (PSMA), or clusters of differentiation (e.g., CD2, CD3, CD4, CD5, CD11, CD11a/LFA-1, CD15, CD18/ITGB2, CD19, CD20, CD22, CD23/IgE Receptor, CD25, CD28, CD30, CD33, CD38, CD40, CD41, CD44, CD51, CD52, CD62L, CD74, CD80, CD125, CD147/basigin, CD152/CTLA-4, CD154/CD40L, CD195/CCR5, CD319/SLAMF7).

[0123] Suitable reference cell surface molecule, e.g., cell surface molecules for modification, include those described in U.S. Patent No. 8,802,374, which is hereby incorporated by reference. In some embodiments, the reference cell surface molecule is an epidermal growth factor receptor (EGFR), an erbB-2 receptor tyrosine-protein kinase, an erbB-3 receptor tyrosine-protein kinase, an erbB-4 receptor tyrosine-protein kinase, a hepatocyte growth factor receptor (HGFR/c-MET) or an insulin-like growth factor receptor-1 (IGFR-1). In some embodiments, the reference cell surface molecule contains the sequence of amino acids set forth in any of SEQ ID NOs: 49-54 or a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOs: 49-54.

[0124] In some embodiments, the reference cell surface molecule can be one that comprises an epitope recognized by an antibody including, but not limited to, 3F8, abagovomab, abciximab, adecatumumab, afutuzumab, alemtuzumab, altumomab pentetate, anatumomab mafenatox, apolizumab, arcitumomab, aselizumab, atlizumab (=tocilizumab), basiliximab, bectumomab, benralizumab, besilesomab, bivatuzumab mertansine, blinatumomab, brentuximab

vedotin, cantuzumab mertansine, capromab pendetide, catumaxomab, CC49, cedelizumab, celmoleukin, citatuzumab bogatox, clenoliximab, clivatuzumab tetraxetan, CNTO-95, conatumumab, dacetuzumab, daclizumab, daratumumab, detumomab, ecromeximab, edrecolomab, efalizumab, elotuzumab, enlimomab pegol, epitumomab cituxetan, epratuzumab, erlizumab, etaracizumab, fanolesomab, faralimomab, farletuzumab, galiximab, gavilimomab, gemtuzumab ozogamicin, glembatumumab vedotin, gomiliximab, ibalizumab, ibritumomab tiuxetan, igovomab, intetumumab, iratumumab, inolimomab, inotuzumab ozogamicin, ipilimumab, keliximab, labetuzumab, lintuzumab, lexatumumab, lucatumumab, lumiliximab, mapatumumab, maslimomab, milatuzumab, minretumomab, mitumomab, muromonab-CD3, naptumomab estafenatox, natalizumab, ocrelizumab, odulimomab, ofatumumab, olaratumab, oportuzumab monatox, oregovomab, otelixizumab, pemtumomab, priliximab, PRO 140, rituximab, rovelizumab, ruplizumab, satumomab pendetide, siplizumab, sontuzumab, tadocizumab, taplitumomab paptox, teneliximab, teplizumab, TGN1412, ticilimumab (=tremelimumab), tigatuzumab, tocilizumab (=atlizumab), toralizumab, tositumomab, tremelimumab, tucotuzumab, vedolizumab, veltuzumab, visilizumab, vitaxin, volociximab, votumumab, zanolimumab, ziralimumab, zolimomab aritox. Atezolizumab, bevacizumab (Avastin ®), denosumab, dinutuximab, nivolumab, obinutuzumab, pembrolizumab, pidilizumab (CT-011), ramucirumab, siltuximab, ado-trastuzumab emtansine, CEA-scan Fab fragment, OC125 monoclonal antibody, ab75705, B72.3, MPDL3280A, MSB001078C, MEDI4736, or an antigen-binding fragment thereof, analogs or derivatives thereof , or an antigen-binding antibody fragment selected from a Fab fragment, Fab' fragment F(ab)'2 fragment, single chain Fv (scFv) or a disulfide stabilized Fv (dsFv). In some embodiments, the modified cell surface molecule comprises an epitope recognized by any of the above antibodies or an antigen-binding fragment thereof.

[0125] In some embodiments, the reference cell surface molecule is a prostate-specific membrane antigen (PSMA). PSMA is a type II transmembrane protein, which contains a short cytoplasmic amino terminus, a single membrane-spanning domain, and a large extracellular domain. PSMA contains a sequence of amino acids that exhibit similarity to the peptidase family M28 proteins that include co-catalytic metallopeptidases. Wild-type, full-length human PSMA, is a 750-amino acid protein that includes an intracellular portion of 19 amino acid residues, a transmembrane portion of 24 amino acid residues, and an extracellular portion of 707 amino acid residue. In humans, PSMA is encoded by the FOLH1 gene, e.g., described in GenBank Accession No. DD461260 (set forth in SEQ ID NO:96), and isoforms and variants

thereof. Exemplary human PSMA amino acid sequence is set forth in, e.g., UniProt Accession No. Q04609 (set forth in SEQ ID NO:94).

[0126] In some cases, the extracellular portion of PSMA folds into three distinct structural and functional domains: a protease domain (residues 56-116 and 352-590), an apical domain (residues 117-351) and a C-terminal helical domain (residues 592-750), with reference to positions a wild-type human PSMA sequence, e.g., the amino acid sequence set forth in SEQ ID NO:94 (see, e.g., Davis et al., (2005) Proc. Natl. Acad. Sci. 102(17): 5981-5986; Mesters et al., (2006) EMBO Journal 25:1375-1384).

[0127] In some cases, PSMA has enzymatic or catalytic activity. In some aspects, particular domains and/or residues in PSMA are involved in the enzymatic or catalytic activity. PSMA generally contains a binuclear zinc site and can act as glutamate carboxypeptidase or folate hydrolase, catalyzing the hydrolytic cleavage of glutamate from poly-γ-glutamated folates. PSMA also has N-acetylated-alpha-linked-acidic dipeptidase (NAALADase) activity and dipeptidyl-peptidase IV type activity. The enzymatic site contains two zinc ions, and is composed of two pockets, the glutamate-sensing pocket (S1' pocket) and the non-pharmacophore pocket (S1 pocket). Amino acid residues from the three domains generally are involved in substrate recognition, binding, and/or catalytic activity. In some cases, active site residues and/or residues involved in substrate binding and/or catalytic activity in PSMA include amino acid residues at positions 210, 257, 269, 272, 377, 387, 387, 424, 424, 425, 433, 436, 453, 517, 518, 519, 552, 553, 534, 535, 536, 552, 553, 628, 666, 689, 699 and/or 700, with reference to positions a wild-type human PSMA sequence, e.g., the amino acid sequence set forth in SEQ ID NO:94. In some cases, active site residues include one more residues to coordinate the active zinc ions, such as one or more residues corresponding to His377, Asp387, Glu425, Asp453, and/or His553, with reference to position of an exemplary human PSMA sequence, e.g. the amino acid sequence set forth in SEQ ID NO:94. In some embodiments, the N-acetylated-alpha-linked-acidic dipeptidase (NAALADase) domain of PSMA can also be defined as including amino acid residues 274-587, with reference to positions a exemplary human PSMA sequence, e.g., the amino acid sequence set forth in SEQ ID NO:94 (Speno et al., (1999) Molecular Pharmacology 55:179-185).

[0128] In some aspects, the intracellular (N-terminal) portion of PSMA contains amino acid residues involved in cellular internalization, e.g., clathrin-dependent endocytic internalization of the molecule. In some aspects, the cellular internalization is mediated by N-terminal amino acids, such as amino acid residues at positions 1-5 of the exemplary human PSMA amino acid

sequence set forth in SEQ ID NO:94 (e.g., MWNLL; see, e.g., Rajasekaran et al. (2003) Mol. Biol. Cell. 14:4835-4845). In some aspects, the intracellular portion of the PSMA contains motifs or residues that are capable of being phosphorylated and/or interacting with one or more adaptor proteins in a signal transduction pathway or downstream process in the cell upon a molecular or cellular signal, such as for internalization of the molecule. In some embodiments, exemplary motifs include a dileucine-based motif (e.g., LL).

[0129] In some embodiments, the reference cell surface molecule is a PSMA, such as a mammalian PSMA, e.g., human PSMA. In some embodiments, the reference surface molecule is wild-type PSMA, optionally wild-type human PSMA, or an allelic variant or other variant thereof, e.g. alternative isoform or fragment thereof. In some embodiments, the PSMA is a full-length PSMA. In some embodiments, the reference cell surface molecule contains the sequence of amino acids set forth in SEQ ID NO:94 or a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:94. In some embodiments, the PSMA comprises or consists essentially of the sequence set forth in SEQ ID NO:94.

[0130] In some embodiments, the PSMA is encoded by a nucleic acid sequence set forth in SEQ ID NO:96, or a sequence of nucleic acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 96. In some embodiments, the PSMA is encoded by a modified nucleic acid sequence, e.g., a nucleic acid sequence that is modified to be CpG-free and/or is codon optimized. In some embodiments, the modified nucleic acid sequence is codon optimized for expression in human cells. In some aspects, codon optimization involves balancing the percentages of codons selected with the published abundance of human transfer RNAs so that none is overloaded or limiting. In some embodiments, a CpG-free nucleic acid sequence encoding PSMA is or includes modified cDNA sequence that contains no CpG sequences. In some aspects, the CpG-free nucleic acid and/or codon optimized sequence does not does not change the protein sequence, compared to the wild-type or unmodified PSMA. In some embodiments, the reference PSMA is encoded by a nucleic acid sequence set forth in SEQ ID NO:97. In some aspects, the PSMA encoded by the CpG-free PSMA has substantial percent identity to the protein sequence set forth in SEQ ID NO:94.

[0131] In some embodiments, the reference cell surface molecule is a PSMA that comprises an epitope recognized by antibodies or antigen-binding fragment thereof, including, but not limited to, J591, DFO-J591, CYT-356, J415, 3/A12, 3/F11, 3/E7, D2B, 107-1A4, YPSMA-1,

YPSMA-2, 3E6, 2G7, 24.4E6, GCP-02, GCP-04, GCP-05, J533, E99, 1G9, 3C6, 4.40, 026, D7-Fc, D7-CH3, 4D4, A5, or an antigen-binding fragment thereof, analogs or derivatives thereof , or an antigen-binding antibody fragment selected from a Fab fragment, Fab' fragment F(ab)'2 fragment, single chain Fv (scFv) or a disulfide stabilized Fv (dsFv). In some embodiments, exemplary antibody or antigen-binding fragment thereof include those described in, e.g., US 2002/0049712; US 2002/0147312; US 2003/0082187; US 2004/0136998; US 2005/0202020; US 2006/0088539; US 2007/0071759; US 2010/0297653; US 2011/0020273; US 2013/0225541; US 2013/0315830; US 2014/0099257; US 2014/0227180; US 2015/0168413; US 2016/0303253; US 2017/0051074; US 6572856; US 7476513; US 8470330; US 8986655; WO 2006/078892; WO 2010/135431; WO 2014/198223; WO 2015/177360; WO 2016/057917; WO 2016/130819; WO 2016/145139; WO 2016/201300; WO 2017/004144; WO 2017/023761; AU 2002/356844; AU 2006/204913; AU 2006/235421; AU 2006/262231; AU 2006/315500; AU 2010/325969; AU 2013/328619; AU 2015/205574; CA 2353267; EP 1390069; EP 1520588; EP 1581794; EP 1599228; EP 1610818; EP 2906250; Banerjee et al. (2011) Angew Chem Int Ed Engl. 50(39): 9167–9170; Maurer et al. (2016) Nature Reviews Urology 13:226-235; Rowe et al. (2016) Prostate Cancer Prostatic Dis. 19(3):223-230; Mease et al., (2013) Curr Top Med Chem. 13(8):951-962; Osborne et al., (2013) Urol Oncol. 31(2): 144–154; Philipp Wolf (2011), Prostate Specific Membrane Antigen as Biomarker and Therapeutic Target for Prostate Cancer, Prostate Cancer - Diagnostic and Therapeutic Advances, Dr. Philippe E. Spiess (Ed.), Intech, pp.81-100; Ruggiero et al., (2011) J Nucl Med. 52(10): 1608–1615; Liu et al., (1997) Cancer Research 57:3629-3634; Regino et al., (2009) Curr Radiopharm. January ; 2(1): 9–17; Kampmeier et al. (2014) EJNMMI Research 4:13; Wolf et al., (2010) The Prostate 70:562-569; Tykvart et al. (2014) The Prostate 74:1674-1690; Jin et al., (2016) EMJ Urol. 4(1):62-69 and Tino et al. (2000) Hybridoma 19(3):24957, or a fragment thereof, a conjugate thereof or a derivative thereof.

I. Exemplary Modified Cell Surface Molecules

[0132] In some embodiments, the modified cell surface molecule contains one or more amino acid modifications, such as one or more amino acid substitutions, deletions and/or insertions, compared to the reference cell surface molecule. In some embodiments, the modified cell surface molecule, such as a modified cell surface receptor is modified to remove any signaling and/or trafficking domains. In some cases, the modified cell surface molecule lacks a functional intracellular signaling domain and/or is not capable of mediating intracellular

signaling. In some embodiments, the modified cell surface molecule, e.g., a modified cell surface receptor, exhibits altered cellular internalization, cellular trafficking, enzymatic activity and/or ligand binding, compared to the wild-type or unmodified cell surface molecule. In some embodiments, the modified cell surface molecule contains and/or retains epitopes recognized and/or bound by a binding molecule, e.g., antibody or antigen-binding fragment thereof specific for the cell surface molecule and/or a ligand capable of binding the cell surface molecule.

[0133] In some embodiments, the one or more amino acid modifications, such as one or more amino acid substitutions, deletions and/or insertions, including truncations, can be present one or more of the intracellular (e.g., cytoplasmic) and/or extracellular portions of the cell surface molecule. In some embodiments, the modified cell surface molecule is truncated, such by contiguous deletion of a contiguous sequence of C-terminal or N-terminal amino acid residues of a reference cell surface molecule, such as deletion of from or from about 50 to 800 amino acids, such as 50 to 600, for example, at least or about at least 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, or more contiguous amino acids of the reference cell surface molecule. In some aspects, the modified cell surface molecule is truncated, such as by deletion of a contiguous amino acid residues of intracellular (e.g., cytoplasmic) portion of the protein, for example, present in the C-terminus portion of type I membrane proteins or in the N-terminus portion of type II membrane proteins. In some aspects, the modified cell surface molecule is truncated, such as by deletion of a contiguous amino acid residues of an extracellular domain or portion of the protein, for example, present in the N-terminus portion of type I membrane proteins or of the C-terminus portion of type II membrane proteins.

[0134] In some embodiments, the cell surface molecule comprises one or more extracellular domains or regions, and the modification is in the extracellular portion of the cell surface molecule. In some aspects, exemplary modifications of the extracellular portion of the cell surface molecule can remove domains or regions involved in epitope binding, enzymatic activity and/or ligand binding and/or signaling or function. In some aspects, exemplary modifications of the extracellular portion of the cell surface molecule contains and/or retains one or more epitope(s) recognized and/or bound by a binding molecule, e.g., antibody or antigen-binding fragment thereof specific for the cell surface molecule and/or a ligand capable of binding the cell surface molecule. In some aspects, exemplary modifications of the extracellular portion of the cell surface molecule generates a modified cell surface molecule that exhibits altered enzymatic activity and/or ligand binding, compared to the reference cell surface molecule.

[0135] In some embodiments, the cell surface molecule comprises one or more intracellular and/or cytoplasmic domains or regions, and the modification is in the intracellular (e.g., cytoplasmic) portion of the cell surface molecule. In some aspects, modifications, e.g., substitutions, deletions, truncations and/or insertions, of the intracellular (e.g., cytoplasmic) portion of the cell surface molecule can remove domains or regions involved in eliciting, mediating, activating, inhibiting and/or transmitting cellular signaling and/or downstream activities or functions, e.g., gene and protein expression, changes in subcellular localization of molecules, intracellular trafficking, changes in protein-protein interaction, receptor internalization, cellular differentiation, proliferation and/or survival. In some aspects, modifications of the intracellular (e.g., cytoplasmic) portion of the cell surface molecule generates a modified cell surface molecule that lacks a functional intracellular signaling domain and/or is not capable of mediating intracellular signaling and/or exhibits altered function or activity, e.g., altered cellular internalization and/or cellular trafficking. In some embodiments, ability of the modified cell surface molecule to elicit, mediate, activate, inhibit and/or transmit cellular signaling and/or regulating or modulating activity and/or functions associated with the cell surface molecule of the reference cell surface molecule is reduced by greater than or greater than about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more. In some embodiments, the modified cell surface molecule, such as modified cell surface receptor, is inert, such is not able to elicit or mediate an intracellular signal.

[0136] In some embodiments, the modified cell surface molecule retains the transmembrane domain of the reference cell surface molecule and at least one extracellular domain of the reference cell surface molecule. In some embodiments, the modified cell surface molecule contains the sequence of amino acids set forth in any of SEQ ID NOS:49-54 or 94, but lacks, such as is truncated or deleted for, the amino acid residues corresponding to the cytoplasmic domain of any of SEQ ID NOS: 49-54 or 94, respectively.

[0137] In some embodiments, the ability of the modified cell surface molecule of the provided conjugate to bind the native ligand of the reference cell surface molecule is altered. For example, in some embodiments, the ability of the modified cell surface molecule to bind the native ligand of the reference cell surface molecule is reduced and diminished. In some embodiments, the cell surface molecule is modified to contain at least one extracellular domain of the reference cell surface molecule but lacks one or more other extracellular domains recognized by the native ligand of the reference cell surface molecule. In some embodiments, binding of the modified cell surface molecule to the ligand of the reference cell surface molecule

is reduced by greater than or greater than about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more.

[0138] In some embodiments, the modified cell surface molecule, such as modified cell surface receptor, is modified or truncated compared to a reference cell surface molecule to retain a binding domain that contains an extracellular epitope recognized by a known antibody or functional fragment thereof. Thus, in some embodiments, modification of such cell surface molecule is accomplished by keeping an epitope present in the extracellular domain that is recognized by a known antibody or functional fragment thereof and removing any signaling or trafficking domains and/or any extracellular domains unrecognized by a known antibody. A modified cell surface molecule can include any modified cell surface molecule as described that retains binding, e.g. exhibits the same or similar binding as a reference cel surface molecule, to one or more of the exemplary antibodies and antigen-binding fragments described above.

[0139] In some embodiments, the modified cell surface molecule is a modified or truncated tyrosine kinase receptor. Examples of tyrosine kinase receptors that may be modified according to the embodiments described herein include, but are not limited to, members of the endothelial growth factor receptor family (EGRF/ErbB1/HER1; ErbB2/HER2/neu; ErbB3/HER3; ErbB4/HER4), hepatocyte growth factor receptor (HGFR/c-MET) and insulin-like growth factor receptor-1 (IGF-1 R). According to some embodiments, the provided cell surface conjugates contain a modified tyrosine kinase receptor that retains an extracellular epitope recognized by a known antibody or functional fragment thereof, and lacks the cytoplasmic domain or a functional protein thereof containing at least a tyrosine kinase domain. A modified tyrosine kinase receptor which lacks at least a tyrosine kinase domain renders the receptor inert. Commercial antibodies that may be used to recognize a modified tyrosine kinase receptor include, but are not limited to AMG-102, AMG-479, BIIB022OA-5D5, CP-751,871, IMC-A12, R1507, cetuximab, cixutumumab, ertumaxomab, figitumumab, matuzumab, necitumumab, panitumumab, pertuzumab, nimotuzumab, robatumumab, trastuzumab, zalutumumab.

[0140] In some embodiments, the modified cell surface molecule is a modified prostate-specific membrane antigen (PSMA). Antibodies that may be used to recognize a modified tyrosine kinase receptor include, but are not limited to J591, DFO-J591, CYT-356, J415, 3/A12, 3/F11, 3/E7, D2B, 107-1A4, YPSMA-1, YPSMA-2, 3E6, 2G7, 24.4E6, GCP-02, GCP-04, GCP-05, J533, E99, 1G9, 3C6, 4.40, 026, D7-Fc, D7-CH3, 4D4 and A5.

[0141] Non-limiting examples of exemplary cell surface molecules of a cell surface conjugate are set forth in Table 1.

[0142] In some embodiments, the modified cell surface molecule is modified compared to a reference cell surface molecule having the sequence of amino acids set forth in any of SEQ ID NOs: 49-54 or 94, in which the modified cell surface molecule at least contains a portion of the extracellular domain and the transmembrane domain of the reference cell surface molecule but lacks, such as is truncated or deleted for, the amino acid residues corresponding to the cytoplasmic domain of such reference cell surface molecule. In some embodiments, such as modified cell surface molecule also lacks one or more extracellular ligand binding domains for binding to a native ligand of the cell surface molecule, such as a native ligand set forth in Table 1. In some embodiments, such a modified cell surface molecule exhibits reduced (e.g. reduced by greater than 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more) binding for a native ligand of the reference cell surface molecule. In some embodiments, the modified cell surface molecule retains at least one extracellular domain containing an epitope for a known antibody specific to the reference cell surface molecule, such as an epitope of an antibody set forth in Table 1 or described herein, e.g., in Section II.A.

TABLE 1: Exemplary Cell Surface Molecules

Reference Cell Surface Molecule	SEQ ID NO		Native Ligand	Antibody
	Pre-cursor	mature		
HER1/ErbB1/EGFR	64	49	EGF, betacellulin, TGFα, HB-EGF, amphiregulin,epi-regulin, epigen	Cetuximab, panitumumab, matuzumab, necitumumab, nimotuzumab, zalutumumab
HER2/neu/ErbB2	65	50	No ligand binding activity alone Neuregulin (with HER4) EGF with EGFR	Trastuzumab, 2C4, ertumaxomab, pertuzumab
HER3/ErbB3	66	51	Hergulin (NRG-1), NRG-2	Patritumab
HER4/ErbB4	67	52	NRG-2, NRG-3, heparin-binding EGF-like growth factor, betacellulin	
HGFR/c-Met	68	53	HGF	DN30/ OA-5D5/ AMG 102/ emibetuzumab
IGF-1 R	69	54	IGF-1, insulin	CP-751,871, figitumumab, cixutumumab, dalotuzumab, Ganitumab, R1507
PSMA WT (full length)		94	Native substrate: N-aceylaspartylglutamate (NAAG), tri-alpha-glutamate peptides, and poly-γ-glutamyl folic acid	J591, DFO-J591, CYT-356, J415, 3/A12, 3/F11, 3/E7, D2B, 107-1A4, YPSMA-1, YPSMA-2, 3E6, 2G7, 24.4E6, GCP-02, GCP-04, GCP-05, J533, E99, 1G9, 3C6, 4.40, 026, D7-Fc, D7-CH3, 4D4, A5

a. Modified EGFR, e.g. truncated EGFR

[0143] In some embodiments, the cell surface molecule is a modified EGFR that is modified or altered compared to a reference EGFR, such as a reference human EGFR, e.g. a reference EGFR set forth in SEQ ID NO: 64 or the mature sequence thereof set forth in SEQ ID NO: 49. The structure of the reference native EGFR contains four extracellular domains (Domains I-IV, corresponding to residues 35-206, 207-333, 334-499 and 500-645, respectively, of SEQ ID NO:64), a transmembrane domain (corresponding to residues 646-668 of SEQ ID NO:64) and a cytoplasmic domain (corresponding to residues 669-1210 of SEQ ID NO:64) in which is contained therein an EGFR Juxtamembrane Domain (corresponding to residues 669-712 of SEQ ID NO:64), and an EGFR Tyrosine Kinase Domain (corresponding to residues 713-982 of SEQ ID NO:64).

[0144] In one embodiment, the modified cell surface molecule is a truncated EGFR (tEGFR) that lacks the membrane distal EGF-binding domain and the cytoplasmic signaling tail containing the tyrosine kinase domain, but retains the transmembrane domain and the extracellular membrane proximal epitope recognized by a known antibody or functional fragment thereof (e.g., cetuximab, matuzumab, necitumumab, nimotuzumab, zalutumumab, or panitumumab). In some embodiments, the absence of the EGF-binding domains and intracellular signaling domains renders EGFR inactive (inert) when expressed by T cells.

[0145] In some embodiments, the modified EGFR lacks one or more of Domain I, Domain II, the Juxtamembrane Domain and the Tyrosine Kinase Domain of the reference EGFR. In some cases, the modified EGFR lacks all of the Domain I, Domain II, the Juxtamembrane Domain and the Tyrosine Kinase Domain of the reference EGFR. In some cases, the modified EGFR lacks all of the Domain I, Domain II and cytoplasmic domain. In such embodiments, the modified EGFR contains or contains essentially Domain III and IV of the reference EGFR. In some embodiments, such as modified EGFR retains an epitope recognized by a known antibody or functional fragment thereof.

[0146] In some embodiments, the modified EGFR comprises amino acids contained in the sequence of amino acids set forth in SEQ ID NO: 44 or the mature form thereof set forth in SEQ ID NO: 46, or a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 44 or 46, in which the modified EGFR lacks the EGF-binding domains, retains an epitope recognized by a known antibody and lacks all of or a functional portion of the

cytoplasmic signaling domain of such reference EGFR. Exemplary of a binding molecules that can recognize the epitope on the modified EGFR include the FDA-approved anti-EGFR monoclonal antibody (mAb) cetuximab or another anti-EGFR antibody.

[0147] In some embodiments, the modified EGFR, such as tEGFR, is encoded by the sequence of nucleotides set forth in SEQ ID NO: 57 or a sequence that has at least at or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO:57, such as a sequence thereof containing degenerate codons. The encoded modified EGFR can contain a signal peptide for expression as a surface molecule or surface protein. In some embodiments, the modified EGFR, such as tEGFR, is encoded by nucleotides containing a sequence encoding the native signal peptide of the reference EGFR contained in SEQ ID NO: 64. In some embodiments, the modified EGFR, such as tEGFR, is encoded by nucleotides containing a sequence encoding a non-native or heterologous signal peptide, for example, set forth in SEQ ID NO: 48. In some embodiments, the modified EGFR is encoded by the sequence of nucleotides set forth in SEQ ID NO:45 or a sequence that has at least at or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO:45, such as a sequence thereof containing degenerate codons.

b. Modified Her2, e.g. truncated Her2

[0148] In some embodiments, the cell surface molecule is a modified HER2/neu/ErbB2 that is modified or altered compared to a reference HER2/neu/ErbB2, such as a reference human HER2/neu/ErbB2, e.g. a reference HER2/neu/ErbB2 set forth in SEQ ID NO: 65 or the mature sequence thereof set forth in SEQ ID NO: 50. The structure of the reference native HER2/neu/ErbB2 contains an extracellular domain (corresponding to residues 23-652, of SEQ ID NO: 65), a transmembrane domain (corresponding to residues 653-675 of SEQ ID NO:65), and a cytoplasmic domain (corresponding to residues 676-1255 of SEQ ID NO:65). The structure of the reference native HER2/neu/ErbB2 extracellular domain contains Domains I-IV, corresponding to residues 1-195, 196-319, 320-488, and 489-630 respectively, of SEQ ID NO: 50 (U.S. Patent Application Publication No. US2014/0186867 and U.S. Patent No. US 7,449,184).

[0149] In one embodiment, the modified cell surface molecule is a truncated HER2/neu/ErbB2 (HER2t) that lacks the cytoplasmic domain, but retains the transmembrane domain and the extracellular membrane proximal epitope recognized by a known antibody or

functional fragment thereof (e.g., trastuzumab, 2C4, ertumaxomab, pertuzumab). In some embodiments, the absence of the ligand-binding domains and intracellular signaling domains renders HER2/neu/ErbB2 inactive (inert) when expressed by T cells.

[0150] In some embodiments, the modified HER2/neu/ErbB2 lacks one or more of Domain I, Domain II, and Domain III of the reference HER2/neu/ErbB2. In some cases, the modified HER2/neu/ErbB2 lacks all of extracellular domains of the reference HER2/neu/ErbB2. In some cases, the modified HER2/neu/ErbB2 lacks all of the extracellular and cytoplasmic domain. In such embodiments, the modified HER2/neu/ErbB2 contains or contains essentially Domain IV, which is retained and the transmembrane domain of the reference HER2/neu/ErbB2. In some embodiments, such as modified HER2/neu/ErbB2 retains an epitope recognized by a known antibody or functional fragment thereof.

[0151] In some embodiments, the modified HER2/neu/ErbB2 comprises amino acids contained in the sequence of amino acids set forth in in SEQ ID NO: 92, or a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 92, in which the modified HER2/neu/ErbB2 lacks the ligand-binding domains, retains an epitope recognized by a known antibody and lacks all of or a functional portion of the cytoplasmic signaling domain of such reference HER2/neu/ErbB2. In some embodiments, the ligand-binding domains can be bind to EGF, transforming growth factor α (TGFα), amphiregulin, heparin-binding EGF-like growth factor, betacellulin, and epiregulin. Exemplary of a binding molecules that can recognize the epitope on the modified HER2/neu/ErbB2 include the FDA-approved anti-HER2 monoclonal antibody (mAb) trastuzumab, 2C4, ertumaxomab, pertuzumab, or another anti-HER2/neu/ErbB2 antibody. In some embodiments, the binding molecule can recognize an epitope in domain IV of the modified HER2/neu/ErbB2 (trastuzumab) or can recognize an epitope in domain II of the modified HER2/neu/ErbB2 (pertuzumab).

[0152] In some embodiments, the modified HER2/neu/ErbB2, such as HER2t, is encoded by the sequence of nucleotides set forth in SEQ ID NO: 91 or a sequence that has at least at or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 91, such as a sequence thereof containing degenerate codons. The encoded modified HER2/neu/ErbB2 can contain a signal peptide for expression as a surface molecule or surface protein. In some embodiments, the modified HER2/neu/ErbB2, such as HER2t, is encoded by nucleotides containing a sequence encoding the native signal peptide of the reference HER2/neu/ErbB2 contained in SEQ ID NO: 65. In some embodiments, the

modified HER2/neu/ErbB2, such as HER2t, is encoded by nucleotides containing a sequence encoding a non-native or heterologous signal peptide, for example, set forth in SEQ ID NO: 48. In some embodiments, the modified HER2/neu/ErbB2 is encoded by the sequence of nucleotides set forth in SEQ ID NO: 93 or a sequence that has at least at or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 93, such as a sequence thereof containing degenerate codons.

c. Modified PSMA, e.g. truncated PSMA

[0153] In some embodiments, the modified cell surface molecule is a modified prostate-specific membrane antigen (PSMA). In some embodiments, the modified cell surface molecule is modified compared to a reference cell surface molecule that is a PSMA, such as a wild-type or unmodified PSMA, e.g. a human PSMA, e.g., containing the sequence of amino acids set forth in SEQ ID NO:94.. In some embodiments, the modified PSMA contains one or more amino acid modification compared to a reference PSMA, such as one or more amino acid substitutions, deletions, truncations and/or insertions. In some embodiments, the modified PSMA exhibits altered cellular internalization, cellular trafficking, enzymatic activity and/or ligand binding, compared to the reference, wild-type or unmodified PSMA.

[0154] In some embodiments, the modified PSMA comprises all or substantially all of the transmembrane domain of the wild-type or unmodified PSMA; or the modified PSMA comprises a transmembrane domain with the same or at least the same number of amino acids as the transmembrane domain of a wild-type or unmodified PSMA. In some embodiments, the PSMA comprises an extracellular domain containing an epitope recognized by any of the antibodies or an antigen-binding fragment thereof described herein that bind to PSMA.

[0155] In some embodiments, the reference, wild-type or unmodified PSMA is human PSMA and/or comprises the sequence of amino acids set forth in SEQ ID NO:94. In some embodiments, the modified PSMA contains the extracellular domain and/or transmembrane domain of the sequence of amino acids set forth in SEQ ID NO:94 or portion or fragment thereof

[0156] In some embodiments, the modified PSMA comprises at least one amino acid substitution, e.g., at the second amino acid residue, where the tryptophan is substituted by glycine, corresponding to W2G, with reference to positions in PSMA set forth in SEQ ID NO:94. In some embodiments, the modified PSMA comprises at least one amino acid substitution corresponding to W2G or does not comprise W2 or does not comprise any residue at

position 2, with reference to positions in the PSMA sequence set forth in SEQ ID NO:94. For example, in some embodiments, the modified PSMA comprises the sequence of amino acids set forth in SEQ ID NO:95 or a fragment thereof, or a sequence of amino acids that exhibits at least or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NOS:95 or a fragment thereof and comprises the at least one amino acid substitution.

[0157] In some embodiments, the modified PSMA comprises an amino acid substitution at one or more of amino acid residues at position 2, 3, 4, 5, 6, 7, 8, 9, 10 or 14 with reference to positions in the PSMA sequence set forth in SEQ ID NO:94, to alanine.

[0158] In some embodiments, the PSMA is a modified PSMA that comprises a deletion of one or more N-terminal amino acid residues within the intracellular portion, compared to the wild-type or unmodified PSMA. Wild-type, full-length human PSMA, is a 750-amino acid protein that includes an intracellular portion of 19 amino acid residues, a transmembrane portion of 24 amino acid residues, and an extracellular portion of 707 amino acid residue. For example, in some embodiments, the modified PSMA contains a deletion at the N-terminus (corresponding to the 5' end of the coding sequence in the nucleic acid sequence encoding PSMA or modified form thereof), the deletion being within the intracellular portion of PSMA.

[0159] In some aspects, the modified PSMA containing one or more deletions within the intracellular portion is also referred to as a truncated form of PSMA, a truncated PSMA or a tPSMA. In some aspects, the truncated PSMA or tPSMA contains a deletion or truncation of one or more amino acid residues, optionally contiguous amino acid residues, at or near the N-terminal of the wild-type or unmodified PSMA. In some aspects, the modified PSMA contains a deletion or truncation of one or more amino acid residues, e.g., one or more contiguous amino acid residues, within an intracellular portion or domain of the PSMA. In some embodiments, the PSMA protein containing a deletion N-terminal amino acids allows the N-terminally modified PSMA to successfully localize to the cell membrane and centrosome and/or (i) exhibits reduced endogenous signaling; (ii) exhibits increased cell surface expression; and/or (iii) exhibits reduced cellular internalization compared to the wild-type or unmodified PSMA. In some embodiments, the modified PSMA exhibits reduced endogenous signaling or reduced cellular internalization, e.g. reduced by greater than or greater than about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more. In some embodiments, the modified PSMA exhibits increased cell surface expression or increased localization to the cell membrane and centrosome, e.g. increased by greater than or greater than about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or

more. In some aspects, cell surface expression and/or cellular internalization can be assessed using cell imaging techniques, such as confocal microscopy using labeled binding molecules, e.g., antibodies, that specifically bind to PSMA or variant thereof.

[0160] In some embodiments, the modified PSMA contains or retains a methionine as a first residue, which, in some cases, is required for translation. In some embodiments, the PSMA is a modified PSMA that comprises a deletion of one or more N-terminal amino acid residues, optionally contiguous amino acid residues, within the intracellular portion, compared to the wild-type or unmodified PSMA, but does not include a deletion of the initial methionine required for translation.

[0161] In some embodiments, the PSMA or modified PSMA includes a PSMA described in, e.g., International PCT Pub. No. WO2015143029, Rajasekaran et al. (2003) Mol. Biol. Cell. 14:4835-4845, Rajasekaran et al. (2008) Mol Cancer Ther. (2008) 7(7): 2142–2151, Barinka et al. (2004) Eur. J. Biochem. 271:2782–2790, and Davis et al. (2005) Proc. Natl. Acad. Sci. 102(17)-5981-5986.

[0162] In some embodiments, the modified PSMA contains a deletion of or lacks 11 N-terminal amino acids and/or the first 11 amino acids, with reference to positions in a wild-type or unmodified PSMA, e.g., the PSMA sequence set forth in SEQ ID NO:94. In some embodiments, the modified PSMA contains a deletion of or lacks 15 N-terminal amino acids, with reference to positions in a wild-type or unmodified PSMA, e.g., the PSMA sequence set forth in SEQ ID NO:94. In some embodiments, the modified PSMA contains deletion or lacks amino acids N-terminal amino acids 6-14, with reference to positions in a wild-type or unmodified PSMA, e.g., the PSMA sequence set forth in SEQ ID NO:94.

[0163] In some embodiments, the modified PSMA comprises a deletion of one or more C-terminal amino acid residues. In some embodiments, the modified PSMA comprises a deletion of amino acid residues 103-750, 626-750, 721-747 or 736-750, with reference to positions in PSMA set forth in SEQ ID NO:94. In some embodiments, the modified PSMA comprises a deletion of 15 C-terminal amino acid residues, with reference to positions in PSMA set forth in SEQ ID NO:94.

[0164] In some embodiments, the modified PSMA is encoded by a modified nucleic acid sequence, e.g., a nucleic acid sequence that is modified to be CpG-free and/or is codon optimized.

B. Agent (e.g. Affinity Tag)

[0165] In some embodiments of the cell surface conjugate, the cell surface molecule, such as a modified cell surface molecule, is linked to at least one agent. In some embodiments, the agent is a peptide or polypeptide. In some embodiments, the agent is a peptide. In some embodiments, the peptide is artificial, synthetic or is a portion of a longer polypeptide. A peptide is generally greater than or equal to 2 amino acids in length, such as one that is greater than or equal to 2 and less than or equal to 50 or 40 amino acids in length. In some embodiments, the peptide is between 7 and 40 amino acids, 8 and 20 amino acids, 10 and 17 amino acids, 7 and 13 amino acids or 8 and 10 amino acids. In some embodiments, the peptide has a length of between 7 and 20 amino acids. In some embodiments, the peptide has a length of 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids.

[0166] In some embodiments, the agent is an affinity tag that is known to be recognized by a binding molecule. In some embodiments, the affinity tag has enough residues to provide an epitope recognized by an antibody or by a non-antibody binding molecule, yet, in some aspects, is short enough such that it does not interfere with or sterically block an epitope of the cell surface molecule recognized by a known antibody as described above. Suitable tag polypeptides generally have at least 5 or 6 amino acid residues and usually between about 8-50 amino acid residues, typically between 9-30 residues. Such tags are well-known and can be readily synthesized and designed.

[0167] In some embodiments, the agent, such as affinity tag, is a streptavidin binding peptide (e.g. Strep-tag), oligohistidine or polyhistidine (e.g. His tag), MAT tag, a glutathione-S-transferase, immunoglobulin domain, calmodulin or an analog thereof, thioredoxin, chitin binding protein (CBP), calmodulin binding peptide (CBP), a FLAG-peptide, an HA-tag, maltose binding protein (MBP), an HSV epitope (e.g. gd tag), a myc epitope, and/or a biotinylated carrier protein. Exemplary of such agents, such as affinity tags, include, MAT tag (sequence: His-Asn-His-Arg-His-Lys-His-Gly-Gly-Gly-Cys) (SEQ ID NO:63), HA-tag (sequence: Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala) (SEQ ID NO: 20), the VSV-G-tag (sequence: Tyr-Thr-Asp-Ile-Glu-Met-Asn-Arg-Leu-Gly-Lys) (SEQ ID NO: 21), the HSV-tag (sequence: Gln-Pro-Glu-Leu-Ala-Pro-Glu-Asp-Pro-Glu-Asp) (SEQ ID NO: 22), the T7 epitope (Ala-Ser-Met-Thr-Gly-Gly-Gln-Gln-Met-Gly) (SEQ ID NO: 22), maltose binding protein (MBP), the HSV epitope of the sequence Gln-Pro-Glu-Leu-Ala-Pro-Glu-Asp-Pro-Glu-Asp (SEQ ID NO: 24) of herpes simplex virus glycoprotein D, the "myc" epitope of the transcription factor c-myc of the

sequence Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu (SEQ ID NO: 25), the V5-tag (sequence: Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Leu-Gly-Leu-Asp-Ser-Thr) (SEQ ID NO: 26), or glutathione-S-transferase (GST). Exemplary of such agents, e.g. affinity tag, also can include a streptavidin binding peptide (e.g. Strep-tag), such as any comprising a sequence set forth in any of SEQ ID NOS: 7-19. Binding molecules known to recognize such tags are known and include, but are not limited to, antibody 12CA5 for recognition of the influenza hemagglutinin (HA) tag polypeptide (Field et al. (1988) Mol. Cell. Biol. 5:2159-2165); 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies for recognition of the c- myc tag (see, e.g., Evan et al. (1985) Molecular and Cellular Biology 5 :3610-3616); and a known antibody for recognizing the Herpes Simplex virus glycoprotein D (gD) tag and its antibody (Paborsky et al. (1990) Protein Engineering 3:547-553), or any known binding molecule recognizing a streptavidin binding peptide (e.g. Strep-tag), such as antibody molecules or reagents comprising a streptavidin mutein (e.g. Strep-Tactin) .

[0168] Further examples of an agent include, but are not limited to, dinitrophenol or digoxigenin, a lectin, protein A, protein G, a metal, a metal ion, nitrilo triacetic acid derivatives (NT A), RGD-motifs, a dextrane, polyethyleneimine (PEI), a redox polymer, a glycoproteins, an aptamers, a dye, amylose, maltose, cellulose, chitin, glutathione, calmodulin, gelatine, polymyxin, heparin, NAD, NADP, lysine, arginine, benzamidine, poly U, or oligo-dT. Lectins such as Concavalin A are known to bind to polysaccharides and glycosylated proteins. An illustrative example of a dye is a triazine dye such as Cibacron blue F3G-A (CB) or Red HE-3B, which specifically bind NADH-dependent enzymes. Typically, Green A binds to Co A proteins, human serum albumin, and dehydrogenases. In some cases, the dyes 7-aminoactinomycin D and 4',6-diamidino-2-phenylindole bind to DNA.

[0169] In some embodiments, the agent (e.g. a peptide), such as affinity tag, is recognized by a binding molecule with an equilibrium dissociation constant (K_D) of from or from about 10^{-4} to 10^{-10} M or an equilibrium association constant (K_A) of from or from about 10^4 to 10^{10} M^{-1}. In some embodiments, the agent, such as affinity tag, e.g. a peptide, is recognized by a binding molecule with a low binding affinity, such as with a K_D of greater than or greater than about 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M or greater or with a K_A or less than or less than about 10^7 M^{-1}, 10^6 M^{-1}, 10^5 M^{-1}, 10^4 M^{-1} or less.

[0170] In some embodiments, the agent, such as affinity tag, is recognized by a binding molecule that is or comprises a binding reagent having at least one binding site Z that binds to the agent. In some embodiments, the binding site Z is a natural biotin binding site of avidin or streptavidin or a mutein or analog thereof for which there can be up to four binding sites in an

individual molecule (e.g. a tetramer contains four binding sites Z), whereby a homo-tetramer can contain up to 4 binding sites that are the same, i.e. Z1, whereas a hetero-tetramer can contain up to 4 binding sites that may be different, e.g. containing Z1 and Z2.

[0171] In some embodiments, the agent is recognized by a binding molecule that is or comprises a reagent that is an oligomer or polymer. In some embodiments, the oligomer or polymer can be generated by linking directly or indirectly individual molecules of the protein as it exists naturally, either by linking directly or indirectly individual molecules of a monomer or a complex of subunits that make up an individual molecule (e.g. linking directly or indirectly dimers, trimers, tetramers, etc. of a protein as it exists naturally). For example, a tetrameric homodimer or heterodimer of streptavidin or avidin may be referred to as an individual molecule or smallest building block of a respective oligomer or polymer. In some embodiments, the oligomer or polymer can contain linkage of at least 2 individual molecules of the protein (e.g. is a 2-mer), or can be at least a 3-mer, 4-mer, 5-mer, 6-mer, 7-mer, 8-mer, 9-mer, 10-mer, 11-mer, 12-mer, 13-mer, 14-mer, 15-mer, 16-mer, 17-mer, 18-mer, 19-mer, 20-mer, 25-mer, 30-mer, 35-mer, 40-mer, 45-mer or 50-mer of individual molecules of the protein (e.g., monomers, tetramers). In some cases, an oligomer can contain a plurality of binding sites Z1, such as at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 40, 45, 50 or more binding sites Z1. In some embodiments, the oligomer is generated or produced from a plurality of individual molecules that can be hetero-tetramers (e.g. of a streptavidin, streptavidin mutein, avidin or avidin mutein) and/or from a plurality of two or more different individual molecules (e.g. different homo-tetramers of streptavidin, streptavidin mutein, avidin or avidin mutein) that differ in their binding sites Z, e.g. Z1 and Z2, in which case a plurality of different binding sites Z, e.g. Z1 and Z2, may be present in the oligomer. For example, in some cases, an oligomer can contain a plurality of binding sites Z1 and a plurality of binding sites Z2, which, in combination, can include at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 40, 45, 50 or more combined binding sites Z1 and Z2.

[0172] Oligomers can be generated using any methods known in the art, such as any described in published U.S. Patent Application No. US2004/0082012. In some embodiments, the oligomer or polymer contains two or more individual molecules that may be crosslinked, such as by a polysaccharide or a bifunctional linker.

[0173] In some embodiments, the oligomer or polymer is obtained by crosslinking individual molecules or a complex of subunits that make up an individual molecule in the

presence of a polysaccharide. In some embodiments, oligomers or polymers can be prepared by the introduction of carboxyl residues into a polysaccharide, e.g. dextran. In some aspects, individual molecules of the reagent (e.g., monomers, tetramers) can be coupled via primary amino groups of internal lysine residues and/or the free N-terminus to the carboxyl groups in the dextran backbone using conventional carbodiimide chemistry. In some embodiments, the coupling reaction is performed at a molar ratio of about 60 moles of individual molecules of the reagent (e.g., monomers, tetramers) per mole of dextran.

[0174] In some cases, the binding interaction between the agent and the at least one binding site Z is a non-covalent interaction. In some embodiments, the binding interaction, such as non-covalent interaction, between the agent and the at least one binding site Z is reversible. In some embodiments, the binding reagent contains a plurality of binding sites capable of reversibly binding to the agent. Binding molecule reagents that can be used in such reversible systems are described and known in the art, see *e.g.*, U.S. Patent Nos. 5,168,049; 5,506,121; 6,103,493; 7,776,562; 7,981,632; 8,298,782; 8,735,540; 9,023,604; and International published PCT Appl. Nos. WO2013/124474 and WO2014/076277.

[0175] In some embodiments, recognition of the agent by the binding molecule is reversible, such as is competed in the presence of a competition substance. In some embodiments, the agent is one in which reversible association can be mediated in the presence of a competition substance that is or contains a binding site that also is able to be recognized or bound by the reagent. In some aspects, the competition substance can act as a competitor due to a higher binding affinity between it and the binding molecule than the binding affinity between the binding molecule and the agent and/or due to the competition substance being present at higher concentration than the agent, thereby detaching and/or dissociating the interaction between the agent and the binding molecule. In some aspects, reversible binding between the agent and the binding molecule can be carried out by contacting cells expressing the cell surface conjugate and bound by the binding molecule with the competition substance, such by adding the competition substance to such a cell composition.

[0176] In some embodiment the agent is or includes a moiety known to the skilled artisan as an affinity tag. In some such embodiments, the binding molecule is or comprises a reagent that is a corresponding binding partner, for example, an antibody or an antibody fragment, known to bind to the affinity tag. In such embodiments, the complex formed between the one or more binding sites Z of the reagent which may be an antibody or antibody fragment, and the antigen can be disrupted competitively by adding the free antigen, i.e. the free peptide (epitope tag) or

the free protein (such as MBP or CBP). In some embodiments, the affinity tag might also be an oligonucleotide tag. In some cases, such an oligonucleotide tag may, for instance, be used to hybridize to an oligonucleotide with a complementary sequence, linked to or included in the reagent.

[0177] In some cases, the binding molecule is or comprises a reagent that contains at least two chelating groups K that may be capable of binding to a transition metal ion, thereby rendering the reagent capable of binding to an oligohistidine affinity tag, multimeric glutathione-S-transferase, or a biotinylated carrier protein or other agent. Generally, cations of metals such as Ni, Cd, Zn, Co, or Cu, are typically used to bind affinity tags such as an oligohistidine containing sequence, including the hexahistidine or the His-Asn-His-Arg-His-Lys-His-Gly-Gly-Gly-Cys tag (MAT tag; SEQ ID NO:63), and N-methacryloyl-(L)-cysteine methyl ester. In some embodiments the binding between the agent (e.g., peptide), such as an affinity tag, and the one or more binding sites Z of the reagent occurs in the presence of a divalent, a trivalent or a tetravalent cation. In this regard, in some embodiments the reagent includes a divalent, a trivalent or a tetravalent cation, typically held, e.g. complexed, by means of a suitable chelator. In some embodiments, the agent (e.g. peptide), such as an affinity tag, may include a moiety that includes, e.g. complexes, a divalent, a trivalent or a tetravalent cation. In some such embodiments, the binding between the agent and the one or more binding sites Z of the reagent can be disrupted by metal ion chelation. The metal chelation may, for example, be accomplished by addition of EGTA or EDTA. Examples of a respective metal chelator, include, but are not limited to, ethylenediamine, ethylene-diaminetetraacetic acid (EDTA), ethylene glycol tetraacetic acid (EGTA), diethylenetri-aminepentaacetic acid (DTPA), N,N-bis(carboxymethyl)glycine (also called nitrilotriacetic acid, NTA), 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA), 2,3-dimer-capto-1-propanol (dimercaprol), porphine and heme. As an example, EDTA forms a complex with most monovalent, divalent, trivalent and tetravalent metal ions, such as e.g. silver (Ag^{+}), calcium (Ca^{2+}), manganese (Mn^{2+}), copper (Cu^{2+}), iron (Fe^{2+}), cobalt (Co^{+}) and zirconium (Zr^{4+}), while BAPTA is specific for Ca^{2+}. As an illustrative example, a standard method used in the art is the formation of a complex between an oligohistidine tag and copper (Cu^{2+}), nickel (Ni^{2+}), cobalt (Co^{2+}), or zinc (Zn^{2+}) ions, which are presented by means of the chelator nitrilotriacetic acid (NTA).

[0178] In some embodiments, the agent , such as an affinity tag, includes a calmodulin binding peptide and the binding molecule reagent includes multimeric calmodulin as described

in US Patent 5,985,658, for example. In some embodiments, the agent, such as an affinity tag, includes a FLAG peptide and the binding molecule reagent includes an antibody that binds to the FLAG peptide, e.g. the FLAG peptide, which binds to the monoclonal antibody 4E11 as described in US Patent 4,851,341. In one embodiment, the agent, such as an affinity tag, includes an oligohistidine tag and the reagent includes an antibody or a transition metal ion binding the oligohistidine tag. In some cases, the disruption of all these binding complexes may be accomplished by metal ion chelation, e.g. calcium chelation, for instance by adding EDTA or EGTA. In some embodiments, calmodulin, antibodies such as 4E11 or chelated metal ions or free chelators may be multimerized by conventional methods, e.g. by biotinylation and complexation with streptavidin or avidin or oligomers thereof or by the introduction of carboxyl residues into a polysaccharide, e.g. dextran, essentially as described in Noguchi, A, et al. Bioconjugate Chemistry (1992) 3, 132-137 in a first step and linking calmodulin or antibodies or chelated metal ions or free chelators via primary amino groups to the carboxyl groups in the polysaccharide, e.g. dextran, backbone using conventional carbodiimide chemistry in a second step.

[0179] In some cases, the binding molecule is or comprises a reagent that is a streptavidin or avidin or any analog or mutein of streptavidin or an analog or mutein of avidin (e.g. neutravidin). In some embodiments, the binding molecule reagent is capable of binding to an agent that is a streptavidin binding peptide. In some embodiments, disrupting or reversing binding can be carried out with biotin or a biotin analog or mimic. Exemplary of such streptavidin binding peptides and binding molecule reagents known to recognize such agents are described below.

I. Exemplary Streptavidin Binding Peptide Agents and Binding Molecules Thereto

[0180] In some embodiments, the agent (e.g. peptide), such as an affinity tag, is recognized by a reagent that is or that comprises a streptavidin or a streptavidin mutein. In some embodiments, the agent can be a biotin, a biotin derivative or analog, or a streptavidin-binding peptide or other molecule that is able to specifically bind to streptavidin, a streptavidin mutein or analog, avidin or an avidin mutein or analog. In some embodiments, the agent, such as an affinity tag, is a streptavidin binding peptide.

[0181] In some embodiments, the streptavidin binding peptide contains a sequence with the general formula set forth in SEQ ID NO: 9, such as contains the sequence set forth in SEQ ID

NO: 10. In some embodiments, the peptide sequence has the general formula set forth in SEQ ID NO: 11, such as set forth in SEQ ID NO: 12. In one example, the peptide sequence is Trp-Arg-His-Pro-Gln-Phe-Gly-Gly (also called Strep-tag®, set forth in SEQ ID NO: 7). In one example, the peptide sequence is Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO:58) or the minimal sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (also called Strep-tag® II, set forth in SEQ ID NO: 8). In some embodiments, the agent contains a sequential arrangement of at least two streptavidin-binding peptide modules, wherein the distance between the two modules is at least 0 and not greater than 50 amino acids, wherein one binding module has 3 to 8 amino acids and contains at least the sequence His-Pro-Xaa (SEQ ID NO: 9), where Xaa is glutamine, asparagine, or methionine, and wherein the other binding module has the same or different streptavidin peptide ligand, such as set forth in SEQ ID NO: 11 (see e.g. International Published PCT Appl. No. WO02/077018; U.S. Patent No. 7,981,632). In some embodiments, the streptavidin binding peptide contains a sequence having the formula set forth in any of SEQ ID NO: 13 or 14. In some embodiments, the agent can contain twin-strep-tags such as by the sequential arrangement of two streptavidin binding modules, such as is commercially available as Twin-Strep-tag® from IBA GmbH, Göttingen, Germany, for example, containing the sequence (SAWSHPQFEK(GGGS)$_2$GGSAWSHPQFEK)(SEQ ID NO: 16). In some embodiments, the streptavidin binding peptide has the sequence of amino acids set forth in any of SEQ ID NOS: 15-19. In most cases, all these streptavidin binding peptides bind to the same binding site, namely the biotin binding site of streptavidin.

[0182] In some embodiments, the streptavidin binding peptide is recognized by a reagent comprising streptavidin or streptavidin mutein, which exhibits binding affinity for the peptide. In some embodiments, the binding affinity of streptavidin or a streptavidin mutein for a streptavidin binding peptide is with a K_D of less than 1 x 10^{-4} M, 5 x 10^{-4} M, 1 x 10^{-5} M, 5x 10^{-5} M, 1 x 10^{-6} M, 5 x 10^{-6} M or 1 x 10^{-7} M, but generally greater than 1 x 10^{-13} M, 1 x 10^{-12} M or 1 x 10^{-11} M. For example, peptide sequences (Strep-tags), such as disclosed in U.S. Pat. No. 5,506,121, can act as biotin mimics and demonstrate a binding affinity for streptavidin, e.g., with a K_D of approximately between 10^{-4} M and 10^{-5} M. In some cases, the binding affinity can be further improved by making a mutation within the streptavidin molecule, see e.g. U.S. Pat. No. 6,103,493 or International published PCT App. No. WO2014/076277. In some embodiments, binding affinity can be determined by methods known in the art, such as any described below.

[0183] In some embodiments, the streptavidin binding peptide is recognized by a reagent that is or comprises a streptavidin, a streptavidin mutein or analog, avidin, an avidin mutein or

analog (such as neutravidin) or a mixture thereof, in which such reagent contains one or more binding sites Z for reversible association with the agent comprising a streptavidin binding peptide. In some embodiments, the reagent is or contains an analog or mutein of streptavidin or an analog or mutein of avidin that reversibly binds a streptavidin-binding peptide. In some embodiments, the substance (e.g. competitive reagent) can be a biotin, a biotin derivative or analog or a streptavidin-binding peptide capable of competing for binding with the agent for the one or more binding sites Z. In some embodiments, the agent of the conjugate and the substance (e.g. competitive reagent) are different, and the substance (e.g. competitive reagent) exhibits a higher binding affinity for the one or more binding sites Z compared to the affinity of the agent.

[0184] In some embodiments, the binding molecule recognizing the agent, e.g. a streptavidin binding peptide (e.g. a Strep-tag), is or comprises a streptavidin that can be wild-type streptavidin, streptavidin muteins or analogs, such as streptavidin-like polypeptides. In some embodiments, the binding molecule is or comprises an avidin that can be wild-type avidin or muteins or analogs of avidin such as neutravidin, a deglycosylated avidin with modified arginines that typically exhibits a more neutral pi and is available as an alternative to native avidin. Generally, deglycosylated, neutral forms of avidin include those commercially available forms such as "Extravidin", available through Sigma Aldrich, or "NeutrAvidin" available from Thermo Scientific or Invitrogen, for example.

[0185] In some embodiments, the agent, such as a streptavidin binding peptide, is recognized by a binding molecule reagent that is or comprises a streptavidin or a streptavidin mutein or analog. In some embodiments, wild-type streptavidin (wt-streptavidin) has the amino acid sequence disclosed by Argarana et al, Nucleic Acids Res. 14 (1986) 1871-1882 (SEQ ID NO: 1). In general, streptavidin naturally occurs as a tetramer of four identical subunits, i.e. it is a homo-tetramer, where each subunit contains a single binding site for biotin, a biotin derivative or analog or a biotin mimic. An exemplary sequence of a streptavidin subunit is the sequence of amino acids set forth in SEQ ID NO: 1, but such a sequence also can include a sequence present in homologs thereof from other *Streptomyces* species. In particular, each subunit of streptavidin may exhibit a strong binding affinity for biotin with a dissociation constant (K_d) on the order of about 10^{-14} M. In some cases, streptavidin can exist as a monovalent tetramer in which only one of the four binding sites is functional (Howarth *et al.* (2006) *Nat. Methods*, 3:267-73; Zhang *et al.* (2015) *Biochem. Biophys. Res. Commun.*, 463:1059-63)), a divalent tetramer in which two of the four binding sites are functional (Fairhead *et al.* (2013) *J. Mol. Biol.*, 426:199-214), or can

be present in monomeric or dimeric form (Wu *et al.* (2005) *J. Biol. Chem.*, 280:23225-31; Lim *et al.* (2010) *Biochemistry*, 50:8682-91).

[0186] In some embodiments, the streptavidin may be in any form, such as wild-type or unmodified streptavidin, such as a streptavidin from a *Streptomyces* species or a functionally active fragment thereof that includes at least one functional subunit containing a binding site for the agent (e.g. streptavidin binding peptide) and/or for biotin, a biotin derivative or analog or a biotin mimic, such as generally contains at least one functional subunit of a wild-type streptavidin from *Streptomyces avidinii* set forth in SEQ ID NO: 1 or a functionally active fragment thereof. For example, in some embodiments, streptavidin can include a fragment of wild-type streptavidin, which is shortened at the N- and/or C-terminus. Such minimal streptavidins include any that begin N-terminally in the region of amino acid positions 10 to 16 of SEQ ID NO: 1 and terminate C-terminally in the region of amino acid positions 133 to 142 of SEQ ID NO: 1. In some embodiments, a functionally active fragment of streptavidin contains the sequence of amino acids set forth in SEQ ID NO: 2. In some embodiments, streptavidin, such as set forth in SEQ ID NO: 2, can further contain an N-terminal methionine at a position corresponding to Ala13 with numbering set forth in SEQ ID NO: 1. Reference to the position of residues in streptavidin or streptavidin muteins is with reference to numbering of residues in SEQ ID NO: 1.

[0187] In some aspects, streptavidin muteins include polypeptides that are distinguished from the sequence of an unmodified or wild-type streptavidin by one or more amino acid substitutions, deletions, or additions, but that include at least one functional subunit containing a binding site for the agent (e.g. streptavidin binding peptide) and/or biotin, a biotin derivative or analog or a streptavidin-binding peptide. In some aspects, streptavidin-like polypeptides and streptavidin muteins can be polypeptides which essentially are immunologically equivalent to wild-type streptavidin and are in particular capable of binding biotin, biotin derivatives or biotin analogues with the same or different affinity as wt-streptavidin. In some cases, streptavidin-like polypeptides or streptavidin muteins may contain amino acids which are not part of wild-type streptavidin or they may include only a part of wild-type streptavidin. In some embodiments, streptavidin-like polypeptides are polypeptides which are not identical to wild-type streptavidin, since the host does not have the enzymes which are required in order to transform the host-produced polypeptide into the structure of wild-type streptavidin. In some embodiments, streptavidin also may be present as streptavidin tetramers and streptavidin dimers, in particular streptavidin homotetramers, streptavidin homodimers, streptavidin heterotetramers and

streptavidin heterodimers. Generally, each subunit normally has a binding site for biotin or biotin analogues or for streptavidin-binding peptides. Examples of streptavidins or streptavidin muteins are mentioned, for example, in WO 86/02077, DE 19641876 Al, US 6,022,951, WO 98/40396 or WO 96/24606.

[0188] In some embodiments, a streptavidin mutein can contain amino acids that are not part of an unmodified or wild-type streptavidin or can include only a part of a wild-type or unmodified streptavidin. In some embodiments, a streptavidin mutein contains at least one subunit that can have one more amino acid substitutions (replacements) compared to a subunit of an unmodified or wild-type streptavidin, such as compared to the wild-type streptavidin subunit set forth in SEQ ID NO: 1 or a functionally active fragment thereof, e.g. set forth in SEQ ID NO: 2. In some embodiments, at least one subunit of a streptavidin mutein can have at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acid differences compared to a wild-type or unmodified streptavidin and/or contains at least one subunit that comprising an amino acid sequence that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the sequence of amino acids set forth in SEQ ID NO: 1 or 2, where such streptavidin mutein exhibits functional activity to bind the agent (e.g. streptavidin binding peptide) and/or biotin, a biotin derivative or analog or biotin mimic. In some embodiments, the amino acid replacements (substitutions) are conservative or non-conservative mutations. Examples of streptavidin muteins are known in the art, see e.g., U.S. Pat. No. 5,168,049; 5,506,121; 6,022,951; 6,156,493; 6,165,750; 6,103,493; or 6,368,813; or International published PCT App. No. WO2014/076277.

[0189] In some embodiments, streptavidin or a streptavidin mutein includes proteins containing one or more than one functional subunit containing one or more binding sites Z for biotin, a biotin derivative or analog or a streptavidin-binding peptide, such as two or more, three or more, four or more, and, in some cases, 5, 6, 7, 8, 9, 10, 11, 12 or more functional subunits. In some embodiments, streptavidin or streptavidin mutein can include a monomer; a dimer, including a heterodimer or a homodimer; a tetramer, including a homotetramer, a heterotetramer, a monovalent tetramer or a divalent tetramer; or can include higher ordered multimers or oligomers thereof.

[0190] In some embodiments, the binding molecule reagent is or contains a streptavidin mutein. In some embodiments, the streptavidin muteins contain one or more mutations (e.g. amino acid replacements) compared to wild-type streptavidin set forth in SEQ ID NO: 1 or a biologically active portion thereof. For example, biologically active portions of streptavidin can

include streptavidin variants that are shortened at the N- and/or the C-terminus, which in some cases is called a minimal streptavidin. In some embodiments, an N-terminally shortened minimal streptavidin, to which any of the mutations can be made, begins N-terminally in the region of the amino acid positions 10 to 16 and terminates C-terminally in the region of the amino acid positions 133 to 142 compared to the sequence set forth in SEQ ID NO: 1. In some embodiments, an N-terminally shortened streptavidin, to which any of the mutations can be made, contains the amino acid sequence set forth in SEQ ID NO: 2. In some embodiments, the minimal streptavidin contains an amino acid sequence from position Ala13 to Ser139 and optionally has an N-terminal methionine residue instead of Ala13. For purposes herein, the numbering of amino acid positions refers throughout to the numbering of wt-streptavidin set forth in SEQ ID NO: 1 (e.g. Argarana et al., Nucleic Acids Res. 14 (1986), 1871 -1882, cf. also Fig. 3).

[0191] In some embodiments, the streptavidin mutein is a mutant as described in U.S. Pat. No. 6,103,493. In some embodiments, the streptavidin mutein contains at least one mutation within the region of amino acid positions 44 to 53, based on the amino acid sequence of wild-type streptavidin, such as set forth in SEQ ID NO: 1. In some embodiments, the streptavidin mutein contains a mutation at one or more residues 44, 45, 46, and/or 47. In some embodiments, the streptavidin mutein contains a replacement of Glu at position 44 of wild-type streptavidin with a hydrophobic aliphatic amino acid, e.g. Val, Ala, Ile or Leu, any amino acid at position 45, an aliphatic amino acid, such as a hydrophobic aliphatic amino acid at position 46 and/or a replacement of Val at position 47 with a basic amino acid, e.g. Arg or Lys, such as generally Arg. In some embodiments, Ala is at position 46 and/or Arg is at position 47 and/or Val or Ile is at position 44. In some embodiments, the streptavidin mutant contains residues Val^{44}-Thr^{45}-Ala^{46}-Arg^{47}, such as set forth in exemplary streptavidin muteins containing the sequence of amino acids set forth in SEQ ID NO: 3 or SEQ ID NO: 4 (also known as streptavidin mutant 1, SAM1). In some embodiments, the streptavidin mutein contains residues Ile^{44}-Gly^{45}-Ala^{46}-Arg^{47}, such as set forth in exemplary streptavidin muteins containing the sequence of amino acids set forth in SEQ ID NO: 5 or 6 (also known as SAM2). In some cases, such streptavidin mutein are described, for example, in US patent 6,103,493, and are commercially available under the trademark Strep-Tactin®.

[0192] In some embodiment, the streptavidin mutein is a mutant as described in International Published PCT Appl. Nos. WO 2014/076277. In some embodiments, the streptavidin mutein contains at least two cysteine residues in the region of amino acid positions

44 to 53 with reference to amino acid positions set forth in SEQ ID NO: 1. In some embodiments, the cysteine residues are present at positions 45 and 52 to create a disulfide bridge connecting these amino acids. In such an embodiment, amino acid 44 is typically glycine or alanine and amino acid 46 is typically alanine or glycine and amino acid 47 is typically arginine. In some embodiments, the streptavidin mutein contains at least one mutation or amino acid difference in the region of amino acids residues 115 to 121 with reference to amino acid positions set forth in SEQ ID NO: 1. In some embodiments, the streptavidin mutein contains at least one mutation at amino acid position 117, 120 and 121 and/or a deletion of amino acids 118 and 119 and substitution of at least amino acid position 121.

[0193] In some embodiments, the streptavidin mutein contains a mutation at a position corresponding to position 117, which mutation can be to a large hydrophobic residue like Trp, Tyr or Phe or a charged residue like Glu, Asp or Arg or a hydrophilic residue like Asn or Gin, or, in some cases, the hydrophobic residues Leu, Met or Ala, or the polar residues Thr, Ser or His. In some embodiments, the mutation at position 117 is combined with a mutation at a position corresponding to position 120, which mutation can be to a small residue like Ser or Ala or Gly, and a mutation at a position corresponding to position 121, which mutation can be to a hydrophobic residue, such as a bulky hydrophobic residue like Trp, Tyr or Phe. In some embodiments, the mutation at position 117 is combined with a mutation at a position corresponding to position 120 of wildtype streptavidin set forth in SEQ ID NO:1 or a biologically active fragment thereof, which mutation can be a hydrophobic residue such as Leu, Ile, Met, or Val or, generally, Tyr or Phe, and a mutation at a position corresponding to position 121 compared to positions of wildtype streptavidin set forth in SEQ ID NO:1 or a biologically active fragment thereof, which mutation can be to a small residue like Gly, Ala, or Ser, or with Gln, or with a hydrophobic residue like Leu, Val, Ile, Trp, Tyr, Phe, or Met. In some embodiments, such muteins also can contain residues Val44-Thr45-Ala46-Arg47 or residues Ile44-Gly45-Ala46-Arg47. In some embodiments, the streptavidin mutein contains the residues Val44, Thr45, Ala46, Arg47, Glu117, Gly120 and Tyr121. In some embodiments, the mutein streptavidin contains the sequence of amino acids set forth in SEQ ID NO:27 or SEQ ID NO:28, or a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the sequence of amino acids set forth in SEQ ID NO: 27 or SEQ ID NO: 28, contains the residues Val44, Thr45, Ala46, Arg47, Glu117, Gly120 and Tyr121 and exhibits functional activity to bind to biotin, a biotin analog or a streptavidin-binding peptide.

[0194] In some embodiments, a streptavidin mutein can contain any of the above mutations in any combination, and the resulting streptavidin mutein may exhibit a binding affinity with a K_D that is less than 2.7 x 10^{-4} M for the streptavidin binding peptide, such as one comprising amino acids Trp Arg His Pro Gln Phe Gly Gly; also called Strep-tag® (set forth in SEQ ID NO: 7) and/or with a K_D that is less than 1.4 x 10^{-4} M for the streptavidin binding peptide, such as one comprising amino acids Trp Ser His Pro Gln Phe Glu Lys; also called Strep-tag® II (set forth in SEQ ID NO: 8 or SEQ ID NO:58) and/or with a K_D that is less than 1 x 10^{-4} M, 5 x 10^{-4} M, 1 x 10^{-5} M, 5x 10^{-5} M, 1 x 10^{-6} M, 5 x 10^{-6} M or 1 x 10^{-7} M, but generally greater than 1 x 10^{-13} M, 1 x 10^{-12} M or 1 x 10^{-11} M for any of the streptavidin binding peptides set forth in any of SEQ ID NOS:7-19 or 58.

[0195] In some embodiments, the streptavidin mutein exhibits the sequence of amino acids set forth in any of SEQ ID NOs: 3-6, 27 or 28, or a sequence of amino acids that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the sequence of amino acids set forth in any of SEQ ID NO: 3-6, 27 or 28, and exhibits a binding affinity with a K_D that is less than 2.7 x 10^{-4} M for the streptavidin binding peptide, such as one comprising amino acids Trp Arg His Pro Gln Phe Gly Gly; also called Strep-tag® (set forth in SEQ ID NO: 7) and/or with a K_D that is less than 1.4 x 10^{-4} M for the streptavidin binding peptide, such as one comprising amino acids Trp Ser His Pro Gln Phe Glu Lys; also called Strep-tag® II (set forth in SEQ ID NO: 8 or 58) and/or with a K_D that is less than 1 x 10^{-4} M, 5 x 10^{-4} M, 1 x 10^{-5} M, 5x 10^{-5} M, 1 x 10^{-6} M, 5 x 10^{-6} M or 1 x 10^{-7} M, but generally greater than 1 x 10^{-13} M, 1 x 10^{-12} M or 1 x 10^{-11} M for any of the peptide ligands set forth in any of SEQ ID NOS:7-19 or 58.

[0196] In some embodiments, the streptavidin mutein also exhibits binding to other streptavidin ligands, such as but not limited to, biotin, iminobiotin, lipoic acid, desthiobiotin, diaminobiotin, HABA (hydroxyazobenzene-benzoic acid) and/or dimethyl-HABA. In some embodiments, the streptavidin mutein exhibits a binding affinity for another streptavidin ligand, such as biotin or desthiobiotin, that is greater than the binding affinity of the streptavidin mutein for a streptavidin peptide ligand, such as set forth in any of SEQ ID NOS: 7-19 or 58. Thus, in some embodiments, biotin or a biotin analog or derivative (e.g. desthiobiotin) can be employed as a competition reagent in the provided methods. For example, as an example, the interaction of a mutein streptavidin designated Strep-tactin® (e.g. containing the sequence set forth in SEQ ID NO: 4) with the streptaviding peptide designated Strep-tag® II (e.g. containing amino acids set forth in SEQ ID NO: 8 or 58) is characterized by a binding affinity with a K_D of

approximately 10^{-6} M compared to approximately 10^{-13} M for the bitoin-streptavidin interaction. In some cases, biotin, which can bind with high affinity to the Strep-tactin® with a K_D of between or between about 10^{-10} and 10^{-13} M, can compete with Strep-tag® II for the binding site.

[0197] In some embodiments, the binding molecule is a reagent that is an oligomer or a polymer of one or more streptavidin or avidin or of any analog or mutein of streptavidin or an analog or mutein of avidin (e.g. neutravidin). In some embodiments, the oligomer is generated or produced from a plurality of individual molecules (e.g. a plurality of homo-tetramers) of the same streptavidin, streptavidin mutein, avidin or avidin mutein, in which case each binding site Z, e.g. Z1, of the oligomer is the same.

[0198] In some embodiments the binding molecule reagent is an oligomer or a polymer of one or more streptavidin or avidin or of any analog or mutein of streptavidin or an analog or mutein of avidin (e.g. neutravidin). In some embodiments, the oligomer is generated or produced from a plurality of individual molecules (e.g. a plurality of homo-tetramers) of the same streptavidin, streptavidin mutein, avidin or avidin mutein, in which case each binding site Z, e.g. Z1, of the oligomer is the same. For example, in some cases, an oligomer can contain a plurality of binding sites Z1, such as at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 40, 45, 50 or more binding sites Z1. In some embodiments, the oligomer is generated or produced from a plurality of individual molecules that can be hetero-tetramers of a streptavidin, streptavidin mutein, avidin or avidin mutein and/or from a plurality of two or more different individual molecules (e.g. different homo-tetramers) of streptavidin, streptavidin mutein, avidin or avidin mutein that differ in their binding sites Z, e.g. Z1 and Z2, in which case a plurality of different binding sites Z, e.g. Z1 and Z2, may be present in the oligomer. For example, in some cases, an oligomer can contain a plurality of binding sites Z1 and a plurality of binding sites Z, which, in combination, can include at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 40, 45, 50 or more combined binding sites Z1 and Z2.

[0199] In some cases, the respective oligomer or polymer may be crosslinked by a polysaccharide. In one embodiment, oligomers or polymers of streptavidin or of avidin or of analogs of streptavidin or of avidin (e.g., neutravidin) can be prepared by the introduction of carboxyl residues into a polysaccharide, e. g. dextran, essentially as described in Noguchi, A, et al, Bioconjugate Chemistry (1992) 3,132-137 in a first step. In some such aspects, streptavidin or avidin or analogs thereof then may be linked via primary amino groups of internal lysine residue and/or the free N-terminus to the carboxyl groups in the dextran backbone using

conventional carbodiimide chemistry in a second step. In some cases, cross-linked oligomers or polymers of streptavidin or avidin or of any analog of streptavidin or avidin may also be obtained by crosslinking via bifunctional molecules, serving as a linker, such as glutardialdehyde or by other methods described in the art.

[0200] In some embodiments, the oligomer or polymer is obtained by crosslinking individual molecules or a complex of subunits that make up an individual molecule using a bifunctional linker or other chemical linker, such as glutardialdehyde or by other methods known in the art. In some aspects, cross-linked oligomers or polymers of streptavidin or avidin or of any mutein or analog of streptavidin or avidin may be obtained by crosslinking individual streptavidin or avidin molecules via bifunctional molecules, serving as a linker, such as glutardialdehyde or by other methods described in the art. It is, for example, possible to generate oligomers of streptavidin muteins by introducing thiol groups into the streptavidin mutein (this can, for example, be done by reacting the streptavidin mutein with 2-iminothiolan (Trauts reagent) and by activating, for example in a separate reaction, amino groups available in the streptavidin mutein. In some embodiments, this activation of amino groups can be achieved by reaction of the streptavidin mutein with a commercially available heterobifunctional crosslinker such as sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo SMCC) or Succinimidyl-6-[(β-maleimidopropionamido)hexanoate (SMPH). In some such embodiments, the two reaction products so obtained are mixed together, typically leading to the reaction of the thiol groups contained in the one batch of modified streptavidin mutein with the activated (such as by maleimide functions) amino acids of the other batch of modified streptavidin mutein. In some cases, by this reaction, multimers/oligomers of the streptavidin mutein are formed. These oligomers can have any suitable number of individual molecules, such as at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 40, 45, 50 or more, and the oligomerization degree can be varied according to the reaction condition.

[0201] In some embodiments, the oligomeric or polymeric reagent can be isolated via size exclusion chromatography and any desired fraction can be used as the binding molecule reagent. For example, in some embodiments, after reacting the modified streptavidin mutein, in the presence of 2-iminothiolan and a heterobifunctional crosslinker such as sulfo SMCC, the oligomeric or polymeric reagent can be isolated via size exclusion chromatography and any desired fraction can be used as the reagent. In some embodiments, the oligomers do not have (and do not need to have) a single molecular weight but they may observe a statistical weight

distribution such as Gaussian distribution. In some cases, any oligomer with more than three streptavidin or mutein tetramers, e.g., homotetramers or heterotetramers, can be used as a soluble reagent, such as generally 3 to 50 tetramers, e.g., homotetramers or heterotetramers, 10 to 40 tetramers, e.g., homotetramers or heterotetramers, or 25 to 35 tetramers, e.g., homotetramers or heterotetramers. The oligomers might have, for example, from 3 to 25 streptavidin mutein tetramers, e.g., homotetramers or heterotetramers. In some aspects, with a molecular weight of about 50 kDa for streptavidin muteins, the soluble oligomers can have a molecular weight from about 150 kDa to about 2000 kDa, about 150 kDa to about 1500 kDa, about 150 kDa to about 1250 kDa, about 150 kDa to 1000 kDa, about 150 kDa to about 500 kDa or about 150 kDa to about 300 kDa, about 300 kDa to about 2000 kDa, about 300 kDa to about 1500 kDa, about 300 kDa to about 1250 kDa, about 300 kDa to 1000 kDa, about 300 kDa to about 500 kDa, about 500 kDa to about 2000 kDa, about 500 kDa to about 1500 kDa, about 500 kDa to about 1250 kDa, about 500 kDa to 1000 kDa, about 1000 kDa to about 2000 kDa, about 1000 kDa to about 1500 kDa, about 1000 kDa to about 1250 kDa, about 1250 kDa to about 2000 kDa or about 1500 kDa to about 2000 kDa. Generally, because each streptavidin molecule/mutein has four biotin binding sites, such a reagent can provide 12 to 160 binding sites Z, such as 12 to 100 binding sites Z.

[0202] In some embodiments, the binding molecule reagent, such as any of the described streptavidin or streptavidin mutein (e.g. Strep-Tactin®) reagents, can be labeled with one or more detectable markers. In some embodiments, the reagent is labeled with a fluorescent marker. Exemplary labeled Strep-Tactin® reagents are known or are commercially available including, for example, Strep-Tactin-HRP, Strep-Tactin AP, Strep-Tactin Chromeo 488, Strep-Tactin Chromeo 546, or Strep-Tactin Oyster 645, each available from IBA (Goettingen Germany).

[0203] In some embodiments, a streptavidin binding peptide (e.g. Strep-tag, such as Strep-tag® II or twin-Strep-tag) can be recognized by an antibody or antigen-binding fragment. In some embodiments, the antibody contains at least one binding site that can specifically bind an epitope or region of the agent of the cell surface conjugate. Antibodies against such streptavidin binding peptides are known, including antibodies against the peptide sequence SAWSHPQFEK (SEQ ID NO:58) or the minimal sequence WSHPQFEK (SEQ ID NO:8), such as present in Strep-tag® II or twin-strep-tag (Schmidt T. & Skerra A., Nature protocols, 2007; international patent application publication number WO2015067768). In some embodiments, a streptavidin binding peptide (e.g. Strep-tag, such as Strep-tag® II or twin-Strep-tag) can be detected using

for example, the commercially available StrepMAB-Classic (IBA, Goettingen Germany), StrepMAB-lmmo (IBA), anti-Streptag II antibody (Genscript), or Strep-tag antibody (Qiagen). In some embodiments, the antibodies or binding partners are labeled with one or more detectable marker, to facilitate purification, selection and/or detection of engineered cells. For example, separation may be based on binding to fluorescently labeled antibodies.

C. Exemplary Conjugates

[0204] In some embodiments, the conjugate contains a modified EGFR and at least one agent (e.g. affinity tag) that is capable of binding streptavidin or a streptavidin mutein. In some embodiments, the modified EGFR is any as described above, such as the EGFRt set forth in SEQ ID NO: 46. In some embodiments, the conjugate contains a modified HER2 and at least one agent (e.g. affinity tag) that is capable of binding streptavidin or a streptavidin mutein. In some embodiments, the modified HER2/neu/ErbB2 is any as described above, such as the HER2t set forth in SEQ ID NO: 92. In some such embodiments, the agent is a streptavidin binding peptide, such as a Strep-tag®, Strep-Tag® II or twin-strep-Tag, including any described above and set forth in SEQ ID NO:7, 8, 15-19 or 58.

[0205] In some embodiments, the streptavidin binding peptide is fused to the N-terminal part of the cell surface molecule. In some embodiments, the provided cell surface conjugate comprises an amino acid sequence containing amino acid residues in which the N-terminal to C-terminal order comprises: the streptavidin binding peptide (e.g. Strep-tag®, Strep-Tag® II or twin-strep-Tag, such as set forth in any of SEQ ID NOS: 7, 8, 15-19 or 58) and a modified EGFR (e.g. EGFRt, such as set forth in SEQ ID NO: 46). In some instances, the streptavidin-binding peptide is directly fused to the modified EGFR. In some instances, the streptavidin-binding peptide is indirectly fused or joined to the modified EGFR, such as via at least one polypeptide linker as described (e.g. set forth in any one of SEQ ID NO: 55, 56, 59-62, 98 or 99). For example, in some aspects, the streptavidin binding peptide is connected to a first polypeptide linker that is attached to the modified EGFR. In some aspects the cell surface conjugate containing the EGFRt and streptavidin binding peptide is a fusion protein.

[0206] In some embodiments, the provided cell surface conjugate comprises an amino acid sequence containing amino acid residues in which the N-terminal to C-terminal order comprises: the streptavidin binding peptide (e.g. Strep-tag®, Strep-Tag® II or twin-strep-Tag, such as set forth in any of SEQ ID NOS: 7, 8, 15-19 or 58) and a modified HER2/neu/ErbB2 (e.g. HEr2t, such as set forth in SEQ ID NO: 92). In some instances, the streptavidin-binding peptide is

directly fused to the modified HER2/neu/ErbB2. In some instances, the streptavidin-binding peptide is indirectly fused or joined to the modified EGFR, such as via at least one polypeptide linker as described (e.g. set forth in any one of SEQ ID NO: 55, 56, 59-62, 98 or 99). For example, in some aspects, the streptavidin binding peptide is connected to a first polypeptide linker that is attached to the modified HER2/neu/ErbB2. In some aspects the cell surface conjugate containing the HER2t and streptavidin binding peptide is a fusion protein.

[0207] In some embodiments, the conjugate comprises in N-terminal to C-terminal order: (1) at least one an agent (e.g. Strep-tag®) that has the sequence of amino acids set forth in any of SEQ ID NOs: 8, 15-19 or 58 or a sequence that has at least at or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to any of SEQ ID NOs: 8, 15-19 or 58; (2) optionally, at least one peptide linker, such as a peptide linker set forth in SEQ ID NO: 55, 56, 59-62, 98 or 99; and (3) a modified EGFR that has the sequence of amino acids set forth in SEQ ID NO: 46 or a sequence that has at least at or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NOs: 46.

[0208] In some embodiments, the conjugate comprises in N-terminal to C-terminal order: (1) at least one an agent (e.g. Strep-tag®) that has the sequence of amino acids set forth in any of SEQ ID NOS: 8, 15-19 or 58 or a sequence that has at least at or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to any of SEQ ID NOS: 8, 15-19 or 58; (2) optionally, at least one peptide linker, such as a peptide linker set forth in SEQ ID NOS: 55, 56, 59-62, 98 or 99; and (3) a modified HER2/neu/ErbB2 that has the sequence of amino acids set forth in SEQ ID NO: 92 or a sequence that has at least at or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO: 92.

[0209] In some embodiments, the conjugate contains a PSMA or a modified form thereof, e.g., a modified PSMA, and at least one agent (e.g. affinity tag) that is capable of binding streptavidin or a streptavidin mutein. In some embodiments, the modified PSMA is any as described above, such as set forth in SEQ ID NO: 95 or an N-terminal truncation of SEQ ID NO:94. In some such embodiments, the agent is a streptavidin binding peptide, such as a Strep-tag®, Strep-Tag® II or twin-strep-Tag, including any described above and set forth in SEQ ID NOS: 7, 8, 15-19, or 58.

[0210] In some embodiments, the conjugate comprises in N-terminal to C-terminal order: (1) a PSMA or a modified PSMA that has the sequence of amino acids set forth in any of SEQ

ID NOS: 94 or 95 or a sequence that has at least at or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity toany of SEQ ID NOS: 94 or 95; (2) optionally, at least one peptide linker, such as a peptide linker set forth in SEQ ID NOS: 55, 56, 59-62, 98 or 99; and (3) at least one an agent (e.g. Strep-tag®) that has the sequence of amino acids set forth in any of SEQ ID NOS: 8, 15-19 or 58 or a sequence that has at least at or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to any of SEQ ID NOS: 8, 15-19 or 58.

[0211] In some embodiments, the cell surface conjugate protein further contains at its N-terminus a signal peptide for targeting the expressed conjugate to the secretory pathway for insertion into the membrane as a surface or membrane protein. In some embodiments, the signal peptide is the native signal peptide of the cell surface molecule, e.g. EGFR contained in SEQ ID NO:64. In some embodiments, the signal peptide is a non-native or heterologous signal peptide. In some embodiments, the signal peptide is derived from Granulocyte macrophage colony-stimulating factor receptor (GMCSFR) alpha chain that has the sequence of amino acids set forth in SEQ ID NO: 48, such as is encoded by the sequence set forth in SEQ ID NO: 47 or a sequence with degenerate codons thereof.

III. ENGINEERED CELLS

[0212] Provided herein are engineered cells that express any of the provided cell surface conjugates. In some embodiments, the engineered cells co-express the cell surface conjugate and one or more recombinant antigen receptor. In some embodiments, the cells can include cells genetically engineered with a recombinant receptor, such as a chimeric antigen receptor.

A. Recombinant Antigen Receptors

[0213] Provided are engineered cells, such as T cells, that express a recombinant receptor, including chimeric receptors containing ligand-binding domains or binding fragments thereof, such as functional non-TCR antigen receptors, such as chimeric antigen receptors (CARs), and also including T cell receptors (TCRs), such as transgenic TCRs, and components thereof. The chimeric receptor, such as a CAR, generally includes the extracellular antigen (or ligand) binding domain linked to one or more intracellular signaling components, in some aspects via linkers and/or transmembrane domain(s).

I. Chimeric Antigen Receptors

[0214] In some embodiments, engineered cells, such as T cells, are provided that express a CAR with specificity for a particular antigen (or marker or ligand), such as an antigen expressed on the surface of a particular cell type. In some embodiments, the antigen is a polypeptide. In some embodiments, it is a carbohydrate or other molecule. In some embodiments, the antigen is selectively expressed or overexpressed on cells of the disease or condition, e.g., the tumor or pathogenic cells, as compared to normal or non-targeted cells or tissues. In other embodiments, the antigen is expressed on normal cells and/or is expressed on the engineered cells.

[0215] In particular embodiments, the recombinant receptor, such as chimeric receptor, contains an intracellular signaling domain or region, which includes an activating or stimulating cytoplasmic signaling domain or region (also interchangeably called an activating or stimulating intracellular signaling domain or region), such as an activating or stimulating cytoplasmic (intracellular) domain or region capable of inducing a primary activation signal in a T cell, for example, a cytoplasmic signaling domain or region of a T cell receptor (TCR) component (e.g. a cytoplasmic signaling domain or region of a zeta chain of a CD3-zeta (CD3ζ) chain or a functional variant or signaling portion thereof) and/or that comprises an immunoreceptor tyrosine-based activation motif (ITAM).

[0216] In some embodiments, the chimeric receptor further contains an extracellular ligand-binding domain that specifically binds to a ligand (e.g. antigen) antigen. In some embodiments, the chimeric receptor is a CAR that contains an extracellular antigen-recognition domain that specifically binds to an antigen. In some embodiments, the ligand, such as an antigen, is a protein expressed on the surface of cells. In some embodiments, the CAR is a TCR-like CAR and the antigen is a processed peptide antigen, such as a peptide antigen of an intracellular protein, which, like a TCR, is recognized on the cell surface in the context of a major histocompatibility complex (MHC) molecule.

[0217] Exemplary antigen receptors, including CARs, and methods for engineering and introducing such receptors into cells, include those described, for example, in international patent application publication numbers WO200014257, WO2013126726, WO2012/129514, WO2014031687, WO2013/166321, WO2013/071154, WO2013/123061 U.S. patent application publication numbers US2002131960, US2013287748, US20130149337, U.S. Patent Nos.: 6,451,995, 7,446,190, 8,252,592, 8,339,645, 8,398,282, 7,446,179, 6,410,319, 7,070,995, 7,265,209, 7,354,762, 7,446,191, 8,324,353, and 8,479,118, and European patent application

number EP2537416,and/or those described by Sadelain et al., Cancer Discov. 2013 April; 3(4): 388–398; Davila et al. (2013) PLoS ONE 8(4): e61338; Turtle et al., Curr. Opin. Immunol., 2012 October; 24(5): 633-39; Wu et al., Cancer, 2012 March 18(2): 160-75. In some aspects, the antigen receptors include a CAR as described in U.S. Patent No.: 7,446,190, and those described in International Patent Application Publication No.: WO/2014055668 A1. Examples of the CARs include CARs as disclosed in any of the aforementioned publications, such as WO2014031687, US 8,339,645, US 7,446,179, US 2013/0149337, U.S. Patent No.: 7,446,190, US Patent No.: 8,389,282, Kochenderfer et al., 2013, Nature Reviews Clinical Oncology, 10, 267-276 (2013); Wang et al. (2012) J. Immunother. 35(9): 689-701; and Brentjens et al., Sci Transl Med. 2013 5(177). See also WO2014031687, US 8,339,645, US 7,446,179, US 2013/0149337, U.S. Patent No.: 7,446,190, and US Patent No.: 8,389,282.

[0218] In some embodiments, the CAR is constructed with a specificity for a particular antigen (or marker or ligand), such as an antigen expressed in a particular cell type to be targeted by adoptive therapy, *e.g.*, a cancer marker, and/or an antigen intended to induce a dampening response, such as an antigen expressed on a normal or non-diseased cell type. Thus, the CAR typically includes in its extracellular portion one or more antigen binding molecules, such as one or more antigen-binding fragment, domain, or portion, or one or more antibody variable domains, and/or antibody molecules. In some embodiments, the CAR includes an antigen-binding portion or portions of an antibody molecule, such as a single-chain antibody fragment (scFv) derived from the variable heavy (VH) and variable light (VL) chains of a monoclonal antibody (mAb).

[0219] In some embodiments, the antibody or antigen-binding portion thereof is expressed on cells as part of a recombinant receptor, such as an antigen receptor. Among the antigen receptors are functional non-TCR antigen receptors, such as chimeric antigen receptors (CARs). Generally, a CAR containing an antibody or antigen-binding fragment that exhibits TCR-like specificity directed against peptide-MHC complexes also may be referred to as a TCR-like CAR. In some embodiments, the extracellular antigen binding domain specific for an MHC-peptide complex of a TCR-like CAR is linked to one or more intracellular signaling components, in some aspects via linkers and/or transmembrane domain(s). In some embodiments, such molecules can typically mimic or approximate a signal through a natural antigen receptor, such as a TCR, and, optionally, a signal through such a receptor in combination with a costimulatory receptor.

[0220] In some embodiments, the recombinant receptor, such as a chimeric receptor (e.g. CAR), includes a ligand-binding domain that binds, such as specifically binds, to an antigen (or a ligand). Among the antigens targeted by the chimeric receptors are those expressed in the context of a disease, condition, or cell type to be targeted via the adoptive cell therapy. Among the diseases and conditions are proliferative, neoplastic, and malignant diseases and disorders, including cancers and tumors, including hematologic cancers, cancers of the immune system, such as lymphomas, leukemias, and/or myelomas, such as B, T, and myeloid leukemias, lymphomas, and multiple myelomas.

[0221] In some embodiments, the antigen (or a ligand) is a polypeptide. In some embodiments, it is a carbohydrate or other molecule. In some embodiments, the antigen (or a ligand) is selectively expressed or overexpressed on cells of the disease or condition, *e.g.*, the tumor or pathogenic cells, as compared to normal or non-targeted cells or tissues. In other embodiments, the antigen is expressed on normal cells and/or is expressed on the engineered cells.

[0222] In some embodiments, the CAR contains an antibody or an antigen-binding fragment (*e.g.* scFv) that specifically recognizes an antigen, such as an intact antigen, expressed on the surface of a cell.

[0010] In some embodiments, the antigen (or a ligand) is a tumor antigen or cancer marker. In some embodiments, the antigen (or a ligand) is or includes αvβ6 integrin (avb6 integrin), B cell maturation antigen (BCMA), B7-H3, B7-H6, carbonic anhydrase 9 (CA9, also known as CAIX or G250), a cancer-testis antigen, cancer/testis antigen 1B (CTAG, also known as NY-ESO-1 and LAGE-2), carcinoembryonic antigen (CEA), a cyclin, cyclin A2, C-C Motif Chemokine Ligand 1 (CCL-1), CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD138, CD171, epidermal growth factor protein (EGFR), truncated epidermal growth factor protein (tEGFR), type III epidermal growth factor receptor mutation (EGFR vIII), epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), ephrinB2, ephrine receptor A2 (EPHa2), estrogen receptor, Fc receptor like 5 (FCRL5; also known as Fc receptor homolog 5 or FCRH5), fetal acetylcholine receptor (fetal AchR), a folate binding protein (FBP), folate receptor alpha, ganglioside GD2, O-acetylated GD2 (OGD2), ganglioside GD3, glycoprotein 100 (gp100), G Protein Coupled Receptor 5D (GPCR5D), Her2/neu (receptor tyrosine kinase erb-B2), Her3 (erb-B3), Her4 (erb-B4), erbB dimers, Human high molecular weight-melanoma-associated antigen (HMW-MAA), hepatitis B surface antigen, Human leukocyte antigen A1 (HLA-A1), Human leukocyte antigen A2 (HLA-A2), IL-22

receptor alpha(IL-22Ra), IL-13 receptor alpha 2 (IL-13Ra2), kinase insert domain receptor (kdr), kappa light chain, L1 cell adhesion molecule (L1-CAM), CE7 epitope of L1-CAM, Leucine Rich Repeat Containing 8 Family Member A (LRRC8A), Lewis Y, Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, mesothelin, c-Met, murine cytomegalovirus (CMV), mucin 1 (MUC1), MUC16, natural killer group 2 member D (NKG2D) ligands, melan A (MART-1), neural cell adhesion molecule (NCAM), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME), progesterone receptor, a prostate specific antigen, prostate stem cell antigen (PSCA), prostate specific membrane antigen (PSMA), Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1), survivin, Trophoblast glycoprotein (TPBG also known as 5T4), tumor-associated glycoprotein 72 (TAG72), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor receptor 2 (VEGFR2), Wilms Tumor 1 (WT-1), a pathogen-specific antigen, or an antigen associated with a universal tag, and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV or other pathogens. Antigens targeted by the receptors in some embodiments include antigens associated with a B cell malignancy, such as any of a number of known B cell marker. In some embodiments, the antigen is or includes CD20, CD19, CD22, ROR1, CD45, CD21, CD5, CD33, Igkappa, Iglambda, CD79a, CD79b or CD30.

[0223] In some embodiments, the antigen (or a ligand) is a tumor antigen or cancer marker. In some embodiments, the antigen (or a ligand) is or includes orphan tyrosine kinase receptor RORl, tEGFR, Her2, Ll-CAM, CD19, CD20, CD22, BCMA, mesothelin, CEA, and hepatitis B surface antigen, anti-folate receptor, CD23, CD24, CD30, CD33, CD38, CD44, EGFR, EGP-2, EGP-4, EPHa2, ErbB2, 3, or 4, FBP, fetal acethycholine e receptor, GD2, GD3, HMW-MAA, IL-22R-alpha, IL-13R-alpha2, kdr, kappa light chain, Lewis Y, L1-cell adhesion molecule, MAGE-A1, mesothelin, MUC1, MUC16, PSCA, NKG2D Ligands, NY-ESO-1, MART-1, gp100, oncofetal antigen, TAG72, VEGF-R2, carcinoembryonic antigen (CEA), prostate specific antigen, PSMA, Her2/neu, estrogen receptor, progesterone receptor, ephrinB2, CD123, CS-1, c-Met, GD-2, and MAGE A3, CE7, Wilms Tumor 1 (WT-1), a cyclin, such as cyclin A1 (CCNA1), and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV or other pathogens. Antigens targeted by the receptors in some embodiments include antigens associated with a B cell malignancy, such as any of a number of known B cell marker. In some embodiments, the antigen targeted by the receptor is CD20, CD19, CD22, ROR1, CD45, CD21, CD5, CD33, Igkappa, Iglambda, CD79a, CD79b or CD30.

[0224] In some embodiments, the antigen is a pathogen-specific or pathogen-expressed antigen. In some embodiments, the antigen is a viral antigen (such as a viral antigen from HIV, HCV, HBV, etc.), bacterial antigens, and/or parasitic antigens.

[0225] In some embodiments, the CAR contains a TCR-like antibody, such as an antibody or an antigen-binding fragment (*e.g.* scFv) that specifically recognizes an intracellular antigen, such as a tumor-associated antigen, presented on the cell surface as a MHC-peptide complex. In some embodiments, an antibody or antigen-binding portion thereof that recognizes an MHC-peptide complex can be expressed on cells as part of a recombinant receptor, such as an antigen receptor. Among the antigen receptors are functional non-TCR antigen receptors, such as chimeric antigen receptors (CARs). Generally, a CAR containing an antibody or antigen-binding fragment that exhibits TCR-like specificity directed against peptide-MHC complexes also may be referred to as a TCR-like CAR.

[0226] Reference to "Major histocompatibility complex" (MHC) refers to a protein, generally a glycoprotein, that contains a polymorphic peptide binding site or binding groove that can, in some cases, complex with peptide antigens of polypeptides, including peptide antigens processed by the cell machinery. In some cases, MHC molecules can be displayed or expressed on the cell surface, including as a complex with peptide, *i.e.* MHC-peptide complex, for presentation of an antigen in a conformation recognizable by an antigen receptor on T cells, such as a TCRs or TCR-like antibody. Generally, MHC class I molecules are heterodimers having a membrane spanning α chain, in some cases with three α domains, and a non-covalently associated β2 microglobulin. Generally, MHC class II molecules are composed of two transmembrane glycoproteins, α and β, both of which typically span the membrane. An MHC molecule can include an effective portion of an MHC that contains an antigen binding site or sites for binding a peptide and the sequences necessary for recognition by the appropriate antigen receptor. In some embodiments, MHC class I molecules deliver peptides originating in the cytosol to the cell surface, where a MHC-peptide complex is recognized by T cells, such as generally $CD8^+$ T cells, but in some cases CD4+ T cells. In some embodiments, MHC class II molecules deliver peptides originating in the vesicular system to the cell surface, where they are typically recognized by $CD4^+$ T cells. Generally, MHC molecules are encoded by a group of linked loci, which are collectively termed H-2 in the mouse and human leukocyte antigen (HLA) in humans. Hence, typically human MHC can also be referred to as human leukocyte antigen (HLA).

[0227] The term "MHC-peptide complex" or "peptide-MHC complex" or variations thereof, refers to a complex or association of a peptide antigen and an MHC molecule, such as, generally, by non-covalent interactions of the peptide in the binding groove or cleft of the MHC molecule. In some embodiments, the MHC-peptide complex is present or displayed on the surface of cells. In some embodiments, the MHC-peptide complex can be specifically recognized by an antigen receptor, such as a TCR, TCR-like CAR or antigen-binding portions thereof.

[0228] In some embodiments, a peptide, such as a peptide antigen or epitope, of a polypeptide can associate with an MHC molecule, such as for recognition by an antigen receptor. Generally, the peptide is derived from or based on a fragment of a longer biological molecule, such as a polypeptide or protein. In some embodiments, the peptide typically is about 8 to about 24 amino acids in length. In some embodiments, a peptide has a length of from or from about 9 to 22 amino acids for recognition in the MHC Class II complex. In some embodiments, a peptide has a length of from or from about 8 to 13 amino acids for recognition in the MHC Class I complex. In some embodiments, upon recognition of the peptide in the context of an MHC molecule, such as MHC-peptide complex, the antigen receptor, such as TCR or TCR-like CAR, produces or triggers an activation signal to the T cell that induces a T cell response, such as T cell proliferation, cytokine production, a cytotoxic T cell response or other response.

[0229] In some embodiments, a TCR-like antibody or antigen-binding portion, are known or can be produced by methods known in the art (see e.g. US Published Application Nos. US 2002/0150914; US 2003/0223994; US 2004/0191260; US 2006/0034850; US 2007/00992530; US20090226474; US20090304679; and International PCT Publication No. WO 03/068201).

[0230] In some embodiments, an antibody or antigen-binding portion thereof that specifically binds to a MHC-peptide complex, can be produced by immunizing a host with an effective amount of an immunogen containing a specific MHC-peptide complex. In some cases, the peptide of the MHC-peptide complex is an epitope of antigen capable of binding to the MHC, such as a tumor antigen, for example a universal tumor antigen, myeloma antigen or other antigen as described below. In some embodiments, an effective amount of the immunogen is then administered to a host for eliciting an immune response, wherein the immunogen retains a three-dimensional form thereof for a period of time sufficient to elicit an immune response against the three-dimensional presentation of the peptide in the binding groove of the MHC molecule. Serum collected from the host is then assayed to determine if desired antibodies that

recognize a three-dimensional presentation of the peptide in the binding groove of the MHC molecule is being produced. In some embodiments, the produced antibodies can be assessed to confirm that the antibody can differentiate the MHC-peptide complex from the MHC molecule alone, the peptide of interest alone, and a complex of MHC and irrelevant peptide. The desired antibodies can then be isolated.

[0231] In some embodiments, an antibody or antigen-binding portion thereof that specifically binds to an MHC-peptide complex can be produced by employing antibody library display methods, such as phage antibody libraries. In some embodiments, phage display libraries of mutant Fab, scFV or other antibody forms can be generated, for example, in which members of the library are mutated at one or more residues of a CDR or CDRs. Exemplary of such methods are known in the art (see *e.g.* US published application No. US20020150914, US2014/0294841; and Cohen CJ. *et al.* (2003) *J Mol. Recogn.* 16:324-332).

[0232] The term "antibody" herein is used in the broadest sense and includes polyclonal and monoclonal antibodies, including intact antibodies and functional (antigen-binding) antibody fragments, including fragment antigen binding (Fab) fragments, $F(ab')_2$ fragments, Fab' fragments, Fv fragments, recombinant IgG (rIgG) fragments, variable heavy chain (V_H) regions capable of specifically binding the antigen, single chain antibody fragments, including single chain variable fragments (scFv), and single domain antibodies (*e.g.*, sdAb, sdFv, nanobody) fragments. The term encompasses genetically engineered and/or otherwise modified forms of immunoglobulins, such as intrabodies, peptibodies, chimeric antibodies, fully human antibodies, humanized antibodies, and heteroconjugate antibodies, multispecific, *e.g.*, bispecific, antibodies, diabodies, triabodies, and tetrabodies, tandem di-scFv, tandem tri-scFv. Unless otherwise stated, the term "antibody" should be understood to encompass functional antibody fragments thereof. The term also encompasses intact or full-length antibodies, including antibodies of any class or sub-class, including IgG and sub-classes thereof, IgM, IgE, IgA, and IgD.

[0233] In some embodiments, the antigen-binding proteins, antibodies and antigen binding fragments thereof specifically recognize an antigen of a full-length antibody. In some embodiments, the heavy and light chains of an antibody can be full-length or can be an antigen-binding portion (a Fab, F(ab')2, Fv or a single chain Fv fragment (scFv)). In other embodiments, the antibody heavy chain constant region is chosen from, *e.g.*, IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD, and IgE, particularly chosen from, *e.g.*, IgG1, IgG2, IgG3, and IgG4, more particularly, IgG1 (*e.g.*, human IgG1). In another embodiment, the antibody light chain constant region is chosen from, *e.g.*, kappa or lambda, particularly kappa.

[0234] Among the provided antibodies are antibody fragments. An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, $F(ab')_2$; diabodies; linear antibodies; variable heavy chain (V_H) regions, single-chain antibody molecules such as scFvs and single-domain V_H single antibodies; and multispecific antibodies formed from antibody fragments. In particular embodiments, the antibodies are single-chain antibody fragments comprising a variable heavy chain region and/or a variable light chain region, such as scFvs.

[0235] The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (V_H and V_L, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three CDRs. (See, *e.g.*, Kindt et al. Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007). A single V_H or V_L domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a V_H or V_L domain from an antibody that binds the antigen to screen a library of complementary V_L or V_H domains, respectively. See, *e.g.*, Portolano et al., J. Immunol. 150:880-887 (1993); Clarkson et al., Nature 352:624-628 (1991).

[0236] Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody. In some embodiments, the CAR comprises an antibody heavy chain domain that specifically binds the antigen, such as a cancer marker or cell surface antigen of a cell or disease to be targeted, such as a tumor cell or a cancer cell, such as any of the target antigens described herein or known in the art.

[0237] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells. In some embodiments, the antibodies are recombinantly-produced fragments, such as fragments comprising arrangements that do not occur naturally, such as those with two or more antibody regions or chains joined by synthetic linkers, *e.g.*, peptide linkers, and/or that are may not be produced by enzyme digestion of a naturally-occurring intact antibody. In some embodiments, the antibody fragments are scFvs.

[0238] A "humanized" antibody is an antibody in which all or substantially all CDR amino acid residues are derived from non-human CDRs and all or substantially all FR amino acid residues are derived from human FRs. A humanized antibody optionally may include at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of a non-human antibody, refers to a variant of the non-human antibody that has undergone humanization, typically to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (*e.g.*, the antibody from which the CDR residues are derived), *e.g.*, to restore or improve antibody specificity or affinity.

[0239] Thus, in some embodiments, the chimeric antigen receptor, including TCR-like CARs, includes an extracellular portion containing an antibody or antibody fragment. In some embodiments, the antibody or fragment includes an scFv. In some aspects, the chimeric antigen receptor includes an extracellular portion containing the antibody or fragment and an intracellular signaling domain.

[0240] In some embodiments, the recombinant receptor such as the CAR, such as the antibody portion thereof, further includes a spacer, which may be or include at least a portion of an immunoglobulin constant region or variant or modified version thereof, such as a hinge region, e.g., an IgG4 hinge region, and/or a CH1/CL and/or Fc region. In some embodiments, the constant region or portion is of a human IgG, such as IgG4 or IgG1. In some aspects, the portion of the constant region serves as a spacer region between the antigen-recognition component, e.g., scFv, and transmembrane domain. The spacer can be of a length that provides for increased responsiveness of the cell following antigen binding, as compared to in the absence of the spacer. In some examples, the spacer is at or about 12 amino acids in length or is no more than 12 amino acids in length. Exemplary spacers include those having at least about 10 to 229 amino acids, about 10 to 200 amino acids, about 10 to 175 amino acids, about 10 to 150 amino acids, about 10 to 125 amino acids, about 10 to 100 amino acids, about 10 to 75 amino acids, about 10 to 50 amino acids, about 10 to 40 amino acids, about 10 to 30 amino acids, about 10 to 20 amino acids, or about 10 to 15 amino acids, and including any integer between the endpoints of any of the listed ranges. In some embodiments, a spacer region has about 12 amino acids or less, about 119 amino acids or less, or about 229 amino acids or less. Exemplary spacers include IgG4 hinge alone, IgG4 hinge linked to CH2 and CH3 domains, or IgG4 hinge linked to the CH3 domain. Exemplary spacers include, but are not limited to, those described in Hudecek

et al. (2013) *Clin. Cancer Res.*, 19:3153 or international patent application publication number WO2014031687. In some embodiments, the spacer has the sequence set forth in SEQ ID NO: 70, and is encoded by the sequence set forth in SEQ ID NO: 71. In some embodiments, the spacer has the sequence set forth in SEQ ID NO: 72. In some embodiments, the spacer has the sequence set forth in SEQ ID NO: 73.

[0241] In some embodiments, the constant region or portion is of IgD. In some embodiments, the spacer has the sequence set forth in SEQ ID NO: 74. In some embodiments, the spacer has a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 70, 72, 73 and 74.

[0242] The antigen recognition domain generally is linked to one or more intracellular signaling components, such as signaling components that mimic activation through an antigen receptor complex, such as a TCR complex, in the case of a CAR, and/or signal via another cell surface receptor. Thus, in some embodiments, the antigen binding component (e.g., antibody) is linked to one or more transmembrane and intracellular signaling domains. In some embodiments, the transmembrane domain is fused to the extracellular domain. In one embodiment, a transmembrane domain that naturally is associated with one of the domains in the receptor, e.g., CAR, is used. In some instances, the transmembrane domain is selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex.

[0243] The transmembrane domain in some embodiments is derived either from a natural or from a synthetic source. Where the source is natural, the domain in some aspects is derived from any membrane-bound or transmembrane protein. Transmembrane regions include those derived from (i.e. comprise at least the transmembrane region(s) of) the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154. Alternatively the transmembrane domain in some embodiments is synthetic. In some aspects, the synthetic transmembrane domain comprises predominantly hydrophobic residues such as leucine and valine. In some aspects, a triplet of phenylalanine, tryptophan and valine will be found at each end of a synthetic transmembrane domain. In some embodiments, the linkage is by linkers, spacers, and/or transmembrane domain(s).

[0244] Among the intracellular signaling domains are those that mimic or approximate a signal through a natural antigen receptor, a signal through such a receptor in combination with a costimulatory receptor, and/or a signal through a costimulatory receptor alone. In some embodiments, a short oligo- or polypeptide linker, for example, a linker of between 2 and 10 amino acids in length, such as one containing glycines and serines, e.g., glycine-serine doublet, is present and forms a linkage between the transmembrane domain and the cytoplasmic signaling domain of the CAR.

[0245] The receptor, e.g., the CAR, generally includes at least one intracellular signaling component or components. In some embodiments, the receptor includes an intracellular component of a TCR complex, such as a TCR CD3 chain that mediates T-cell activation and cytotoxicity, e.g., CD3 zeta chain. Thus, in some aspects, the ROR1-binding antibody is linked to one or more cell signaling modules. In some embodiments, cell signaling modules include CD3 transmembrane domain, CD3 intracellular signaling domains, and/or other CD transmembrane domains. In some embodiments, the receptor, e.g., CAR, further includes a portion of one or more additional molecules such as Fc receptor γ, CD8, CD4, CD25, or CD16. For example, in some aspects, the CAR includes a chimeric molecule between CD3-zeta (CD3-ζ) or Fc receptor γ and CD8, CD4, CD25 or CD16.

[0246] In some embodiments, upon ligation of the CAR, the cytoplasmic domain or intracellular signaling domain of the CAR activates at least one of the normal effector functions or responses of the immune cell, e.g., T cell engineered to express the CAR. For example, in some contexts, the CAR induces a function of a T cell such as cytolytic activity or T-helper activity, such as secretion of cytokines or other factors. In some embodiments, a truncated portion of an intracellular signaling domain of an antigen receptor component or costimulatory molecule is used in place of an intact immunostimulatory chain, for example, if it transduces the effector function signal. In some embodiments, the intracellular signaling domain or domains include the cytoplasmic sequences of the T cell receptor (TCR), and in some aspects also those of co-receptors that in the natural context act in concert with such receptor to initiate signal transduction following antigen receptor engagement, and/or any derivative or variant of such molecules, and/or any synthetic sequence that has the same functional capability.

[0247] In the context of a natural TCR, full activation generally requires not only signaling through the TCR, but also a costimulatory signal. Thus, in some embodiments, to promote full activation, a component for generating secondary or co-stimulatory signal is also included in the CAR. In other embodiments, the CAR does not include a component for generating a

costimulatory signal. In some aspects, an additional CAR is expressed in the same cell and provides the component for generating the secondary or costimulatory signal.

[0248] T cell activation is in some aspects described as being mediated by two classes of cytoplasmic signaling sequences: those that initiate antigen-dependent primary activation through the TCR (primary cytoplasmic signaling sequences), and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal (secondary cytoplasmic signaling sequences). In some aspects, the CAR includes one or both of such signaling components.

[0249] In some aspects, the CAR includes a primary cytoplasmic signaling sequence that regulates primary activation of the TCR complex. Primary cytoplasmic signaling sequences that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs. Examples of ITAM containing primary cytoplasmic signaling sequences include those derived from TCR or CD3 zeta, FcR gamma or FcR beta. In some embodiments, cytoplasmic signaling molecule(s) in the CAR contain(s) a cytoplasmic signaling domain, portion thereof, or sequence derived from CD3 zeta.

[0250] In some embodiments, the CAR includes a signaling domain and/or transmembrane portion of a costimulatory receptor, such as CD28, 4-1BB, OX40, DAP10, and ICOS. In some aspects, the same CAR includes both the activating and costimulatory components.

[0251] In some embodiments, the activating domain is included within one CAR, whereas the costimulatory component is provided by another CAR recognizing another antigen. In some embodiments, the CARs include activating or stimulatory CARs, and costimulatory CARs, both expressed on the same cell (*see* WO2014/055668).

[0252] In certain embodiments, the intracellular signaling domain comprises a CD28 transmembrane and signaling domain linked to a CD3 (e.g., CD3-zeta) intracellular domain. In some embodiments, the intracellular signaling domain comprises a chimeric CD28 and CD137 (4-1BB, TNFRSF9) co-stimulatory domains, linked to a CD3 zeta intracellular domain.

[0253] In some embodiments, the CAR encompasses one or more, e.g., two or more, costimulatory domains and an activation domain, e.g., primary activation domain, in the cytoplasmic portion. Exemplary CARs include intracellular components of CD3-zeta, CD28, and 4-1BB.

[0254] In some cases, CARs are referred to as first, second, and/or third generation CARs. In some aspects, a first generation CAR is one that solely provides a CD3-chain induced signal upon antigen binding; in some aspects, a second-generation CARs is one that provides such a

signal and costimulatory signal, such as one including an intracellular signaling domain from a costimulatory receptor such as CD28 or CD137; in some aspects, a third generation CAR in some aspects is one that includes multiple costimulatory domains of different costimulatory receptors.

[0255] In some embodiments, the chimeric antigen receptor includes an extracellular portion containing the antibody or fragment described herein. In some aspects, the chimeric antigen receptor includes an extracellular portion containing the antibody or fragment described herein and an intracellular signaling domain. In some embodiments, the antibody or fragment includes an scFv or a single-domain V_H antibody and the intracellular domain contains an ITAM. In some aspects, the intracellular signaling domain includes a signaling domain of a zeta chain of a CD3-zeta (CD3ζ) chain. In some embodiments, the chimeric antigen receptor includes a transmembrane domain linking the extracellular domain and the intracellular signaling domain.

[0256] In some aspects, the transmembrane domain contains a transmembrane portion of CD28. The extracellular domain and transmembrane can be linked directly or indirectly. In some embodiments, the extracellular domain and transmembrane are linked by a spacer, such as any described herein. In some embodiments, the chimeric antigen receptor contains an intracellular domain of a T cell costimulatory molecule, such as between the transmembrane domain and intracellular signaling domain. In some aspects, the T cell costimulatory molecule is CD28 or 41BB.

[0257] In some embodiments, the CAR contains an antibody, e.g., an antibody fragment, a transmembrane domain that is or contains a transmembrane portion of CD28 or a functional variant thereof, and an intracellular signaling domain containing a signaling portion of CD28 or functional variant thereof and a signaling portion of CD3 zeta or functional variant thereof. In some embodiments, the CAR contains an antibody, e.g., antibody fragment, a transmembrane domain that is or contains a transmembrane portion of CD28 or a functional variant thereof, and an intracellular signaling domain containing a signaling portion of a 4-1BB or functional variant thereof and a signaling portion of CD3 zeta or functional variant thereof. In some such embodiments, the receptor further includes a spacer containing a portion of an Ig molecule, such as a human Ig molecule, such as an Ig hinge, e.g. an IgG4 hinge, such as a hinge-only spacer.

[0258] In some embodiments, the transmembrane domain of the receptor, e.g., the CAR is a transmembrane domain of human CD28 or variant thereof, e.g., a 27-amino acid transmembrane domain of a human CD28 (Accession No.: P10747.1), or is a transmembrane domain that comprises the sequence of amino acids set forth in SEQ ID NO: 77 or a sequence of amino acids

that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:77; in some embodiments, the transmembrane-domain containing portion of the recombinant receptor comprises the sequence of amino acids set forth in SEQ ID NO: 78 or a sequence of amino acids having at least at or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto.

[0259] In some embodiments, the chimeric antigen receptor contains an intracellular domain of a T cell costimulatory molecule. In some aspects, the T cell costimulatory molecule is CD28 or 41BB.

[0260] In some embodiments, the intracellular signaling domain comprises an intracellular costimulatory signaling domain of human CD28 or functional variant or portion thereof thereof, such as a 41 amino acid domain thereof and/or such a domain with an LL to GG substitution at positions 186-187 of a native CD28 protein. In some embodiments, the intracellular signaling domain can comprise the sequence of amino acids set forth in SEQ ID NO: 79 or 80 or a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 79 or 80. In some embodiments, the intracellular domain comprises an intracellular costimulatory signaling domain of 41BB or functional variant or portion thereof, such as a 42-amino acid cytoplasmic domain of a human 4-1BB (Accession No. Q07011.1) or functional variant or portion thereof, such as the sequence of amino acids set forth in SEQ ID NO: 81 or a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 81.

[0261] In some embodiments, the intracellular signaling domain comprises a human CD3 zeta stimulatory signaling domain or functional variant thereof, such as an 112 AA cytoplasmic domain of isoform 3 of human CD3ζ (Accession No.: P20963.2) or a CD3 zeta signaling domain as described in U.S. Patent No.: 7,446,190 or U.S. Patent No. 8,911,993. In some embodiments, the intracellular signaling domain comprises the sequence of amino acids set forth in SEQ ID NO: 82, 83 or 84 or a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 82, 83 or 84.

[0262] In some aspects, the spacer contains only a hinge region of an IgG, such as only a hinge of IgG4 or IgG1, such as the hinge only spacer set forth in SEQ ID NO:70. In other embodiments, the spacer is an Ig hinge, e.g., and IgG4 hinge, linked to a CH2 and/or CH3

domains. In some embodiments, the spacer is an Ig hinge, e.g., an IgG4 hinge, linked to CH2 and CH3 domains, such as set forth in SEQ ID NO:73. In some embodiments, the spacer is an Ig hinge, e.g., an IgG4 hinge, linked to a CH3 domain only, such as set forth in SEQ ID NO:72. In some embodiments, the spacer is or comprises a glycine-serine rich sequence or other flexible linker such as known flexible linkers.

2. T cell Receptors

[0263] In some embodiments, engineered cells, such as T cells, are provided that express a T cell receptor (TCR) or antigen-binding portion thereof that recognizes an peptide epitope or T cell epitope of a target polypeptide, such as an antigen of a tumor, viral or autoimmune protein.

[0264] In some embodiments, a "T cell receptor" or "TCR" is a molecule that contains a variable α and β chains (also known as TCRα and TCRβ, respectively) or a variable γ and δ chains (also known as TCRα and TCRβ, respectively), or antigen-binding portions thereof, and which is capable of specifically binding to a peptide bound to an MHC molecule. In some embodiments, the TCR is in the αβ form. Typically, TCRs that exist in αβ and γδ forms are generally structurally similar, but T cells expressing them may have distinct anatomical locations or functions. A TCR can be found on the surface of a cell or in soluble form. Generally, a TCR is found on the surface of T cells (or T lymphocytes) where it is generally responsible for recognizing antigens bound to major histocompatibility complex (MHC) molecules.

[0265] Unless otherwise stated, the term "TCR" should be understood to encompass full TCRs as well as antigen-binding portions or antigen-binding fragments thereof. In some embodiments, the TCR is an intact or full-length TCR, including TCRs in the αβ form or γδ form. In some embodiments, the TCR is an antigen-binding portion that is less than a full-length TCR but that binds to a specific peptide bound in an MHC molecule, such as binds to an MHC-peptide complex. In some cases, an antigen-binding portion or fragment of a TCR can contain only a portion of the structural domains of a full-length or intact TCR, but yet is able to bind the peptide epitope, such as MHC-peptide complex, to which the full TCR binds. In some cases, an antigen-binding portion contains the variable domains of a TCR, such as variable α chain and variable β chain of a TCR, sufficient to form a binding site for binding to a specific MHC-peptide complex. Generally, the variable chains of a TCR contain complementarity determining regions involved in recognition of the peptide, MHC and/or MHC-peptide complex.

[0266] In some embodiments, the variable domains of the TCR contain hypervariable loops, or complementarity determining regions (CDRs), which generally are the primary contributors to antigen recognition and binding capabilities and specificity. In some embodiments, a CDR of a TCR or combination thereof forms all or substantially all of the antigen-binding site of a given TCR molecule. The various CDRs within a variable region of a TCR chain generally are separated by framework regions (FRs), which generally display less variability among TCR molecules as compared to the CDRs (see, e.g., Jores et al., Proc. Nat'l Acad. Sci. U.S.A. 87:9138, 1990; Chothia et al., EMBO J. 7:3745, 1988; see also Lefranc et al., Dev. Comp. Immunol. 27:55, 2003). In some embodiments, CDR3 is the main CDR responsible for antigen binding or specificity, or is the most important among the three CDRs on a given TCR variable region for antigen recognition, and/or for interaction with the processed peptide portion of the peptide-MHC complex. In some contexts, the CDR1 of the alpha chain can interact with the N-terminal part of certain antigenic peptides. In some contexts, CDR1 of the beta chain can interact with the C-terminal part of the peptide. In some contexts, CDR2 contributes most strongly to or is the primary CDR responsible for the interaction with or recognition of the MHC portion of the MHC-peptide complex. In some embodiments, the variable region of the β-chain can contain a further hypervariable region (CDR4 or HVR4), which generally is involved in superantigen binding and not antigen recognition (Kotb (1995) Clinical Microbiology Reviews, 8:411-426).

[0267] In some embodiments, a TCR also can contain a constant domain, a transmembrane domain and/or a short cytoplasmic tail (see, e.g., Janeway et al., Immunobiology: The Immune System in Health and Disease, 3rd Ed., Current Biology Publications, p. 4:33, 1997). In some aspects, each chain of the TCR can possess one N-terminal immunoglobulin variable domain, one immunoglobulin constant domain, a transmembrane region, and a short cytoplasmic tail at the C-terminal end. In some embodiments, a TCR is associated with invariant proteins of the CD3 complex involved in mediating signal transduction.

[0268] In some embodiments, a TCR chain contains one or more constant domain. For example, the extracellular portion of a given TCR chain (e.g., α-chain or β-chain) can contain two immunoglobulin-like domains, such as a variable domain (e.g., Vα or Vβ; typically amino acids 1 to 116 based on Kabat numbering Kabat et al., "Sequences of Proteins of Immunological Interest, US Dept. Health and Human Services, Public Health Service National Institutes of Health, 1991, 5th ed.) and a constant domain (e.g., α-chain constant domain or Cα, typically positions 117 to 259 of the chain based on Kabat numbering or β chain constant domain or C_β,

typically positions 117 to 295 of the chain based on Kabat) adjacent to the cell membrane. For example, in some cases, the extracellular portion of the TCR formed by the two chains contains two membrane-proximal constant domains, and two membrane-distal variable domains, which variable domains each contain CDRs. The constant domain of the TCR may contain short connecting sequences in which a cysteine residue forms a disulfide bond, thereby linking the two chains of the TCR. In some embodiments, a TCR may have an additional cysteine residue in each of the α and β chains, such that the TCR contains two disulfide bonds in the constant domains.

[0269] In some embodiments, the TCR chains contain a transmembrane domain. In some embodiments, the transmembrane domain is positively charged. In some cases, the TCR chain contains a cytoplasmic tail. In some cases, the structure allows the TCR to associate with other molecules like CD3 and subunits thereof. For example, a TCR containing constant domains with a transmembrane region may anchor the protein in the cell membrane and associate with invariant subunits of the CD3 signaling apparatus or complex. The intracellular tails of CD3 signaling subunits (e.g. CD3γ, CD3δ, CD3ε and CD3ζ chains) contain one or more immunoreceptor tyrosine-based activation motif or ITAM that are involved in the signaling capacity of the TCR complex.

[0270] In some embodiments, the TCR may be a heterodimer of two chains α and β (or optionally γ and δ) or it may be a single chain TCR construct. In some embodiments, the TCR is a heterodimer containing two separate chains (α and β chains or γ and δ chains) that are linked, such as by a disulfide bond or disulfide bonds.

[0271] In some embodiments, the TCR can be generated from a known TCR sequence(s), such as sequences of Vα,β chains, for which a substantially full-length coding sequence is readily available. Methods for obtaining full-length TCR sequences, including V chain sequences, from cell sources are well known. In some embodiments, nucleic acids encoding the TCR can be obtained from a variety of sources, such as by polymerase chain reaction (PCR) amplification of TCR-encoding nucleic acids within or isolated from a given cell or cells, or synthesis of publicly available TCR DNA sequences.

[0272] In some embodiments, the TCR is obtained from a biological source, such as from cells such as from a T cell (e.g. cytotoxic T cell), T-cell hybridomas or other publicly available source. In some embodiments, the T-cells can be obtained from in vivo isolated cells. In some embodiments, the TCR is a thymically selected TCR. In some embodiments, the TCR is a neoepitope-restricted TCR. In some embodiments, the T- cells can be a cultured T-cell

hybridoma or clone. In some embodiments, the TCR or antigen-binding portion thereof can be synthetically generated from knowledge of the sequence of the TCR.

[0273] In some embodiments, the TCR is generated from a TCR identified or selected from screening a library of candidate TCRs against a target polypeptide antigen, or target T cell epitope thereof. TCR libraries can be generated by amplification of the repertoire of Vα and Vβ from T cells isolated from a subject, including cells present in PBMCs, spleen or other lymphoid organ. In some cases, T cells can be amplified from tumor-infiltrating lymphocytes (TILs). In some embodiments, TCR libraries can be generated from CD4+ or CD8+ cells. In some embodiments, the TCRs can be amplified from a T cell source of a normal of healthy subject, i.e. normal TCR libraries. In some embodiments, the TCRs can be amplified from a T cell source of a diseased subject, i.e. diseased TCR libraries. In some embodiments, degenerate primers are used to amplify the gene repertoire of Vα and Vβ, such as by RT-PCR in samples, such as T cells, obtained from humans. In some embodiments, scTv libraries can be assembled from naïve Vα and Vβ libraries in which the amplified products are cloned or assembled to be separated by a linker. Depending on the source of the subject and cells, the libraries can be HLA allele-specific. Alternatively, in some embodiments, TCR libraries can be generated by mutagenesis or diversification of a parent or scaffold TCR molecule. In some aspects, the TCRs are subjected to directed evolution, such as by mutagenesis, e.g., of the α or β chain. In some aspects, particular residues within CDRs of the TCR are altered. In some embodiments, selected TCRs can be modified by affinity maturation. In some embodiments, antigen-specific T cells may be selected, such as by screening to assess CTL activity against the peptide. In some aspects, TCRs, e.g. present on the antigen-specific T cells, may be selected, such as by binding activity, e.g., particular affinity or avidity for the antigen.

[0274] In some embodiments, the TCR or antigen-binding portion thereof is one that has been modified or engineered. In some embodiments, directed evolution methods are used to generate TCRs with altered properties, such as with higher affinity for a specific MHC-peptide complex. In some embodiments, directed evolution is achieved by display methods including, but not limited to, yeast display (Holler et al. (2003) Nat Immunol, 4, 55-62; Holler et al. (2000) Proc Natl Acad Sci U S A, 97, 5387-92), phage display (Li et al. (2005) Nat Biotechnol, 23, 349-54), or T cell display (Chervin et al. (2008) J Immunol Methods, 339, 175-84). In some embodiments, display approaches involve engineering, or modifying, a known, parent or reference TCR. For example, in some cases, a wild-type TCR can be used as a template for producing mutagenized TCRs in which in one or more residues of the CDRs are mutated, and

mutants with an desired altered property, such as higher affinity for a desired target antigen, are selected.

[0275] In some embodiments, peptides of a target polypeptide for use in producing or generating a TCR of interest are known or can be readily identified by a skilled artisan. In some embodiments, peptides suitable for use in generating TCRs or antigen-binding portions can be determined based on the presence of an HLA-restricted motif in a target polypeptide of interest, such as a target polypeptide described below. In some embodiments, peptides are identified using computer prediction models known to those of skill in the art. In some embodiments, for predicting MHC class I binding sites, such models include, but are not limited to, ProPred1 (Singh and Raghava (2001) Bioinformatics 17(12):1236-1237, and SYFPEITHI (see Schuler et al. (2007) Immunoinformatics Methods in Molecular Biology, 409(1): 75-93 2007). In some embodiments, the MHC-restricted epitope is HLA-A0201, which is expressed in approximately 39-46% of all Caucasians and therefore, represents a suitable choice of MHC antigen for use preparing a TCR or other MHC-peptide binding molecule.

[0276] HLA-A0201-binding motifs and the cleavage sites for proteasomes and immune-proteasomes using computer prediction models are known to those of skill in the art. For predicting MHC class I binding sites, such models include, but are not limited to, ProPred1 (described in more detail in Singh and Raghava, ProPred: prediction of HLA-DR binding sites. BIOINFORMATICS 17(12):1236-1237 2001), and SYFPEITHI (see Schuler et al. SYFPEITHI, Database for Searching and T-Cell Epitope Prediction. in Immunoinformatics Methods in Molecular Biology, vol 409(1): 75-93 2007)

[0277] In some embodiments, the TCR or antigen binding portion thereof may be a recombinantly produced natural protein or mutated form thereof in which one or more property, such as binding characteristic, has been altered. In some embodiments, a TCR may be derived from one of various animal species, such as human, mouse, rat, or other mammal. A TCR may be cell-bound or in soluble form. In some embodiments, for purposes of the provided methods, the TCR is in cell-bound form expressed on the surface of a cell.

[0278] In some embodiments, the TCR is a full-length TCR. In some embodiments, the TCR is an antigen-binding portion. In some embodiments, the TCR is a dimeric TCR (dTCR). In some embodiments, the TCR is a single-chain TCR (sc-TCR). In some embodiments, a dTCR or scTCR have the structures as described in WO 03/020763, WO 04/033685, WO2011/044186.

[0279] In some embodiments, the TCR contains a sequence corresponding to the transmembrane sequence. In some embodiments, the TCR does contain a sequence corresponding to cytoplasmic sequences. In some embodiments, the TCR is capable of forming a TCR complex with CD3. In some embodiments, any of the TCRs, including a dTCR or scTCR, can be linked to signaling domains that yield an active TCR on the surface of a T cell. In some embodiments, the TCR is expressed on the surface of cells.

[0280] In some embodiments a dTCR contains a first polypeptide wherein a sequence corresponding to a TCR α chain variable region sequence is fused to the N terminus of a sequence corresponding to a TCR α chain constant region extracellular sequence, and a second polypeptide wherein a sequence corresponding to a TCR β chain variable region sequence is fused to the N terminus a sequence corresponding to a TCR β chain constant region extracellular sequence, the first and second polypeptides being linked by a disulfide bond. In some embodiments, the bond can correspond to the native inter-chain disulfide bond present in native dimeric αβ TCRs. In some embodiments, the interchain disulfide bonds are not present in a native TCR. For example, in some embodiments, one or more cysteines can be incorporated into the constant region extracellular sequences of dTCR polypeptide pair. In some cases, both a native and a non-native disulfide bond may be desirable. In some embodiments, the TCR contains a transmembrane sequence to anchor to the membrane.

[0281] In some embodiments, a dTCR contains a TCR α chain containing a variable α domain, a constant α domain and a first dimerization motif attached to the C-terminus of the constant α domain, and a TCR β chain comprising a variable β domain, a constant β domain and a first dimerization motif attached to the C-terminus of the constant β domain, wherein the first and second dimerization motifs easily interact to form a covalent bond between an amino acid in the first dimerization motif and an amino acid in the second dimerization motif linking the TCR α chain and TCR β chain together.

[0282] In some embodiments, the TCR is a scTCR. Typically, a scTCR can be generated using methods known to those of skill in the art, See e.g., Soo Hoo, W. F. et al. PNAS (USA) 89, 4759 (1992); Wülfing, C. and Plückthun, A., J. Mol. Biol. 242, 655 (1994); Kurucz, I. et al. PNAS (USA) 90 3830 (1993); International published PCT Nos. WO 96/13593, WO 96/18105, WO99/60120, WO99/18129, WO 03/020763, WO2011/044186; and Schlueter, C. J. et al. J. Mol. Biol. 256, 859 (1996). In some embodiments, a scTCR contains an introduced non-native disulfide interchain bond to facilitate the association of the TCR chains (see e.g. International published PCT No. WO 03/020763). In some embodiments, a scTCR is a non-disulfide linked

truncated TCR in which heterologous leucine zippers fused to the C-termini thereof facilitate chain association (see e.g. International published PCT No. WO99/60120). In some embodiments, a scTCR contain a TCRα variable domain covalently linked to a TCRβ variable domain via a peptide linker (see e.g., International published PCT No. WO99/18129).

[0283] In some embodiments, a scTCR contains a first segment constituted by an amino acid sequence corresponding to a TCR α chain variable region, a second segment constituted by an amino acid sequence corresponding to a TCR β chain variable region sequence fused to the N terminus of an amino acid sequence corresponding to a TCR β chain constant domain extracellular sequence, and a linker sequence linking the C terminus of the first segment to the N terminus of the second segment.

[0284] In some embodiments, a scTCR contains a first segment constituted by an α chain variable region sequence fused to the N terminus of an α chain extracellular constant domain sequence, and a second segment constituted by a β chain variable region sequence fused to the N terminus of a sequence β chain extracellular constant and transmembrane sequence, and, optionally, a linker sequence linking the C terminus of the first segment to the N terminus of the second segment.

[0285] In some embodiments, a scTCR contains a first segment constituted by a TCR β chain variable region sequence fused to the N terminus of a β chain extracellular constant domain sequence, and a second segment constituted by an α chain variable region sequence fused to the N terminus of a sequence α chain extracellular constant and transmembrane sequence, and, optionally, a linker sequence linking the C terminus of the first segment to the N terminus of the second segment.

[0286] In some embodiments, the linker of a scTCRs that links the first and second TCR segments can be any linker capable of forming a single polypeptide strand, while retaining TCR binding specificity. In some embodiments, the linker sequence may, for example, have the formula -P-AA-P- wherein P is proline and AA represents an amino acid sequence wherein the amino acids are glycine and serine. In some embodiments, the first and second segments are paired so that the variable region sequences thereof are orientated for such binding. Hence, in some cases, the linker has a sufficient length to span the distance between the C terminus of the first segment and the N terminus of the second segment, or vice versa, but is not too long to block or reduces bonding of the scTCR to the target ligand. In some embodiments, the linker can contain from or from about 10 to 45 amino acids, such as 10 to 30 amino acids or 26 to 41 amino acids residues, for example 29, 30, 31 or 32 amino acids. In some embodiments, the

linker has the formula -PGGG-(SGGGG)$_5$-P- wherein P is proline, G is glycine and S is serine (SEQ ID NO:89). In some embodiments, the linker has the sequence GSADDAKKDAAKKDGKS (SEQ ID NO:90)

[0287] In some embodiments, the scTCR contains a covalent disulfide bond linking a residue of the immunoglobulin region of the constant domain of the α chain to a residue of the immunoglobulin region of the constant domain of the β chain. In some embodiments, the interchain disulfide bond in a native TCR is not present. For example, in some embodiments, one or more cysteines can be incorporated into the constant region extracellular sequences of the first and second segments of the scTCR polypeptide. In some cases, both a native and a non-native disulfide bond may be desirable.

[0288] In some embodiments of a dTCR or scTCR containing introduced interchain disulfide bonds, the native disulfide bonds are not present. In some embodiments, the one or more of the native cysteines forming a native interchain disulfide bonds are substituted to another residue, such as to a serine or alanine. In some embodiments, an introduced disulfide bond can be formed by mutating non-cysteine residues on the first and second segments to cysteine. Exemplary non-native disulfide bonds of a TCR are described in published International PCT No. WO2006/000830.

[0289] In some embodiments, the TCR or antigen-binding fragment thereof exhibits an affinity with an equilibrium binding constant for a target antigen of between or between about 10-5 and 10-12 M and all individual values and ranges therein. In some embodiments, the target antigen is an MHC-peptide complex or ligand.

[0290] In some embodiments, nucleic acid or nucleic acids encoding a TCR, such as α and β chains, can be amplified by PCR, cloning or other suitable means and cloned into a suitable expression vector or vectors. The expression vector can be any suitable recombinant expression vector, and can be used to transform or transfect any suitable host. Suitable vectors include those designed for propagation and expansion or for expression or both, such as plasmids and viruses.

[0291] In some embodiments, the vector can a vector of the pUC series (Fermentas Life Sciences), the pBluescript series (Stratagene, LaJolla, Calif.), the pET series (Novagen, Madison, Wis.), the pGEX series (Pharmacia Biotech, Uppsala, Sweden), or the pEX series (Clontech, Palo Alto, Calif.). In some cases, bacteriophage vectors, such as λG10, λGT11, λZapII (Stratagene), λEMBL4, and λNM1149, also can be used. In some embodiments, plant expression vectors can be used and include pBI01, pBI101.2, pBI101.3, pBI121 and pBIN19

(Clontech). In some embodiments, animal expression vectors include pEUK-Cl, pMAM and pMAMneo (Clontech). In some embodiments, a viral vector is used, such as a retroviral vector.

[0292] In some embodiments, the recombinant expression vectors can be prepared using standard recombinant DNA techniques. In some embodiments, vectors can contain regulatory sequences, such as transcription and translation initiation and termination codons, which are specific to the type of host (e.g., bacterium, fungus, plant, or animal) into which the vector is to be introduced, as appropriate and taking into consideration whether the vector is DNA- or RNA-based. In some embodiments, the vector can contain a nonnative promoter operably linked to the nucleotide sequence encoding the TCR or antigen-binding portion (or other MHC-peptide binding molecule). In some embodiments, the promoter can be a non-viral promoter or a viral promoter, such as a cytomegalovirus (CMV) promoter, an SV40 promoter, an RSV promoter, and a promoter found in the long-terminal repeat of the murine stem cell virus. Other promoters known to a skilled artisan also are contemplated.

[0293] In some embodiments, to generate a vector encoding a TCR, the α and β chains are PCR amplified from total cDNA isolated from a T cell clone expressing the TCR of interest and cloned into an expression vector. In some embodiments, the α and β chains are cloned into the same vector. In some embodiments, the α and β chains are cloned into different vectors. In some embodiments, the generated α and β chains are incorporated into a retroviral, e.g. lentiviral, vector.

3. Chimeric Auto-Antibody Receptors (CAARs)

[0294] In some embodiments, the recombinant receptor is a chimeric autoantibody receptor (CAAR). In some embodiments, the CAAR is specific for an autoantibody. In some embodiments, a cell expressing the CAAR, such as a T cell engineered to express a CAAR, can be used to specifically bind to and kill autoantibody-expressing cells, but not normal antibody expressing cells. In some embodiments, CAAR-expressing cells can be used to treat an autoimmune disease associated with expression of self-antigens, such as autoimmune diseases. In some embodiments, CAAR-expressing cells can target B cells that ultimately produce the autoantibodies and display the autoantibodies on their cell surfaces, mark these B cells as disease-specific targets for therapeutic intervention. In some embodiments, CAAR-expressing cells can be used to efficiently targeting and killing the pathogenic B cells in autoimmune diseases by targeting the disease-causing B cells using an antigen-specific chimeric autoantibody

receptor. In some embodiments, the recombinant receptor is a CAAR, such as any described in U.S. Patent Application Pub. No. US 2017/0051035.

[0295] In some embodiments, the CAAR comprises an autoantibody binding domain, a transmembrane domain, and an intracellular signaling region. In some embodiments, the intracellular signaling region comprises an intracellular signaling domain. In some embodiments, the intracellular signaling domain is or comprises a primary signaling domain, a signaling domain that is capable of inducing a primary activation signal in a T cell, a signaling domain of a T cell receptor (TCR) component, and/or a signaling domain comprising an immunoreceptor tyrosine-based activation motif (ITAM). In some embodiments, the intracellular signaling region comprises a secondary or costimulatory signaling region (secondary intracellular signaling regions).

[0296] In some embodiments, the autoantibody binding domain comprises an autoantigen or a fragment thereof. The choice of autoantigen can depend upon the type of autoantibody being targeted. For example, the autoantigen may be chosen because it recognizes an autoantibody on a target cell, such as a B cell, associated with a particular disease state, e.g. an autoimmune disease, such as an autoantibody-mediated autoimmune disease. In some embodiments, the autoimmune disease includes pemphigus vulgaris (PV). Exemplary autoantigens include desmoglein 1 (Dsg1) and Dsg3.

B. Nucleic Acids and Vectors

[0297] Provided are polynucleotides (nucleic acid molecules) encoding the cell surface conjugates and recombinant receptors, vectors for genetically engineering cells to express such conjugates and receptors and methods for producing the engineered cells.

[0298] In some embodiments, provided are polynucleotides that encode any of the cell surface conjugates provided herein. In some aspects, the polynucleotide contains a single coding sequence, such as only a coding sequence encoding the cell surface conjugate. In other instances, the polynucleotide contains at least two different coding sequences, such as a first nucleic acid sequence encoding the cell surface conjugate and a second nucleic acid sequence encoding a recombinant receptor. In some aspects, the recombinant receptor is or contains a chimeric antigen receptor (CAR). In some aspects, the recombinant receptor is or contains a T cell receptor (TCR), e.g., a transgenic TCR. In some aspects, the recombinant receptor is or contains a chimeric autoantibody receptor (CAAR). In some embodiments, the polynucleotides and vectors are used for co-expression in cells of the cell surface conjugate and the recombinant

receptor. In some embodiments, the polynucleotide encodes a cell surface conjugate that is capable of being expressed on the surface of a cell. In some embodiments, the nucleic acid encoding the cell surface conjugates encode a cell surface molecule comprising an extracellular portion and a transmembrane portion.

[0299] In some cases, the nucleic acid sequence encoding the conjugate contains a signal sequence that encodes a signal peptide. In some aspects, the signal sequence may encode a signal peptide derived from the native cell surface molecule. In other aspects, the signal sequence may encode a heterologous or non-native signal peptide, such as the exemplary signal peptide of the GMCSFR alpha chain set forth in SEQ ID NO: 48 and encoded by the nucleotide sequence set forth in SEQ ID NO:47.

[0300] In some cases, the nucleic acid sequence encoding the chimeric antigen receptor (CAR) contains a signal sequence that encodes a signal peptide. Non-limiting exemplary examples of signal peptides include, for example, the GMCSFR alpha chain signal peptide set forth in SEQ ID NO: 48 or the CD8 alpha signal peptide set forth in SEQ ID NO:75.

[0301] In some embodiments, the polynucleotide encoding the cell surface conjugate and/or recombinant receptor contains at least one promoter that is operatively linked to control expression of the cell surface conjugate and/or recombinant receptor. In some examples, the polynucleotide contains two, three, or more promoters operatively linked to control expression of the cell surface conjugate and/or recombinant receptor.

[0302] In certain cases where nucleic acid molecules encode two or more different polypeptide chains, each of the polypeptide chains can be encoded by a separate nucleic acid molecule. For example, two separate nucleic acids are provided, and each can be individually transferred or introduced into the cell for expression in the cell.

[0303] In some embodiments, such as those where the polynucleotide contains a first and second nucleic acid sequence, the coding sequences encoding each of the different polypeptide chains can be operatively linked to a promoter, which can be the same or different. In some embodiments, the nucleic acid molecule can contain a promoter that drives the expression of two or more different polypeptide chains. In some embodiments, such nucleic acid molecules can be multicistronic (bicistronic or tricistronic, see *e.g.*, U.S. Patent No. 6,060,273). In some embodiments, transcription units can be engineered as a bicistronic unit containing an IRES (internal ribosome entry site), which allows coexpression of gene products ((*e.g.* encoding the conjugate and encoding the recombinant receptor) by a message from a single promoter. Alternatively, in some cases, a single promoter may direct expression of an RNA that contains,

in a single open reading frame (ORF), two or three genes (*e.g.* encoding the conjugate and encoding the recombinant receptor) separated from one another by sequences encoding a self-cleavage peptide (*e.g.*, 2A sequences) or a protease recognition site (*e.g.*, furin). The ORF thus encodes a single polypeptide, which, either during (in the case of 2A) or after translation, is processed into the individual proteins. In some cases, the peptide, such as T2A, can cause the ribosome to skip (ribosome skipping) synthesis of a peptide bond at the C-terminus of a 2A element, leading to separation between the end of the 2A sequence and the next peptide downstream (*see*, for example, de Felipe. *Genetic Vaccines and Ther.* 2:13 (2004) and deFelipe et al. *Traffic* 5:616-626 (2004)). Many 2A elements are known in the art. Examples of 2A sequences that can be used in the methods and system disclosed herein, without limitation, 2A sequences from the foot-and-mouth disease virus (F2A, *e.g.*, SEQ ID NO: 88), equine rhinitis A virus (E2A, *e.g.*, SEQ ID NO: 87), Thosea asigna virus (T2A, *e.g.*, SEQ ID NO: 43 or SEQ ID NO:76), and porcine teschovirus-1 (P2A, *e.g.*, SEQ ID NO: 85 or 86) as described in U.S. Patent Publication No. 20070116690.

[0304] In some embodiments, the polynucleotide encoding the cell surface conjugate and/or recombinant receptor is introduced into a composition containing cultured cells, such as by retroviral transduction, transfection, or transformation.

[0305] Also provided are sets or combinations of polynucleotides. In some embodiments, the set or combination comprises a first polynucleotide comprising a nucleic acid encoding a cell surface conjugate, such as any described herein, and a second polynucleotide comprising a nucleic acid encoding a recombinant receptor. Also provided are compositions containing such set or combination of polynucleotides. In some embodiments, the set or combination of polynucleotides, are used together for engineering of cells. In some embodiments, the first and the second polynucleotides in the set are introduced simultaneously or sequentially, in any order into a cell for engineering. In some embodiments, there is a set of polynucleotides comprising a first polynucleotide comprising a nucleic acid encoding a cell surface conjugate, such as any described herein, and a second polynucleotide comprising a nucleic acid encoding a chimeric receptor and/or a recombinant antigen receptor.

[0306] Also provided are vectors or constructs containing such nucleic acid molecules. In some embodiments, the vectors or constructs contain one or more promoters operatively linked to the nucleotide encoding the polypeptide or receptor to drive expression thereof. In some embodiments, the promoter is operatively linked to one or more than one nucleic acid molecule. Thus, also provided are vectors, such as those that contain any of the polynucleotides provided

herein. In some cases, the vector is a viral vector, such as a retroviral vector, e.g., a lentiviral vector or a gammaretroviral vector.

[0307] Also provided a set or combination of vectors. In some embodiments, the set or combination of vectors comprises a first vector and a second vector, wherein the first vector comprises the first polynucleotide, e.g., a first polynucleotide encoding a cell surface conjugate, and the second vector comprises the second polynucleotide encoding a recombinant receptor, e.g., CAR. Also provided are compositions containing such set or combination of vectors. In some embodiments, the set or combination of vectors, are used together for engineering of cells. In some embodiments, the first and the second vectors in the set are introduced simultaneously or sequentially, in any order into a cell for engineering.

[0308] In some embodiments, the vectors include viral vectors, e.g., retroviral or lentiviral, non-viral vectors or transposons, e.g. *Sleeping Beauty* transposon system, vectors derived from simian virus 40 (SV40), adenoviruses, adeno-associated virus (AAV), lentiviral vectors or retroviral vectors, such as gamma-retroviral vectors, retroviral vector derived from the Moloney murine leukemia virus (MoMLV), myeloproliferative sarcoma virus (MPSV), murine embryonic stem cell virus (MESV), murine stem cell virus (MSCV), spleen focus forming virus (SFFV) or adeno-associated virus (AAV).

[0309] Any of the cell surface conjugate and/or recombinant receptors described herein can be encoded by polynucleotides containing one or more nucleic acid sequences encoding cell surface conjugate and/or recombinant receptors, in any combinations or arrangements. For example, one, two, three or more polynucleotides can encode one, two, three or more different polypeptides, e.g., cell surface conjugate and/or recombinant receptors. In some embodiments, one vector or construct contains a nucleic acid sequence encoding cell surface conjugate, and a separate vector or construct contains a nucleic acid sequence encoding a recombinant receptor, e.g., CAR. In some embodiments, the nucleic acid encoding the cell surface conjugate and the nucleic acid encoding the recombinant receptor are operably linked to two different promoters. In some embodiments, the nucleic acid encoding the recombinant receptor is present downstream of the nucleic acid encoding the cell surface conjugate.

C. Cells and Preparation of Cells for Engineering

[0310] Also provided are cells, such as cells that contain the cell surface conjugate and/or an engineered recombinant receptor, such as described herein. Also provided are populations of such cells, compositions containing such cells and/or enriched for such cells, such as in which

cells expressing the cell surface conjugate and/or recombinant receptor, e.g. chimeric receptor, make up at least 50, 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or more percent of the total cells in the composition or cells of a certain type such as T cells or CD8+ or CD4+ cells. Among the compositions are pharmaceutical compositions and formulations for administration, such as for adoptive cell therapy. Also provided are therapeutic methods for administering the cells and compositions to subjects, e.g., patients.

[0311] Thus, also provided are genetically engineered cells expressing the cell surface conjugates and/or recombinant receptors e.g., CARs. The cells generally are eukaryotic cells, such as mammalian cells, and typically are human cells. In some embodiments, the cells are derived from the blood, bone marrow, lymph, or lymphoid organs, are cells of the immune system, such as cells of the innate or adaptive immunity, e.g., myeloid or lymphoid cells, including lymphocytes, typically T cells and/or NK cells. Other exemplary cells include stem cells, such as multipotent and pluripotent stem cells, including induced pluripotent stem cells (iPSCs). The cells typically are primary cells, such as those isolated directly from a subject and/or isolated from a subject and frozen. In some embodiments, the cells include one or more subsets of T cells or other cell types, such as whole T cell populations, CD4+ cells, CD8+ cells, and subpopulations thereof, such as those defined by function, activation state, maturity, potential for differentiation, expansion, recirculation, localization, and/or persistence capacities, antigen-specificity, type of antigen receptor, presence in a particular organ or compartment, marker or cytokine secretion profile, and/or degree of differentiation. With reference to the subject to be treated, the cells may be allogeneic and/or autologous. Among the methods include off-the-shelf methods. In some aspects, such as for off-the-shelf technologies, the cells are pluripotent and/or multipotent, such as stem cells, such as induced pluripotent stem cells (iPSCs). In some embodiments, the methods include isolating cells from the subject, preparing, processing, culturing, and/or engineering them, as described herein, and re-introducing them into the same patient, before or after cryopreservation.

[0312] Among the sub-types and subpopulations of T cells and/or of CD4+ and/or of CD8+ T cells are naïve T (TN) cells, effector T cells (TEFF), memory T cells and sub-types thereof, such as stem cell memory T (TSCM), central memory T (TCM), effector memory T (TEM), or terminally differentiated effector memory T cells, tumor-infiltrating lymphocytes (TIL), immature T cells, mature T cells, helper T cells, cytotoxic T cells, mucosa-associated invariant T (MAIT) cells, naturally occurring and adaptive regulatory T (Treg) cells, helper T cells, such as

TH1 cells, TH2 cells, TH3 cells, TH17 cells, TH9 cells, TH22 cells, follicular helper T cells, alpha/beta T cells, and delta/gamma T cells.

[0313] In some embodiments, the cells are natural killer (NK) cells. In some embodiments, the cells are monocytes or granulocytes, e.g., myeloid cells, macrophages, neutrophils, dendritic cells, mast cells, eosinophils, and/or basophils.

[0314] In some embodiments, the cells include one or more nucleic acids introduced via genetic engineering, and thereby express recombinant or genetically engineered products of such nucleic acids. In some embodiments, the nucleic acids are heterologous, i.e., normally not present in a cell or sample obtained from the cell, such as one obtained from another organism or cell, which for example, is not ordinarily found in the cell being engineered and/or an organism from which such cell is derived. In some embodiments, the nucleic acids are not naturally occurring, such as a nucleic acid not found in nature, including one comprising chimeric combinations of nucleic acids encoding various domains from multiple different cell types.

[0315] In some embodiments, preparation of the engineered cells includes one or more culture and/or preparation steps. The cells for introduction of the cells surface conjugate and/or recombinant receptor, e.g., CAR, may be isolated from a sample, such as a biological sample, e.g., one obtained from or derived from a subject. In some embodiments, the subject from which the cell is isolated is one having the disease or condition or in need of a cell therapy or to which cell therapy will be administered. The subject in some embodiments is a human in need of a particular therapeutic intervention, such as the adoptive cell therapy for which cells are being isolated, processed, and/or engineered.

[0316] Accordingly, the cells in some embodiments are primary cells, e.g., primary human cells. The samples include tissue, fluid, and other samples taken directly from the subject, as well as samples resulting from one or more processing steps, such as separation, centrifugation, genetic engineering (e.g. transduction with viral vector), washing, and/or incubation. The biological sample can be a sample obtained directly from a biological source or a sample that is processed. Biological samples include, but are not limited to, body fluids, such as blood, plasma, serum, cerebrospinal fluid, synovial fluid, urine and sweat, tissue and organ samples, including processed samples derived therefrom.

[0317] In some aspects, the sample from which the cells are derived or isolated is blood or a blood-derived sample, or is or is derived from an apheresis or leukapheresis product. Exemplary samples include whole blood, peripheral blood mononuclear cells (PBMCs), leukocytes, bone

marrow, thymus, tissue biopsy, tumor, leukemia, lymphoma, lymph node, gut associated lymphoid tissue, mucosa associated lymphoid tissue, spleen, other lymphoid tissues, liver, lung, stomach, intestine, colon, kidney, pancreas, breast, bone, prostate, cervix, testes, ovaries, tonsil, or other organ, and/or cells derived therefrom. Samples include, in the context of cell therapy, e.g., adoptive cell therapy, samples from autologous and allogeneic sources.

[0318] In some embodiments, the cells are derived from cell lines, e.g., T cell lines. The cells in some embodiments are obtained from a xenogeneic source, for example, from mouse, rat, non-human primate, or pig.

[0319] In some embodiments, isolation of the cells includes one or more preparation and/or non-affinity based cell separation steps. In some examples, cells are washed, centrifuged, and/or incubated in the presence of one or more reagents, for example, to remove unwanted components, enrich for desired components, lyse or remove cells sensitive to particular reagents. In some examples, cells are separated based on one or more property, such as density, adherent properties, size, sensitivity and/or resistance to particular components.

[0320] In some examples, cells from the circulating blood of a subject are obtained, e.g., by apheresis or leukapheresis. The samples, in some aspects, contain lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and/or platelets, and in some aspects contains cells other than red blood cells and platelets.

[0321] In some embodiments, the blood cells collected from the subject are washed, e.g., to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In some embodiments, the cells are washed with phosphate buffered saline (PBS). In some embodiments, the wash solution lacks calcium and/or magnesium and/or many or all divalent cations. In some aspects, a washing step is accomplished a semi-automated "flow-through" centrifuge (for example, the Cobe 2991 cell processor, Baxter) according to the manufacturer's instructions. In some aspects, a washing step is accomplished by tangential flow filtration (TFF) according to the manufacturer's instructions. In some embodiments, the cells are resuspended in a variety of biocompatible buffers after washing, such as, for example, Ca++/Mg++ free PBS. In certain embodiments, components of a blood cell sample are removed and the cells directly resuspended in culture media.

[0322] In some embodiments, the methods include density-based cell separation methods, such as the preparation of white blood cells from peripheral blood by lysing the red blood cells and centrifugation through a Percoll or Ficoll gradient.

[0323] In some embodiments, the isolation methods include the separation of different cell types based on the expression or presence in the cell of one or more specific molecules, such as surface markers, e.g., surface molecules or surface proteins, intracellular markers, or nucleic acid. In some embodiments, any known method for separation based on such markers may be used. In some embodiments, the separation is affinity- or immunoaffinity-based separation. For example, the isolation in some aspects includes separation of cells and cell populations based on the cells' expression or expression level of one or more markers, typically cell surface markers, surface molecules or surface proteins, for example, by incubation with an antibody or binding partner that specifically binds to such markers, followed generally by washing steps and separation of cells having bound the antibody or binding partner, from those cells having not bound to the antibody or binding partner.

[0324] Such separation steps can be based on positive selection, in which the cells having bound the reagents are retained for further use, and/or negative selection, in which the cells having not bound to the antibody or binding partner are retained. In some examples, both fractions are retained for further use. In some aspects, negative selection can be particularly useful where no antibody is available that specifically identifies a cell type in a heterogeneous population, such that separation is best carried out based on markers expressed by cells other than the desired population.

[0325] The separation need not result in 100% enrichment or removal of a particular cell population or cells expressing a particular marker. For example, positive selection of or enrichment for cells of a particular type, such as those expressing a marker, refers to increasing the number or percentage of such cells, but need not result in a complete absence of cells not expressing the marker. Likewise, negative selection, removal, or depletion of cells of a particular type, such as those expressing a marker, refers to decreasing the number or percentage of such cells, but need not result in a complete removal of all such cells.

[0326] In some examples, multiple rounds of separation steps are carried out, where the positively or negatively selected fraction from one step is subjected to another separation step, such as a subsequent positive or negative selection. In some examples, a single separation step can deplete cells expressing multiple markers simultaneously, such as by incubating cells with a plurality of antibodies or binding partners, each specific for a marker targeted for negative selection. Likewise, multiple cell types can simultaneously be positively selected by incubating cells with a plurality of antibodies or binding partners expressed on the various cell types.

[0327] For example, in some aspects, specific subpopulations of T cells, such as cells positive or expressing high levels of one or more surface markers, e.g., CD28+, CD62L+, CCR7+, CD27+, CD127+, CD4+, CD8+, CD45RA+, and/or CD45RO+ T cells, are isolated by positive or negative selection techniques.

[0328] For example, CD3+, CD28+ T cells can be positively selected using anti-CD3/anti-CD28 conjugated magnetic beads (e.g., DYNABEADS® M-450 CD3/CD28 T Cell Expander).

[0329] In some embodiments, isolation is carried out by enrichment for a particular cell population by positive selection, or depletion of a particular cell population, by negative selection. In some embodiments, positive or negative selection is accomplished by incubating cells with one or more antibodies or other binding agent that specifically bind to one or more surface markers expressed or expressed (marker+) at a relatively higher level (markerhigh) on the positively or negatively selected cells, respectively.

[0330] In some embodiments, T cells are separated from a PBMC sample by negative selection of markers expressed on non-T cells, such as B cells, monocytes, or other white blood cells, such as CD14. In some aspects, a CD4+ or CD8+ selection step is used to separate CD4+ helper and CD8+ cytotoxic T cells. Such CD4+ and CD8+ populations can be further sorted into sub-populations by positive or negative selection for markers expressed or expressed to a relatively higher degree on one or more naive, memory, and/or effector T cell subpopulations.

[0331] In some embodiments, CD8+ cells are further enriched for or depleted of naive, central memory, effector memory, and/or central memory stem cells, such as by positive or negative selection based on surface antigens associated with the respective subpopulation. In some embodiments, enrichment for central memory T (TCM) cells is carried out to increase efficacy, such as to improve long-term survival, expansion, and/or engraftment following administration, which in some aspects is particularly robust in such sub-populations. See Terakura et al. (2012) Blood.1:72–82; Wang et al. (2012) J Immunother. 35(9):689-701. In some embodiments, combining TCM-enriched CD8+ T cells and CD4+ T cells further enhances efficacy.

[0332] In embodiments, memory T cells are present in both CD62L+ and CD62L- subsets of CD8+ peripheral blood lymphocytes. PBMC can be enriched for or depleted of CD62L-CD8+ and/or CD62L+CD8+ fractions, such as using anti-CD8 and anti-CD62L antibodies.

[0333] In some embodiments, the enrichment for central memory T (TCM) cells is based on positive or high surface expression of CD45RO, CD62L, CCR7, CD28, CD3, and/or CD 127; in some aspects, it is based on negative selection for cells expressing or highly expressing

CD45RA and/or granzyme B. In some aspects, isolation of a CD8+ population enriched for TCM cells is carried out by depletion of cells expressing CD4, CD14, CD45RA, and positive selection or enrichment for cells expressing CD62L. In one aspect, enrichment for central memory T (TCM) cells is carried out starting with a negative fraction of cells selected based on CD4 expression, which is subjected to a negative selection based on expression of CD14 and CD45RA, and a positive selection based on CD62L. Such selections in some aspects are carried out simultaneously and in other aspects are carried out sequentially, in either order. In some aspects, the same CD4 expression-based selection step used in preparing the CD8+ cell population or subpopulation, also is used to generate the CD4+ cell population or sub-population, such that both the positive and negative fractions from the CD4-based separation are retained and used in subsequent steps of the methods, optionally following one or more further positive or negative selection steps.

[0334] In a particular example, a sample of PBMCs or other white blood cell sample is subjected to selection of CD4+ cells, where both the negative and positive fractions are retained. The negative fraction then is subjected to negative selection based on expression of CD14 and CD45RA or ROR1, and positive selection based on a marker characteristic of central memory T cells, such as CD62L or CCR7, where the positive and negative selections are carried out in either order.

[0335] CD4+ T helper cells are sorted into naïve, central memory, and effector cells by identifying cell populations that have cell surface antigens. CD4+ lymphocytes can be obtained by standard methods. In some embodiments, naive CD4+ T lymphocytes are CD45RO-, CD45RA+, CD62L+, CD4+ T cells. In some embodiments, central memory CD4+ cells are CD62L+ and CD45RO+. In some embodiments, effector CD4+ cells are CD62L- and CD45RO-.

[0336] In one example, to enrich for CD4+ cells by negative selection, a monoclonal antibody cocktail typically includes antibodies to CD14, CD20, CD11b, CD16, HLA-DR, and CD8. In some embodiments, the antibody or binding partner is bound to a solid support or matrix, such as a magnetic bead or paramagnetic bead, to allow for separation of cells for positive and/or negative selection. For example, in some embodiments, the cells and cell populations are separated or isolated using immunomagnetic (or affinitymagnetic) separation techniques (reviewed in Methods in Molecular Medicine, vol. 58: Metastasis Research Protocols, Vol. 2: Cell Behavior In Vitro and In Vivo, p 17-25 Edited by: S. A. Brooks and U. Schumacher © Humana Press Inc., Totowa, NJ).

[0337] In some aspects, the sample or composition of cells to be separated is incubated with small, magnetizable or magnetically responsive material, such as magnetically responsive particles or microparticles, such as paramagnetic beads (e.g., such as Dynalbeads or MACS beads). The magnetically responsive material, e.g., particle, generally is directly or indirectly attached to a binding partner, e.g., an antibody, that specifically binds to a molecule, e.g., surface marker, present on the cell, cells, or population of cells that it is desired to separate, e.g., that it is desired to negatively or positively select.

[0338] In some embodiments, the magnetic particle or bead comprises a magnetically responsive material bound to a specific binding member, such as an antibody or other binding partner. There are many well-known magnetically responsive materials used in magnetic separation methods. Suitable magnetic particles include those described in Molday, U.S. Pat. No. 4,452,773, and in European Patent Specification EP 452342 B, which are hereby incorporated by reference. Colloidal sized particles, such as those described in Owen U.S. Pat. No. 4,795,698, and Liberti et al., U.S. Pat. No. 5,200,084 are other examples.

[0339] The incubation generally is carried out under conditions whereby the antibodies or binding partners, or molecules, such as secondary antibodies or other reagents, which specifically bind to such antibodies or binding partners, which are attached to the magnetic particle or bead, specifically bind to cell surface molecules if present on cells within the sample.

[0340] In some aspects, the sample is placed in a magnetic field, and those cells having magnetically responsive or magnetizable particles attached thereto will be attracted to the magnet and separated from the unlabeled cells. For positive selection, cells that are attracted to the magnet are retained; for negative selection, cells that are not attracted (unlabeled cells) are retained. In some aspects, a combination of positive and negative selection is performed during the same selection step, where the positive and negative fractions are retained and further processed or subject to further separation steps.

[0341] In certain embodiments, the magnetically responsive particles are coated in primary antibodies or other binding partners, secondary antibodies, lectins, enzymes, or streptavidin. In certain embodiments, the magnetic particles are attached to cells via a coating of primary antibodies specific for one or more markers. In certain embodiments, the cells, rather than the beads, are labeled with a primary antibody or binding partner, and then cell-type specific secondary antibody- or other binding partner (e.g., streptavidin)-coated magnetic particles, are added. In certain embodiments, streptavidin-coated magnetic particles are used in conjunction with biotinylated primary or secondary antibodies.

[0342] In some embodiments, the magnetically responsive particles are left attached to the cells that are to be subsequently incubated, cultured and/or engineered; in some aspects, the particles are left attached to the cells for administration to a patient. In some embodiments, the magnetizable or magnetically responsive particles are removed from the cells. Methods for removing magnetizable particles from cells are known and include, e.g., the use of competing non-labeled antibodies, magnetizable particles or antibodies conjugated to cleavable linkers, etc. In some embodiments, the magnetizable particles are biodegradable.

[0343] In some embodiments, the affinity-based selection is via magnetic-activated cell sorting (MACS) (Miltenyi Biotec, Auburn, CA). Magnetic Activated Cell Sorting (MACS) systems are capable of high-purity selection of cells having magnetized particles attached thereto. In certain embodiments, MACS operates in a mode wherein the non-target and target species are sequentially eluted after the application of the external magnetic field. That is, the cells attached to magnetized particles are held in place while the unattached species are eluted. Then, after this first elution step is completed, the species that were trapped in the magnetic field and were prevented from being eluted are freed in some manner such that they can be eluted and recovered. In certain embodiments, the non-target cells are labelled and depleted from the heterogeneous population of cells.

[0344] In certain embodiments, the isolation or separation is carried out using a system, device, or apparatus that carries out one or more of the isolation, cell preparation, separation, processing, incubation, culture, and/or formulation steps of the methods. In some aspects, the system is used to carry out each of these steps in a closed or sterile environment, for example, to minimize error, user handling and/or contamination. In one example, the system is a system as described in International Patent Application, Publication Number WO2009/072003, or US 20110003380 A1.

[0345] In some embodiments, the system or apparatus carries out one or more, e.g., all, of the isolation, processing, engineering, and formulation steps in an integrated or self-contained system, and/or in an automated or programmable fashion. In some aspects, the system or apparatus includes a computer and/or computer program in communication with the system or apparatus, which allows a user to program, control, assess the outcome of, and/or adjust various aspects of the processing, isolation, engineering, and formulation steps.

[0346] In some aspects, the separation and/or other steps is carried out using CliniMACS system (Miltenyi Biotec), for example, for automated separation of cells on a clinical-scale level in a closed and sterile system. Components can include an integrated microcomputer, magnetic

separation unit, peristaltic pump, and various pinch valves. The integrated computer in some aspects controls all components of the instrument and directs the system to perform repeated procedures in a standardized sequence. The magnetic separation unit in some aspects includes a movable permanent magnet and a holder for the selection column. The peristaltic pump controls the flow rate throughout the tubing set and, together with the pinch valves, ensures the controlled flow of buffer through the system and continual suspension of cells.

[0347] The CliniMACS system in some aspects uses antibody-coupled magnetizable particles that are supplied in a sterile, non-pyrogenic solution. In some embodiments, after labelling of cells with magnetic particles the cells are washed to remove excess particles. A cell preparation bag is then connected to the tubing set, which in turn is connected to a bag containing buffer and a cell collection bag. The tubing set consists of pre-assembled sterile tubing, including a pre-column and a separation column, and are for single use only. After initiation of the separation program, the system automatically applies the cell sample onto the separation column. Labelled cells are retained within the column, while unlabeled cells are removed by a series of washing steps. In some embodiments, the cell populations for use with the methods described herein are unlabeled and are not retained in the column. In some embodiments, the cell populations for use with the methods described herein are labeled and are retained in the column. In some embodiments, the cell populations for use with the methods described herein are eluted from the column after removal of the magnetic field, and are collected within the cell collection bag.

[0348] In certain embodiments, separation and/or other steps are carried out using the CliniMACS Prodigy system (Miltenyi Biotec). The CliniMACS Prodigy system in some aspects is equipped with a cell processing unity that permits automated washing and fractionation of cells by centrifugation. The CliniMACS Prodigy system can also include an onboard camera and image recognition software that determines the optimal cell fractionation endpoint by discerning the macroscopic layers of the source cell product. For example, peripheral blood may be automatically separated into erythrocytes, white blood cells and plasma layers. The CliniMACS Prodigy system can also include an integrated cell cultivation chamber which accomplishes cell culture protocols such as, e.g., cell differentiation and expansion, antigen loading, and long-term cell culture. Input ports can allow for the sterile removal and replenishment of media and cells can be monitored using an integrated microscope. See, e.g., Klebanoff et al. (2012) J Immunother. 35(9): 651–660, Terakura et al. (2012) Blood.1:72–82, and Wang et al. (2012) J Immunother. 35(9):689-701.

[0349] In some embodiments, a cell population described herein is collected and enriched (or depleted) via flow cytometry, in which cells stained for multiple cell surface markers are carried in a fluidic stream. In some embodiments, a cell population described herein is collected and enriched (or depleted) via preparative scale (FACS)-sorting. In certain embodiments, a cell population described herein is collected and enriched (or depleted) by use of microelectromechanical systems (MEMS) chips in combination with a FACS-based detection system (see, e.g., WO 2010/033140, Cho et al. (2010) Lab Chip 10, 1567-1573; and Godin et al. (2008) J Biophoton. 1(5):355–376. In both cases, cells can be labeled with multiple markers, allowing for the isolation of well-defined T cell subsets at high purity.

[0350] In some embodiments, the antibodies or binding partners are labeled with one or more detectable marker, to facilitate separation for positive and/or negative selection. For example, separation may be based on binding to fluorescently labeled antibodies. In some examples, separation of cells based on binding of antibodies or other binding partners specific for one or more cell surface markers are carried in a fluidic stream, such as by fluorescence-activated cell sorting (FACS), including preparative scale (FACS) and/or microelectromechanical systems (MEMS) chips, e.g., in combination with a flow-cytometric detection system. Such methods allow for positive and negative selection based on multiple markers simultaneously.

[0351] In some embodiments, the preparation methods include steps for freezing, e.g., cryopreserving, the cells, either before or after isolation, incubation, and/or engineering. In some embodiments, the freeze and subsequent thaw step removes granulocytes and, to some extent, monocytes in the cell population. In some embodiments, the cells are suspended in a freezing solution, e.g., following a washing step to remove plasma and platelets. Any of a variety of known freezing solutions and parameters in some aspects may be used. One example involves using PBS containing 20% DMSO and 8% human serum albumin (HSA), or other suitable cell freezing media. This is then diluted 1:1 with media so that the final concentration of DMSO and HSA are 10% and 4%, respectively. The cells are then frozen to −80° C. at a rate of 1° per minute and stored in the vapor phase of a liquid nitrogen storage tank.

[0352] In some embodiments, the provided methods include cultivation, incubation, culture, and/or genetic engineering steps. For example, in some embodiments, provided are methods for incubating and/or engineering the depleted cell populations and culture-initiating compositions.

[0353] Thus, in some embodiments, the cell populations are incubated in a culture-initiating composition. The incubation and/or engineering may be carried out in a culture vessel, such as a

unit, chamber, well, column, tube, tubing set, valve, vial, culture dish, bag, or other container for culture or cultivating cells.

[0354] In some embodiments, the cells are incubated and/or cultured prior to or in connection with genetic engineering. The incubation steps can include culture, cultivation, stimulation, activation, and/or propagation. In some embodiments, the compositions or cells are incubated in the presence of stimulating conditions or a stimulatory agent. Such conditions include those designed to induce proliferation, expansion, activation, and/or survival of cells in the population, to mimic antigen exposure, and/or to prime the cells for genetic engineering, such as for the introduction of a recombinant antigen receptor.

[0355] The conditions can include one or more of particular media, temperature, oxygen content, carbon dioxide content, time, agents, e.g., nutrients, amino acids, antibiotics, ions, and/or stimulatory factors, such as cytokines, chemokines, antigens, binding partners, fusion proteins, recombinant soluble receptors, and any other agents designed to activate the cells.

[0356] In some embodiments, the stimulating conditions or agents include one or more agent, e.g., ligand, which is capable of activating an intracellular signaling domain of a TCR complex. In some aspects, the agent turns on or initiates TCR/CD3 intracellular signaling cascade in a T cell. Such agents can include antibodies, such as those specific for a TCR component and/or costimulatory receptor, e.g., anti-CD3, anti-CD28, for example, bound to solid support such as a bead, and/or one or more cytokines. Optionally, the expansion method may further comprise the step of adding anti-CD3 and/or anti CD28 antibody to the culture medium (e.g., at a concentration of at least about 0.5 ng/ml). In some embodiments, the stimulating agents include IL-2 and/or IL-15, for example, an IL-2 concentration of at least about 10 units/mL.

[0357] In some aspects, incubation is carried out in accordance with techniques such as those described in US Patent No. 6,040,177 to Riddell et al., Klebanoff et al.(2012) J Immunother. 35(9): 651–660, Terakura et al. (2012) Blood.1:72–82, and/or Wang et al. (2012) J Immunother. 35(9):689-701.

[0358] In some embodiments, the T cells are expanded by adding to the culture-initiating composition feeder cells, such as non-dividing peripheral blood mononuclear cells (PBMC), (e.g., such that the resulting population of cells contains at least about 5, 10, 20, or 40 or more PBMC feeder cells for each T lymphocyte in the initial population to be expanded); and incubating the culture (e.g. for a time sufficient to expand the numbers of T cells). In some aspects, the non-dividing feeder cells can comprise gamma-irradiated PBMC feeder cells. In

some embodiments, the PBMC are irradiated with gamma rays in the range of about 3000 to 3600 rads to prevent cell division. In some aspects, the feeder cells are added to culture medium prior to the addition of the populations of T cells.

[0359] In some embodiments, the stimulating conditions include temperature suitable for the growth of human T lymphocytes, for example, at least about 25 degrees Celsius, generally at least about 30 degrees, and generally at or about 37 degrees Celsius. Optionally, the incubation may further comprise adding non-dividing EBV-transformed lymphoblastoid cells (LCL) as feeder cells. LCL can be irradiated with gamma rays in the range of about 6000 to 10,000 rads. The LCL feeder cells in some aspects is provided in any suitable amount, such as a ratio of LCL feeder cells to initial T lymphocytes of at least about 10:1.

[0360] In embodiments, antigen-specific T cells, such as antigen-specific CD4+ and/or CD8+ T cells, are obtained by stimulating naive or antigen specific T lymphocytes with antigen. For example, antigen-specific T cell lines or clones can be generated to cytomegalovirus antigens by isolating T cells from infected subjects and stimulating the cells in vitro with the same antigen.

D. Vectors and Methods for Genetic Engineering

[0361] Various methods for the introduction of genetically engineered components, e.g., cell surface conjugates and recombinant receptors, e.g., CARs or TCRs, are well known and may be used with the provided methods and compositions. Exemplary methods include those for transfer of nucleic acids encoding the polypeptides or receptors, including via viral vectors, e.g., retroviral or lentiviral, non-viral vectors or transposons, e.g. *Sleeping Beauty* transposon system. Methods of gene transfer can include transduction, electroporation or other method that results into gene transfer into the cell.

[0362] In some embodiments, gene transfer is accomplished by first stimulating the cell, such as by combining it with a stimulus that induces a response such as proliferation, survival, and/or activation, e.g., as measured by expression of a cytokine or activation marker, followed by transduction of the activated cells, and expansion in culture to numbers sufficient for clinical applications.

[0363] In some contexts, it may be desired to safeguard against the potential that overexpression of a stimulatory factor (for example, a lymphokine or a cytokine) could potentially result in an unwanted outcome or lower efficacy in a subject, such as a factor associated with toxicity in a subject. Thus, in some contexts, the engineered cells include gene

segments that cause the cells to be susceptible to negative selection in vivo, such as upon administration in adoptive immunotherapy. For example in some aspects, the cells are engineered so that they can be eliminated as a result of a change in the in vivo condition of the patient to which they are administered. The negative selectable phenotype may result from the insertion of a gene that confers sensitivity to an administered agent, for example, a compound. Negative selectable genes include the Herpes simplex virus type I thymidine kinase (HSV-I TK) gene (Wigler et al., Cell 2 :223, 1977) which confers ganciclovir sensitivity; the cellular hypoxanthine phosphribosyltransferase (HPRT) gene, the cellular adenine phosphoribosyltransferase (APRT) gene, bacterial cytosine deaminase, (Mullen et al., Proc. Natl. Acad. Sci. USA. 89:33 (1992)).

[0364] In some embodiments, recombinant nucleic acids are transferred into cells using recombinant infectious virus particles, such as, e.g., vectors derived from simian virus 40 (SV40), adenoviruses, adeno-associated virus (AAV). In some embodiments, recombinant nucleic acids are transferred into T cells using recombinant lentiviral vectors or retroviral vectors, such as gamma-retroviral vectors (see, e.g., Koste et al. (2014) Gene Therapy 2014 Apr 3. doi: 10.1038/gt.2014.25; Carlens et al. (2000) Exp Hematol 28(10): 1137-46; Alonso-Camino et al. (2013) Mol Ther Nucl Acids 2, e93; Park et al., Trends Biotechnol. 2011 November 29(11): 550–557.

[0365] In some embodiments, the retroviral vector has a long terminal repeat sequence (LTR), e.g., a retroviral vector derived from the Moloney murine leukemia virus (MoMLV), myeloproliferative sarcoma virus (MPSV), murine embryonic stem cell virus (MESV), murine stem cell virus (MSCV), spleen focus forming virus (SFFV), or adeno-associated virus (AAV). Most retroviral vectors are derived from murine retroviruses. In some embodiments, the retroviruses include those derived from any avian or mammalian cell source. The retroviruses typically are amphotropic, meaning that they are capable of infecting host cells of several species, including humans. In one embodiment, the gene to be expressed replaces the retroviral gag, pol and/or env sequences. A number of illustrative retroviral systems have been described (e.g., U.S. Pat. Nos. 5,219,740; 6,207,453; 5,219,740; Miller and Rosman (1989) BioTechniques 7:980-990; Miller, A. D. (1990) Human Gene Therapy 1:5-14; Scarpa et al. (1991) Virology 180:849-852; Burns et al. (1993) Proc. Natl. Acad. Sci. USA 90:8033-8037; and Boris-Lawrie and Temin (1993) Cur. Opin. Genet. Develop. 3:102-109.

[0366] Methods of lentiviral transduction are known. Exemplary methods are described in, e.g., Wang et al. (2012) *J. Immunother.* 35(9): 689-701; Cooper et al. (2003) *Blood.* 101:1637–

1644; Verhoeyen et al. (2009) *Methods Mol Biol.* 506: 97-114; and Cavalieri et al. (2003) Blood. 102(2): 497-505.

[0367] In some embodiments, recombinant nucleic acids are transferred into T cells via electroporation (*see*, e.g., Chicaybam et al, (2013) *PLoS ONE* 8(3): e60298 and Van Tedeloo et al. (2000) *Gene Therapy* 7(16): 1431-1437). In some embodiments, recombinant nucleic acids are transferred into T cells via transposition (see, e.g., Manuri et al. (2010) Hum Gene Ther 21(4): 427-437; Sharma et al. (2013) *Molec Ther Nucl Acids* 2, e74; and Huang et al. (2009) *Methods Mol Biol* 506: 115-126). Other methods of introducing and expressing genetic material in immune cells include calcium phosphate transfection (e.g., as described in Current Protocols in Molecular Biology, John Wiley & Sons, New York. N.Y.), protoplast fusion, cationic liposome-mediated transfection; tungsten particle-facilitated microparticle bombardment (Johnston, Nature, 346: 776-777 (1990)); and strontium phosphate DNA co-precipitation (Brash et al., Mol. Cell Biol., 7: 2031-2034 (1987)).

[0368] Other approaches and vectors for transfer of the nucleic acids encoding the recombinant products are those described, e.g., in international patent application, Publication No.: WO2014055668, and U.S. Patent No. 7,446,190.

[0369] In some embodiments, the cells, e.g., T cells, may be transfected either during or after expansion, e.g. with a cell surface conjugate, a T cell receptor (TCR), or a chimeric antigen receptor (CAR). This transfection for the introduction of the gene of the desired polypeptide or receptor can be carried out with any suitable retroviral vector, for example. The genetically modified cell population can then be liberated from the initial stimulus (the CD3/CD28 stimulus, for example) and subsequently be stimulated with a second type of stimulus e.g. via a de novo introduced receptor). This second type of stimulus may include an antigenic stimulus in form of a peptide/MHC molecule, the cognate (cross-linking) ligand of the genetically introduced receptor (e.g. natural ligand of a CAR) or any ligand (such as an antibody) that directly binds within the framework of the new receptor (e.g. by recognizing constant regions within the receptor). See, for example, Cheadle et al, "Chimeric antigen receptors for T-cell based therapy" Methods Mol Biol. 2012; 907:645-66 or Barrett et al., Chimeric Antigen Receptor Therapy for Cancer Annual Review of Medicine Vol. 65: 333-347 (2014).

[0370] Among additional nucleic acids, e.g., genes for introduction are those to improve the efficacy of therapy, such as by promoting viability and/or function of transferred cells; genes to provide a genetic marker for selection and/or evaluation of the cells, such as to assess *in vivo* survival or localization; genes to improve safety, for example, by making the cell susceptible to

negative selection in vivo as described by Lupton S. D. et al., *Mol. and Cell Biol.,* 11:6 (1991); and Riddell et al., *Human Gene Therapy* 3:319-338 (1992); see also the publications of PCT/US91/08442 and PCT/US94/05601 by Lupton et al. describing the use of bifunctional selectable fusion genes derived from fusing a dominant positive selectable marker with a negative selectable marker. *See,* e.g., Riddell et al., US Patent No. 6,040,177, at columns 14-17.

[0371] In some embodiments, the cells are incubated and/or cultured prior to or in connection with genetic engineering. The incubation steps can include culture, cultivation, stimulation, activation, and/or propagation. The incubation and/or engineering may be carried out in a culture vessel, such as a unit, chamber, well, column, tube, tubing set, valve, vial, culture dish, bag, or other container for culture or cultivating cells. In some embodiments, the compositions or cells are incubated in the presence of stimulating conditions or a stimulatory agent. Such conditions include those designed to induce proliferation, expansion, activation, and/or survival of cells in the population, to mimic antigen exposure, and/or to prime the cells for genetic engineering, such as for the introduction of a recombinant antigen receptor.

[0372] The conditions can include one or more of particular media, temperature, oxygen content, carbon dioxide content, time, agents, e.g., nutrients, amino acids, antibiotics, ions, and/or stimulatory factors, such as cytokines, chemokines, antigens, binding partners, fusion proteins, recombinant soluble receptors, and any other agents designed to activate the cells.

[0373] In some embodiments, the stimulating conditions or agents include one or more agent, e.g., ligand, which is capable of activating an intracellular signaling domain of a TCR complex. In some aspects, the agent turns on or initiates TCR/CD3 intracellular signaling cascade in a T cell. Such agents can include antibodies, such as those specific for a TCR, e.g. anti-CD3. In some embodiments, the stimulating conditions include one or more agent, e.g. ligand, which is capable of stimulating a costimulatory receptor, e.g., anti-CD28. In some embodiments, such agents and/or ligands may be, bound to solid support such as a bead, and/or one or more cytokines. Optionally, the expansion method may further comprise the step of adding anti-CD3 and/or anti CD28 antibody to the culture medium (e.g., at a concentration of at least about 0.5 ng/ml). In some embodiments, the stimulating agents include IL-2, IL-15 and/or IL-7. In some aspects, the IL-2 concentration is at least about 10 units/mL.

[0374] In some aspects, incubation is carried out in accordance with techniques such as those described in US Patent No. 6,040,177 to Riddell et al., Klebanoff et al.(2012) J Immunother. 35(9): 651–660, Terakura et al. (2012) Blood.1:72–82, and/or Wang et al. (2012) J Immunother. 35(9):689-701.

[0375] In some embodiments, the T cells are expanded by adding to a culture-initiating composition feeder cells, such as non-dividing peripheral blood mononuclear cells (PBMC), (e.g., such that the resulting population of cells contains at least about 5, 10, 20, or 40 or more PBMC feeder cells for each T lymphocyte in the initial population to be expanded); and incubating the culture (e.g. for a time sufficient to expand the numbers of T cells). In some aspects, the non-dividing feeder cells can comprise gamma-irradiated PBMC feeder cells. In some embodiments, the PBMC are irradiated with gamma rays in the range of about 3000 to 3600 rads to prevent cell division. In some aspects, the feeder cells are added to culture medium prior to the addition of the populations of T cells.

[0376] In some embodiments, the stimulating conditions include temperature suitable for the growth of human T lymphocytes, for example, at least about 25 degrees Celsius, generally at least about 30 degrees, and generally at or about 37 degrees Celsius. Optionally, the incubation may further comprise adding non-dividing EBV-transformed lymphoblastoid cells (LCL) as feeder cells. LCL can be irradiated with gamma rays in the range of about 6000 to 10,000 rads. The LCL feeder cells in some aspects is provided in any suitable amount, such as a ratio of LCL feeder cells to initial T lymphocytes of at least about 10:1.

[0377] In some embodiments, the preparation methods include steps for freezing, e.g., cryopreserving, the cells, either before or after isolation, incubation, and/or engineering. In some embodiments, the freeze and subsequent thaw step removes granulocytes and, to some extent, monocytes in the cell population. In some embodiments, the cells are suspended in a freezing solution, e.g., following a washing step to remove plasma and platelets. Any of a variety of known freezing solutions and parameters in some aspects may be used. One example involves using PBS containing 20% DMSO and 8% human serum albumin (HSA), or other suitable cell freezing media. This is then diluted 1:1 with media so that the final concentration of DMSO and HSA are 10% and 4%, respectively. The cells are generally then frozen to –80° C. at a rate of 1° per minute and stored in the vapor phase of a liquid nitrogen storage tank.

IV. METHODS OF SELECTING OR DETECTING TRANSDUCED CELLS

[0378] Provided are methods of targeting the agent (e.g. affinity tag, such as a strep-tag) of the cell surface conjugate in connection with manufacturing, such as preparing and processing, genetically engineered cells. In some embodiments, the cell surface conjugate containing a cell surface molecule and at least one agent is used for detection of cells transduced with the cell surface conjugate. In further embodiments, the cell detection of cells transduced with the cell

surface conjugate is followed by isolation and identification of cells transduced with the cell surface conjugate.

[0379] In some aspects, provided are methods of detecting, selecting or isolating gene modified cells before, during or after one or more steps of gene transfer, cell processing, incubation, culture, and/or formulation steps of the methods of engineering cells, such as during any of the process steps as described above. In some aspects, during production and further processing of gene modified cells (e.g. T cells), it is of interest to specifically select and further process only those cells that are positive for the transgene. In the provided methods, detection and selection of gene modified cells is carried out by detection of the agent (e.g. peptide), such as affinity tag, of the cell surface conjugate, such as by detection of the streptavidin binding protein (e.g. Strep-tag). In some aspects, detection of the cell surface conjugate is a surrogate marker for the recombinant receptor co-introduced and/or co-expressed with the cell surface conjugate.

[0380] In some aspects, the compositions containing cells for detection include samples resulting from one or more processing steps, such as separation, centrifugation, genetic engineering (e.g. transduction with viral vector), washing, and/or incubation. In some embodiments, cells or a composition of cells obtained before, during or after one or more steps of gene transfer (e.g. transduction with a viral vector), cell processing, incubation, culture, washing and/or formulation steps of the methods of engineering cells, such as any described herein, are contacted with the binding molecule specific for the agent of the conjugate. In certain embodiments, the contacting is under conditions permissive for binding of the binding molecule to the agent of the cell surface conjugate present in cells of the composition. In certain embodiments, the methods further include detecting whether a complex is formed between the binding molecule and the agent of the conjugate in the sample, and/or detecting the presence or absence or level of such binding. In some embodiments, the binding molecule is detectably labeled, such as labeled with a fluorescent moiety.

[0381] In some aspects of the provided methods, detection is carried out using an antibody or antigen-binding fragment that is capable of specifically binding the agent (e.g. peptide), such as affinity tag, of the cell surface conjugate. Any of the known antibody or antigen-binding fragments against an affinity tag of the cell surface conjugate can be used, such as any as described above. In some embodiments, the cell surface conjugate contains a streptavidin binding peptide as described, such as a Strep-tag (e.g. Strep-tag® II or a twin-strep tag), and the

antibody or antigen-binding fragment specifically binds the streptavidin binding peptide. In some embodiments, the antibody is detectably labeled, such as fluorescently labeled.

[0382] In some aspects of the provided methods, detection is carried out using a non-antibody binding molecule reagent. In some embodiments, the cell surface conjugate contains a streptavidin binding peptide as described, such as a Strep-tag (e.g. Strep-tag® II or a twin-strep tag) and the reagent is or comprises a streptavidin or streptavidin mutein or an oligomer of streptavidin or streptavidin mutein. In some embodiments, the binding molecule reagent is or comprises a streptavidin mutein set forth in any of SEQ ID NOS: 3, 4, 5, 6, 27 or 28 or is an oligomer thereof. In some embodiments, the binding molecule reagent is the commercially available reagent known as Strep-Tactin® or Strep-Tacin® XT. In some embodiments, the non-antibody binding molecule reagent is detectably labeled, such as fluorescently labeled.

[0383] In some embodiments, the binding molecules can be used to identify, sort, enrich or isolate cells expressing a cell surface conjugate of this disclosure, such as for isolation of gene modified cells that are positive for the cell surface conjugate (e.g. ST-EGFRt or ST-PSMA) and hence, also positive for the recombinant receptor. In some embodiments, the provided methods include contacting cells or a composition of cells obtained before, during or after one or more steps of gene transfer (e.g. transduction with viral vector), washing, cell processing, incubation, culture, and/or formulation steps with a binding molecule specific for the agent of the conjugate and selecting or isolating cells that are positive for binding of the binding molecule. In some embodiments, the binding molecule is an antibody or an antigen binding fragment that specifically binds the agent (e.g., anti-agent antibodies, such as anti- Strep-Tag® antibody). In some embodiments, the binding molecule is a non-antibody protein reagent that specifically binds an agent (e.g., Strep-Tactin® binding to Strep-tag). In some aspects, matrices, such as magnetic beads, agarose particles, cell culture dishes or other solid surface matrix can be employed, in which a binding molecule specific for the agent of the conjugate (e.g. specific for an affinity tag) has been immobilized, conjugated or bound. In some embodiments, the reagent is comprised on a support, such as a solid support or surface, e.g., bead, or a stationary phase (chromatography matrix). In certain embodiments, such cells are sorted, enriched or isolated using magnetic bead or paramagnetic bead-based separations or by using an affinity column.

[0384] In some embodiments, the binding molecule specific for the agent, such as any antibody or non-antibody reagent (e.g. streptavidin mutein, such as Strept-tacin), is comprised on a support, such as a solid support or surface, e.g., bead, or a stationary phase (chromatography matrix). In some such embodiments, the reagent is reversibly immobilized on

the support. In some cases, the reagent is immobilized to the support via covalent bonds. In some aspects, the reagent is reversibly immobilized to the support non-covalently.

[0385] In some embodiments, the support is a solid support. Any solid support (surface) can be used for the immobilization of the binding molecule, including an antibody or a non-antibody reagent. Illustrative examples of solid supports on which the binding molecule can be immobilized include a magnetic bead, a polymeric bead, a cell culture plate, a microtiter plate, a membrane, or a hollow fiber. In some aspects, hollow fibers can be used as a bioreactor in the Quantum® Cell Expansion System, available from TerumoBCT Inc. (Lakewood, CO, USA). In some embodiments, the binding molecule is covalently attached to the solid support. In other embodiments, non-covalent interactions can also be used for immobilization, for example on plastic substrates.

[0386] In some embodiments, the binding molecule can, for example, be a non-antibody reagent comprising streptavidin or avidin mutein that binds a streptavidin binding peptide as described. Such streptavidin muteins can be covalently attached to any surface, for example, resin (beads) used for chromatography purification and are commercially available in such form from IBA GmbH, Göttingen, for example, as Strep-Tactin® Sepharose, Strep-Tactin® Superflow®, Strep-Tactin® Superflow® high capacity or Strep-Tactin® MacroPrep®.

[0387] Other illustrative examples that are readily commercially available are immobilized metal affinity chromatography (IMAC) resins such as the TALON® resins (Westburg, Leusden, The Netherlands) that can be used for the immobilization of oligo-histidine tagged (his-tagged) proteins, such as for the binding of an oligohistidine tag such as an penta- or hexa-histidine tag. Other examples include calmodulin sepharose available from GE Life Sciences which can be used for binding a conjugate in which the agent (affinity tag) is a calmodulin binding peptide. Further examples include sepharose to which glutathion is coupled, which can be used for binding a conjugate in which the agent (affinity tag) is glutathion-S-transferase.

[0388] In some embodiments, a solid support employed in the present methods may include magnetically attractable matter such as one or more magnetically attractable particles or a ferrofluid. A respective magnetically attractable particle may comprise a reagent with a binding site that is capable of binding a target cell. In some cases, magnetically attractable particles may contain diamagnetic, ferromagnetic, paramagnetic or superparamagnetic material. In general, superparamagnetic material responds to a magnetic field with an induced magnetic field without a resulting permanent magnetization. Magnetic particles based on iron oxide are for example commercially available as Dynabeads® from Dynal Biotech, as magnetic MicroBeads from

Miltenyi Biotec, as magnetic porous glass beads from CPG Inc., as well as from various other sources, such as Roche Applied Science, BIOCLON, BioSource International Inc., micromod, AMBION, Merck, Bangs Laboratories, Polysciences, or Novagen Inc., to name only a few. Magnetic nanoparticles based on superparamagnetic Co and FeCo, as well as ferromagnetic Co nanocrystals have been described, for example by Hutten, A. et al. (J. Biotech. (2004), 112, 47-63). In some embodiments, the cells and cell populations are separated or isolated using immunomagnetic (or affinity magnetic) separation techniques (reviewed in Methods in Molecular Medicine, vol. 58: Metastasis Research Protocols, Vol. 2: Cell Behavior In Vitro and In Vivo, p 17-25 Edited by: S. A. Brooks and U. Schumacher © Humana Press Inc., Totowa, NJ).

[0389] In some embodiments, the support contains a stationary phase. Thus, in some embodiments, the binding molecule is comprised on a stationary phase (also called chromatography matrix). In some such embodiments, the binding molecule is reversibly immobilized on the stationary phase. In some cases, the binding molecule is reversibly immobilized to the stationary phase via covalent bonds. In some aspects, the binding molecule is reversibly immobilized to the stationary phase non-covalently.

[0390] Any material may be employed as a chromatography matrix. In general, a suitable chromatography material is essentially innocuous, i.e. not detrimental to cell viability, such as when used in a packed chromatography column under desired conditions. In some embodiments, the stationary phase remains in a predefined location, such as a predefined position, whereas the location of the sample is being altered. Thus, in some embodiments the stationary phase is the part of a chromatographic system through which the mobile phase flows (either by flow through or in a batch mode) and where distribution of the components contained in the liquid phase (either dissolved or dispersed) between the phases occurs.

[0391] In some embodiments, the chromatography matrix has the form of a solid or semisolid phase, whereas the sample that contains the target cell to be isolated/separated is a fluid phase. The chromatography matrix can be a particulate material (of any suitable size and shape) or a monolithic chromatography material, including a paper substrate or membrane. Thus, in some aspects, the chromatography can be both column chromatography as well as planar chromatography. In some embodiments, in addition to standard chromatography columns, columns allowing a bidirectional flow such as PhyTip® columns available from PhyNexus, Inc. San Jose, CA, U.S.A. or pipette tips can be used for column based/flow through mode based methods. Thus, in some cases, pipette tips or columns allowing a bidirectional flow

are also comprised by chromatography columns useful in the present methods. In some cases, such as where a particulate matrix material is used, the particulate matrix material may, for example, have a mean particle size of about 5 μm to about 200 μm, or from about 5 μm to about 400 μm, or from about 5 μm to about 600 μm. In some aspects, the chromatography matrix may, for example, be or include a polymeric resin or a metal oxide or a metalloid oxide. In some aspects, such as where planar chromatography is used, the matrix material may be any material suitable for planar chromatography, such as conventional cellulose-based or organic polymer based membranes (for example, a paper membrane, a nitrocellulose membrane or a polyvinylidene difluoride (PVDF) membrane) or silica coated glass plates. In one embodiment, the chromatography matrix/stationary phase is a non-magnetic material or non-magnetizable material. In other embodiments, a chromatography matrix employed in the present methods is void of any magnetically attractable matter.

[0392] In some embodiments, non-magnetic or non-magnetizable chromatography stationary phases that are suitable in the present methods include derivatized silica or a crosslinked gel. In some aspects, a crosslinked gel may be based on a natural polymer, such as on a polymer class that occurs in nature. For example, a natural polymer on which a chromatography stationary phase may be based is a polysaccharide. In some cases, a respective polysaccharide is generally crosslinked. An example of a polysaccharide matrix includes, but is not limited to, an agarose gel (for example, Superflow™ agarose or a Sepharose® material such as Superflow™ Sepharose® that are commercially available in different bead and pore sizes) or a gel of crosslinked dextran(s). A further illustrative example is a particulate cross-linked agarose matrix, to which dextran is covalently bonded, that is commercially available (in various bead sizes and with various pore sizes) as Sephadex® or Superdex®, both available from GE Healthcare. Another illustrative example of such a chromatography material is Sephacryl® which is also available in different bead and pore sizes from GE Healthcare.

[0393] In some embodiments, a crosslinked gel may also be based on a synthetic polymer, such as on a polymer class that does not occur in nature. In some aspects, such a synthetic polymer on which a chromatography stationary phase is based is a polymer that has polar monomer units, and which is therefore in itself polar. Thus, in some cases, such a polar polymer is hydrophilic. Hydrophilic molecules, also termed lipophobic, in some aspects contain moieties that can form dipole-dipole interactions with water molecules. In general, hydrophobic molecules, also termed lipophilic, have a tendency to separate from water.

[0394] Illustrative examples of suitable synthetic polymers are polyacrylamide(s), a styrene-divinylbenzene gel and a copolymer of an acrylate and a diol or of an acrylamide and a diol. An illustrative example is a polymethacrylate gel, commercially available as a Fractogel®. A further example is a copolymer of ethylene glycol and methacrylate, commercially available as a Toyopearl®. In some embodiments, a chromatography stationary phase may also include natural and synthetic polymer components, such as a composite matrix or a composite or a co-polymer of a polysaccharide and agarose, e.g. a polyacrylamide/agarose composite, or of a polysaccharide and N,N'-methylenebisacrylamide. An illustrative example of a copolymer of a dextran and N,N'-methylenebisacrylamide is the above-mentioned Sephacryl® series of material. In some embodiments, a derivatized silica may include silica particles that are coupled to a synthetic or to a natural polymer. Examples of such embodiments include, but are not limited to, polysaccharide grafted silica, polyvinylpyrrolidone grafted silica, polyethylene oxide grafted silica, poly(2-hydroxyethylaspartamide) silica and poly(N-isopropylacrylamide) grafted silica.

[0395] In some embodiments, the solid support, such as a bead or chromatography matrix, can be used in enrichment and selection methods as described herein by contacting said solid support (e.g. matrix) with a sample containing cells to be enriched or selected as described. In some embodiments, the selected cells are eluted or released from the solid support (e.g. matrix) by disrupting the interaction of the binding molecule and the agent (e.g. affinity tag).

[0396] In some embodiments, binding of the binding molecule to the agent of the cell surface conjugate is reversible. In some embodiments, disrupting the reversible binding of the binding molecule to the agent is achieved by contacting the cells with a composition comprising a substance capable of reversing the bond between the binding molecule and agent. For example, the substance is s free binding partner and/or is a competition agent (e.g. a biotin, a biotin analog, a biologically active fragment thereof). In some embodiments, the methods include after contacting cells in the sample to the solid support containing the binding molecule bound thereto, applying a competition substance to disrupt the bond between the agent (e.g. affinity tag) of the conjugate and binding molecule, thereby recovering the selected cells from the solid surface. Exemplary competition substances for use in the provided methods are described above and the choice of competition substance depends on the particular agent and binding molecule. In some embodiments, the binding molecule is a streptavidin mutein (e.g. Strep-Tactin) for recognition of a streptavidin binding peptide (e.g. Strep-tag) agent and competition substance is biotin or biotin analog.

[0397] In provided embodiments, selection of transduced cells during the manufacturing process using reversible binding between the binding molecule (e.g. streptavidin mutein reagent, such as Strep-Tactin) and the agent (e.g. Strep-tag) of the cell surface conjugate is advantageous over using antibodies with higher affinity to the cell surface conjugate, which may remain attached to cells in products that are administered to subjects. In some embodiments, a Strep-Tactin® is used as the reagent. In some embodiments, detection of the agent portion of the cell surface conjugate with the reagent is reversible and addition of biotin to the sample can gently release the transduced cells.

[0398] In some aspects, reversibility can be achieved because the bond between the streptavidin binding peptide (e.g. Strep-tag) and streptavidin mutein binding reagent is high, but is less than the binding affinity of the streptavidin binding reagent for biotin or a biotin analog. Hence, in some embodiments, biotin (Vitamin H) or a biotin analog can be added to compete for binding to disrupt the binding interaction between the streptavidin mutein binding reagent on the solid support (e.g. bead or chromatography matrix) and the streptavidin binding peptide (e.g. Strep-tag) of the conjugate. In some embodiments, the interaction can be reversed in the presence of low concentrations of biotin or analog, such as in the presence of 0.1 mM to 10 mM, 0.5 mM to 5 mM or 1 mM to 3 mM, such as generally at least or at least about 1 mM or at least 2 mM, for example at or about 2.5 mM. In some embodiments, incubation in the presence of a competing agent, such as a biotin or biotin analog, releases the selected cell from the solid support, such as chromatography matrix or bead.

[0399] In some embodiments, the method further includes separating or removing one or more of the components remaining after the reversible dissociation of components. In some embodiments, any unbound or residual biotin in the target cells (e.g. gene modified, such as transduced, T cells) can be separated or removed. In some embodiments, the binding molecule reagent is removed or separated from the cells in the target cell composition. In some embodiments, due to the dissociation of the reversibly bound binding molecules (e.g. reagents containing a streptavidin mutein, such as Strep-Tactin reagents) from the cell surface conjugate, the provided method has the added advantage that the isolated cells are free of the binding molecule at the end of the contacting or incubation period. In some embodiments, the composition containing target cells is free of any reactants, which in some aspects is an advantageous for use in connection with diagnostic applications (for example, further FACS™ sorting) or for any cell based therapeutic application.

[0400] In some embodiments, the separation/removal of the binding molecule can be carried out using a second stationary phase. For this purpose, a mixture comprising the target cells and one or more remaining components are exposed, before or after being applied onto the first stationary phase described above, to chromatography on a suitable second stationary phase. This secondary stationary phase may be a gel filtration matrix and/or affinity chromatography matrix, wherein the gel filtration and/or affinity chromatography matrix comprises an affinity reagent. The affinity reagent comprised on the chromatography resin include a binding partner D that (specifically) binds to the binding site Z of the binding molecule reagent (e.g. a streptavidin mutein, such as Strep-Tactin), thereby immobilizing the binding molecule reagent on the stationary phase. If a streptavidin based binding molecule reagent is used, such as Strep-Tactin) and the agent of the conjugate is or comprises a streptavidin binding peptide (e.g. Strep-tag), the binding partner D that is comprised in the affinity reagent of this second stationary phase can be biotin. Any remaining streptavidin or of a streptavidin mutein in the composition then binds to the biotin that is usually covalently coupled to a chromatography matrix such as biotin-sepharose™ that is commercially available. In some such embodiments, the target cells (e.g. gene modified, such as transduced, T cells) can be recovered away from the binding molecule reagent.

[0401] In some embodiments, the competition substance used to disrupt or reverse binding between the agent and binding molecule can be easily removed from the stimulated cell population via a "removal cartridge" (see e.g. described in International patent application WO 2013/124474). In some cases, for example in which the binding molecule is immobilized on a solid support, such as a bioreactor surface or a magnetic bead, it is being held back. Thus, the use of a removal cartridge for removal of the free agent and the competition reagent, can include loading the elution sample (e.g. sample obtained after disruption of the reversible binding) onto a second chromatography column. In some embodiments, this chromatography column has a suitable stationary phase that is both an affinity chromatography matrix and, at the same time, can act as gel permeation matrix. In some aspects, this affinity chromatography matrix has an affinity reagent immobilized thereon. In some embodiments, the affinity reagent may, for instance, be streptavidin, a streptavidin mutein, avidin, an avidin mutein or a mixture thereof.

[0402] In some embodiments, the chromatography matrix is a gel filtration matrix, for example, when used in a removal cartridge as described herein. Generally, a gel filtration can be characterized by the property that it is designed to undergo. Hence, a gel filtration matrix in some aspects allows the separation of cells or other biological entities largely on the basis of

their size. In some such aspects, the respective chromatography matrix is typically a particulate porous material as mentioned above. The chromatography matrix may have a certain exclusion limit, which is typically defined in terms of a molecular weight above which molecules are entirely excluded from entering the pores. In some embodiments, the respective molecular weight defining the size exclusion limit may be selected to be below the weight corresponding to the weight of a target cell. In such an embodiment, the target cell is prevented from entering the pores of the size exclusion chromatography matrix. Likewise, a stationary phase may have pores that are of a size that is smaller than the size of a chosen target cell. In illustrative embodiments chromatography matrix has a mean pore size of 0 to about 500 nm.

[0403] In some embodiments, components present in a sample such as a competition substance may have a size that is below the exclusion limit of the pores and thus can enter the pores of the chromatography matrix. In some aspects, of such components that are able to partially or fully enter the pore volume, larger molecules, with less access to the pore volume can elute first, whereas the smallest molecules typically elute last. In some embodiments, the exclusion limit of the chromatography matrix is selected to be below the maximal width of the target cell. Hence, in some aspects, components that have access to the pore volume can remain longer in/on the chromatography matrix than target cell. Thus, in some cases, target cells can be collected in the eluate of a chromatography column separately from other matter/components of a sample. Therefore, in some aspects, components such as a competition substance, may elute at a later point of time from a gel filtration matrix than the target cell. In some embodiments, this effect can be further increased, such as if the gel permeation matrix contains an affinity reagent (such as covalently bound thereon) that contains binding sites Z that are able to bind a competition substance present in a sample. In some cases, the competition substance can be bound by the binding sites Z of the reagent and thereby immobilized on the matrix. In some aspects, this method is carried out in a removal cartridge.

[0404] In some embodiments, provided is an apparatus that contains at least one arrangement of a first and a second stationary phase, such as chromatography column for selection of target cells (a selection cartridge) and a second chromatography column (a removal cartridge) for removal of reagents. The apparatus may comprise a plurality of arrangements of first and second stationary phases (chromatography columns) being fluidly connected in series. The apparatus may comprise a sample inlet being fluidly connected to the first stationary phase of the first arrangement of the first and second stationary phases. In some embodiments, the apparatus may also comprise a sample outlet for cells, the sample outlet being fluidly connected

to the second stationary phase of the last of the at least one arrangement of a first and second stationary phases for chromatography. In some aspects, the apparatus may also comprise a competition reagent container that is fluidly connected to at least one of the first stationary phases of the arrangements of the first and second stationary phases.

[0405] In some embodiments, the ability to remove the reagent and other components from the composition has the further advantage of being able to avoid any solid support such as magnetic beads. In some embodiments, this means there is no risk or minimal risk of contamination of the target cells (e.g. gene modified, such as transduced, T cells) by such magnetic beads. In some embodiments, this also means that a process that is compliant with GMP standards can be more easily established compared to other methods, such as the use of Dynabeads® in which additional measures have to be taken to ensure that the final T cell population is free of magnetic beads.

[0406] In some embodiments, since no solid phase (e.g. magnetic beads) are present, the present invention also provides for an automated closed system for expansion of the cells that can be integrated into known cell expansion systems such as the Xuri Cell Expansion System W25 and WAVE Bioreactor 2/10 System, available from GE Healthcare (HYPERLINK "http://en.wikipedia.org/wiki/Little_Chalfont" \o "Little Chalfont" Little Chalfont, Buckinghamshire, United Kingdom) or the Quantum® Cell Expansion System, available from TerumoBCT Inc. (Lakewood, CO, USA).

[0407] In some embodiments, the closed system is automated. In some embodiments, components associated with the system can include an integrated microcomputer, peristaltic pump, and various valves, such as pinch valves or stop cocks, to control flow of fluid between the various parts of the system. The integrated computer in some aspects controls all components of the instrument and directs the system to perform repeated procedures in a standardized sequence. In some embodiments, the peristaltic pump controls the flow rate throughout the tubing set and, together with the pinch valves, ensures the controlled flow of buffer through the system.

[0408] In some embodiments, the methods is carried out to select, isolate or enrich cells that express the cell surface conjugate based on detection of the agent (e.g. affinity tag), such as a streptavidin binding peptide, of the conjugate. In some aspects, the isolated, enriched or selected cells represent cells that have been genetically engineered, such as by transduction, with a nucleic acid molecule encoding the cell surface conjugate, and, optionally, a co-expressed recombinant receptor, such as a CAR. In some embodiments, the provided methods produce or

result in a cell composition containing cells enriched for cells expressing the cell surface conjugate, and hence also cells expressing a recombinant receptor.

[0409] In some embodiments, the yield of cells expressing the cell surface conjugate in the enriched composition, i.e. the number of enriched cells in the population compared to the number of the same population of cells in the starting sample, is 10% to 100%, such as 20% to 80%, 20% to 60%, 20% to 40%, 40% to 80%, 40% to 60%, or 60% to 80%.

[0410] In some embodiments, the percentage of the cells expressing the cell surface conjugate in the enriched or isolated composition, i.e. the percentage of cells positive for the selected cell surface conjugate versus total cells in the population of enriched or isolated cells, is at least at or about 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, and is generally at least at or about 95%, 96%, 97%, 98%, 99% or greater.

V. COMPOSITIONS AND FORMULATIONS

[0411] Provided are compositions including cells, such as engineered cells containing the cell surface conjugate and/or additional recombinant receptors, e.g., CAR, for administration. In some aspects, the pharmaceutical compositions and formulations are provided as unit dose form compositions including the number of cells for administration in a given dose or fraction thereof. The pharmaceutical compositions and formulations generally include one or more optional pharmaceutically acceptable carrier or excipient. In some embodiments, the composition includes at least one additional therapeutic agent.

[0412] The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[0413] A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[0414] In some aspects, the choice of carrier is determined in part by the particular cell and/or by the method of administration. Accordingly, there are a variety of suitable formulations. For example, the pharmaceutical composition can contain preservatives. Suitable preservatives may include, for example, methylparaben, propylparaben, sodium benzoate, and benzalkonium chloride. In some aspects, a mixture of two or more preservatives is used. The preservative or mixtures thereof are typically present in an amount of about 0.0001% to about

2% by weight of the total composition. Carriers are described, e.g., by Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980). Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG).

[0415] Buffering agents in some aspects are included in the compositions. Suitable buffering agents include, for example, citric acid, sodium citrate, phosphoric acid, potassium phosphate, and various other acids and salts. In some aspects, a mixture of two or more buffering agents is used. The buffering agent or mixtures thereof are typically present in an amount of about 0.001% to about 4% by weight of the total composition. Methods for preparing administrable pharmaceutical compositions are known. Exemplary methods are described in more detail in, for example, Remington: The Science and Practice of Pharmacy, Lippincott Williams & Wilkins; 21st ed. (May 1, 2005).

[0416] The formulation or composition may also contain more than one active ingredients useful for the particular indication, disease, or condition being treated with the cells, preferably those with activities complementary to the cell, where the respective activities do not adversely affect one another. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended. Thus, in some embodiments, the pharmaceutical composition further includes other pharmaceutically active agents or drugs, such as chemotherapeutic agents, e.g., asparaginase, busulfan, carboplatin, cisplatin, daunorubicin, doxorubicin, fluorouracil, gemcitabine, hydroxyurea, methotrexate, paclitaxel, rituximab, vinblastine, vincristine, etc. In some embodiments, the cells or antibodies are administered in the form of a salt, e.g., a pharmaceutically acceptable salt. Suitable pharmaceutically acceptable

acid addition salts include those derived from mineral acids, such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric, and sulphuric acids, and organic acids, such as tartaric, acetic, citric, malic, lactic, fumaric, benzoic, glycolic, gluconic, succinic, and arylsulphonic acids, for example, p-toluenesulphonic acid.

[0417] Active ingredients may be entrapped in microcapsules, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. In certain embodiments, the pharmaceutical composition is formulated as an inclusion complex, such as cyclodextrin inclusion complex, or as a liposome. Liposomes can serve to target the host cells (e.g., T-cells or NK cells) to a particular tissue. Many methods are available for preparing liposomes, such as those described in, for example, Szoka et al., Ann. Rev. Biophys. Bioeng., 9: 467 (1980), and U.S. Patents 4,235,871, 4,501,728, 4,837,028, and 5,019,369.

[0418] The pharmaceutical composition in some aspects can employ time-released, delayed release, and sustained release delivery systems such that the delivery of the composition occurs prior to, and with sufficient time to cause, sensitization of the site to be treated. Many types of release delivery systems are available and known. Such systems can avoid repeated administrations of the composition, thereby increasing convenience to the subject and the physician.

[0419] The pharmaceutical composition in some embodiments contains engineered cells in amounts effective to treat or prevent the disease or condition, such as a therapeutically effective or prophylactically effective amount. Therapeutic or prophylactic efficacy in some embodiments is monitored by periodic assessment of treated subjects. For repeated administrations over several days or longer, depending on the condition, the treatment is repeated until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful and can be determined. The desired dosage can be delivered by a single bolus administration of the composition, by multiple bolus administrations of the composition, or by continuous infusion administration of the composition.

[0420] The pharmaceutical compositions, such as those containing the engineered cells, may be administered using standard administration techniques, formulations, and/or devices. Provided are formulations and devices, such as syringes and vials, for storage and administration of the compositions. Administration of the engineered cells can be autologous or heterologous. For example, immunoresponsive cells or progenitors can be obtained from one subject, and administered to the same subject or a different, compatible subject. Peripheral blood derived

immunoresponsive cells or their progeny (e.g., in vivo, ex vivo or in vitro derived) can be administered via localized injection, including catheter administration, systemic injection, localized injection, intravenous injection, or parenteral administration. When administering a therapeutic composition (e.g., a pharmaceutical composition containing a genetically modified immunoresponsive cell), it will generally be formulated in a unit dosage injectable form (solution, suspension, emulsion).

[0421] Formulations include those for oral, intravenous, intraperitoneal, subcutaneous, pulmonary, transdermal, intramuscular, intranasal, buccal, sublingual, or suppository administration. In some embodiments, the cell populations are administered parenterally. The term "parenteral," as used herein, includes intravenous, intramuscular, subcutaneous, rectal, vaginal, and intraperitoneal administration. In some embodiments, the cell populations are administered to a subject using peripheral systemic delivery by intravenous, intraperitoneal, or subcutaneous injection.

[0422] Compositions in some embodiments are provided as sterile liquid preparations, e.g., isotonic aqueous solutions, suspensions, emulsions, dispersions, or viscous compositions, which may in some aspects be buffered to a selected pH. Liquid preparations are normally easier to prepare than gels, other viscous compositions, and solid compositions. Additionally, liquid compositions are somewhat more convenient to administer, especially by injection. Viscous compositions, on the other hand, can be formulated within the appropriate viscosity range to provide longer contact periods with specific tissues. Liquid or viscous compositions can comprise carriers, which can be a solvent or dispersing medium containing, for example, water, saline, phosphate buffered saline, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol) and suitable mixtures thereof.

[0423] Sterile injectable solutions can be prepared by incorporating the cells in a solvent, such as in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, dextrose, or the like. The compositions can also be lyophilized. The compositions can contain auxiliary substances such as wetting, dispersing, or emulsifying agents (e.g., methylcellulose), pH buffering agents, gelling or viscosity enhancing additives, preservatives, flavoring agents, colors, and the like, depending upon the route of administration and the preparation desired. Standard texts may in some aspects be consulted to prepare suitable preparations.

[0424] Various additives which enhance the stability and sterility of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added.

Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0425] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules.

[0426] The formulations to be used for in vivo administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

VI. METHODS OF ADMINISTRATION AND TREATMENTAND RELATED METHODS

[0427] Also provided are methods of using and uses of the molecules and compositions, such as containing the engineered cells, in the treatment of diseases, conditions, and disorders in which the antigen recognized by the recombinant receptor (e.g. CAR) is expressed. Also provided are methods and uses for identification, detection or selection of the molecules and compositions, such as containing the engineered cells, by recognition of the cell surface conjugate expressed by the engineered cells. In some embodiments, such methods include diagnostic and prognostic methods as well as, in some cases, suicide or deletion methods of the engineered cells. Included among such methods are methods of monitoring the administered engineered cells and methods of modulating the engineered cells, such as in connection with adoptive cell therapy.

[0428] In some embodiments, the cell surface conjugate containing a cell surface molecule and at least one agent is used for detection of cells transduced with the cell surface conjugate. In some embodiments, the detection is *in vivo or ex vivo*. In some embodiments, the cell surface receptor conjugate is used for targeting engineered cells for suicide killing of engineered cells. In some aspects, killing of cells transduced to express the cell surface conjugate uses binding molecules specific for the cell surface molecule of the expressed cell surface conjugate. In other aspects, provided are methods of killing cells by targeting the agent of the cell surface conjugate using a molecule comprising a binding molecule specific for the agent of the conjugate linked to a cytotoxic agent, such as a toxin.

A. Adoptive Cell Therapy Methods

[0429] Provided are methods of administering the engineered cells and compositions, and uses of such engineered cells and compositions to treat or prevent diseases, conditions, and disorders, including cancers. In some embodiments, the engineered cells and compositions are administered to a subject or patient having the particular disease or condition to be treated, e.g., via adoptive cell therapy, such as adoptive T cell therapy. In some embodiments, provided cells and compositions are administered to a subject, such as a subject having or at risk for the disease or condition. In some aspects, the methods thereby treat, e.g., ameliorate one or more symptom of, the disease or condition, such as by lessening tumor burden in a cancer expressing an antigen recognized by an engineered T cell.

[0430] Methods for administration of engineered cells for adoptive cell therapy are known and may be used in connection with the provided methods and compositions. For example, adoptive T cell therapy methods are described, e.g., in US Patent Application Publication No. 2003/0170238 to Gruenberg et al; US Patent No. 4,690,915 to Rosenberg; Rosenberg (2011) Nat Rev Clin Oncol. 8(10):577-85). See, e.g., Themeli et al. (2013) Nat Biotechnol. 31(10): 928-933; Tsukahara et al. (2013) Biochem Biophys Res Commun 438(1): 84-9; Davila et al. (2013) PLoS ONE 8(4): e61338.

[0431] As used herein, a "subject" is a mammal, such as a human or other animal, and typically is human. In some embodiments, the subject, e.g., patient, to whom the immunomodulatory polypeptides, engineered cells, or compositions are administered, is a mammal, typically a primate, such as a human. In some embodiments, the primate is a monkey or an ape. The subject can be male or female and can be any suitable age, including infant, juvenile, adolescent, adult, and geriatric subjects. In some embodiments, the subject is a non-primate mammal, such as a rodent.

[0432] As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to complete or partial amelioration or reduction of a disease or condition or disorder, or a symptom, adverse effect or outcome, or phenotype associated therewith. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis.

The terms do not imply complete curing of a disease or complete elimination of any symptom or effect(s) on all symptoms or outcomes.

[0433] As used herein, "delaying development of a disease" means to defer, hinder, slow, retard, stabilize, suppress and/or postpone development of the disease (such as cancer). This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease. For example, a late stage cancer, such as development of metastasis, may be delayed.

[0434] "Preventing," as used herein, includes providing prophylaxis with respect to the occurrence or recurrence of a disease in a subject that may be predisposed to the disease but has not yet been diagnosed with the disease. In some embodiments, the provided cells and compositions are used to delay development of a disease or to slow the progression of a disease.

[0435] As used herein, to "suppress" a function or activity is to reduce the function or activity when compared to otherwise same conditions except for a condition or parameter of interest, or alternatively, as compared to another condition. For example, cells that suppress tumor growth reduce the rate of growth of the tumor compared to the rate of growth of the tumor in the absence of the cells.

[0436] An "effective amount" of an agent, e.g., a pharmaceutical formulation, cells, or composition, in the context of administration, refers to an amount effective, at dosages/amounts and for periods of time necessary, to achieve a desired result, such as a therapeutic or prophylactic result.

[0437] A "therapeutically effective amount" of an agent, e.g., a pharmaceutical formulation or engineered cells, refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result, such as for treatment of a disease, condition, or disorder, and/or pharmacokinetic or pharmacodynamic effect of the treatment. The therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the subject, and the immunomodulatory polypeptides or engineered cells administered. In some embodiments, the provided methods involve administering the immunomodulatory polypeptides, engineered cells, or compositions at effective amounts, e.g., therapeutically effective amounts.

[0438] A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically but not

necessarily, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

[0439] The disease or condition that is treated can be any in which expression of an antigen is associated with and/or involved in the etiology of a disease condition or disorder, e.g. causes, exacerbates or otherwise is involved in such disease, condition, or disorder. Exemplary diseases and conditions can include diseases or conditions associated with malignancy or transformation of cells (e.g. cancer), autoimmune or inflammatory disease, or an infectious disease, e.g. caused by a bacterial, viral or other pathogen. Exemplary antigens, which include antigens associated with various diseases and conditions that can be treated, are described above. In particular embodiments, the immunomodulatory polypeptide and/or recombinant receptor, e.g., the chimeric antigen receptor or transgenic TCR, specifically binds to an antigen associated with the disease or condition.

[0440] In some embodiments, the disease or condition is a tumor, such as a solid tumor, lymphoma, leukemia, blood tumor, metastatic tumor, or other cancer or tumor type.

[0441] In some embodiments, the disease or condition is an infectious disease or condition, such as, but not limited to, viral, retroviral, bacterial, and protozoal infections, immunodeficiency, Cytomegalovirus (CMV), Epstein-Barr virus (EBV), adenovirus, BK polyomavirus. In some embodiments, the disease or condition is an autoimmune or inflammatory disease or condition, such as arthritis, e.g., rheumatoid arthritis (RA), Type I diabetes, systemic lupus erythematosus (SLE), inflammatory bowel disease, psoriasis, scleroderma, autoimmune thyroid disease, Grave's disease, Crohn's disease, multiple sclerosis, asthma, and/or a disease or condition associated with transplant.

[0011] In some embodiments, the antigen associated with the disease or disorder is selected from the group consisting of αvβ6 integrin (avb6 integrin), B cell maturation antigen (BCMA), B7-H3, B7-H6, carbonic anhydrase 9 (CA9, also known as CAIX or G250), a cancer-testis antigen, cancer/testis antigen 1B (CTAG, also known as NY-ESO-1 and LAGE-2), carcinoembryonic antigen (CEA), a cyclin, cyclin A2, C-C Motif Chemokine Ligand 1 (CCL-1), CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD138, CD171, epidermal growth factor protein (EGFR), truncated epidermal growth factor protein (tEGFR), type III epidermal growth factor receptor mutation (EGFR vIII), epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), ephrinB2, ephrine receptor A2 (EPHa2), estrogen receptor, Fc receptor like 5 (FCRL5; also known as Fc receptor homolog 5 or FCRH5), fetal acetylcholine receptor (fetal AchR), a folate binding protein (FBP), folate

receptor alpha, ganglioside GD2, O-acetylated GD2 (OGD2), ganglioside GD3, glycoprotein 100 (gp100), G Protein Coupled Receptor 5D (GPCR5D), Her2/neu (receptor tyrosine kinase erb-B2), Her3 (erb-B3), Her4 (erb-B4), erbB dimers, Human high molecular weight-melanoma-associated antigen (HMW-MAA), hepatitis B surface antigen, Human leukocyte antigen A1 (HLA-A1), Human leukocyte antigen A2 (HLA-A2), IL-22 receptor alpha(IL-22Ra), IL-13 receptor alpha 2 (IL-13Ra2), kinase insert domain receptor (kdr), kappa light chain, L1 cell adhesion molecule (L1-CAM), CE7 epitope of L1-CAM, Leucine Rich Repeat Containing 8 Family Member A (LRRC8A), Lewis Y, Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, mesothelin, c-Met, murine cytomegalovirus (CMV), mucin 1 (MUC1), MUC16, natural killer group 2 member D (NKG2D) ligands, melan A (MART-1), neural cell adhesion molecule (NCAM), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME), progesterone receptor, a prostate specific antigen, prostate stem cell antigen (PSCA), prostate specific membrane antigen (PSMA), Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1), survivin, Trophoblast glycoprotein (TPBG also known as 5T4), tumor-associated glycoprotein 72 (TAG72), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor receptor 2 (VEGFR2), Wilms Tumor 1 (WT-1), a pathogen-specific antigen, or an antigen associated with a universal tag, and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV or other pathogens. Antigens targeted by the receptors in some embodiments include antigens associated with a B cell malignancy, such as any of a number of known B cell marker. In some embodiments, the antigen is or includes CD20, CD19, CD22, ROR1, CD45, CD21, CD5, CD33, Igkappa, Iglambda, CD79a, CD79b or CD30.

[0012] In some embodiments, the antigen is or includes a pathogen-specific or pathogen-expressed antigen. In some embodiments, the antigen is a viral antigen (such as a viral antigen from HIV, HCV, HBV, etc.), bacterial antigens, and/or parasitic antigens.

[0442] In some embodiments, the antigen associated with the disease or disorder is selected from the group consisting of orphan tyrosine kinase receptor RORl, tEGFR, HER2, Ll-CAM, CD19, CD20, CD22, mesothelin, CEA, and hepatitis B surface antigen, anti-folate receptor, CD23, CD24, CD30, CD33, CD38, CD44, EGFR, EGP-2, EGP-4, 0EPHa2, ErbB2, 3, or 4, FBP, fetal acethycholine e receptor, GD2, GD3, HMW-MAA, IL-22R-alpha, IL-13R-alpha2, kdr, kappa light chain, Lewis Y, L1-cell adhesion molecule, MAGE-A1, mesothelin, MUC1, MUC16, PSCA, NKG2D Ligands, NY-ESO-1, MART-1, gp100, oncofetal antigen, TAG72, VEGF-R2, carcinoembryonic antigen (CEA), prostate specific antigen, PSMA, HER2/neu,

estrogen receptor, progesterone receptor, ephrinB2, CD123, CS-1, c-Met, GD-2, and MAGE A3, CE7, Wilms Tumor 1 (WT-1), a cyclin, such as cyclin A1 (CCNA1), and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV or other pathogens.

[0443] The provided methods and uses include methods and uses for adoptive cell therapy. In some embodiments, the methods include administration of the engineered cells or a composition containing the cells to a subject, tissue, or cell, such as one having, at risk for, or suspected of having the disease, condition or disorder. In some embodiments, the cells, populations, and compositions are administered to a subject having the particular disease or condition to be treated, e.g., via adoptive cell therapy, such as adoptive T cell therapy. In some embodiments, the cells or compositions are administered to the subject, such as a subject having or at risk for the disease or condition, ameliorate one or more symptom of the disease or condition.

[0444] In some embodiments, the cell therapy, e.g., adoptive T cell therapy, is carried out by autologous transfer, in which the cells are isolated and/or otherwise prepared from the subject who is to receive the cell therapy, or from a sample derived from such a subject. Thus, in some aspects, the cells are derived from a subject, e.g., patient, in need of a treatment and the cells, following isolation and processing are administered to the same subject.

[0445] In some embodiments, the cell therapy, e.g., adoptive T cell therapy, is carried out by allogeneic transfer, in which the cells are isolated and/or otherwise prepared from a subject other than a subject who is to receive or who ultimately receives the cell therapy, e.g., a first subject. In such embodiments, the cells then are administered to a different subject, e.g., a second subject, of the same species. In some embodiments, the first and second subjects are genetically identical. In some embodiments, the first and second subjects are genetically similar. In some embodiments, the second subject expresses the same HLA class or super type as the first subject. The cells can be administered by any suitable means. Dosing and administration may depend in part on whether the administration is brief or chronic. Various dosing schedules include but are not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion.

[0446] In certain embodiments, the cells, or individual populations of sub-types of cells, are administered to the subject at a range of about one million to about 100 billion cells and/or that amount of cells per kilogram of body weight, such as, e.g., 1 million to about 50 billion cells (e.g., about 5 million cells, about 25 million cells, about 500 million cells, about 1 billion cells, about 5 billion cells, about 20 billion cells, about 30 billion cells, about 40 billion cells, or a

range defined by any two of the foregoing values), such as about 10 million to about 100 billion cells (e.g., about 20 million cells, about 30 million cells, about 40 million cells, about 60 million cells, about 70 million cells, about 80 million cells, about 90 million cells, about 10 billion cells, about 25 billion cells, about 50 billion cells, about 75 billion cells, about 90 billion cells, or a range defined by any two of the foregoing values), and in some cases about 100 million cells to about 50 billion cells (e.g., about 120 million cells, about 250 million cells, about 350 million cells, about 450 million cells, about 650 million cells, about 800 million cells, about 900 million cells, about 3 billion cells, about 30 billion cells, about 45 billion cells) or any value in between these ranges and/or per kilogram of body weight. Dosages may vary depending on attributes particular to the disease or disorder and/or patient and/or other treatments.

[0447] In some embodiments, for example, where the subject is a human, the dose includes fewer than about 5 x 10^8 total recombinant receptor (e.g., CAR)-expressing cells, T cells, or peripheral blood mononuclear cells (PBMCs), e.g., in the range of about 1 x 10^6 to 5 x 10^8 such cells, such as 2 x 10^6, 5 x 10^6, 1 x 10^7, 5 x 10^7, 1 x 10^8, or 5 x 10^8 or total such cells, or the range between any two of the foregoing values.

[0448] In some embodiments, the cell therapy comprises administration of a dose comprising a number of cell from or from about 1 x 10^5 to 5 x 10^8 total recombinant receptor-expressing cells, total T cells, or total peripheral blood mononuclear cells (PBMCs), from or from about 5 x 10^5 to 1 x 10^7 total recombinant receptor-expressing cells, total T cells, or total peripheral blood mononuclear cells (PBMCs) or from or from about 1 x 10^6 to 1 x 10^7 total recombinant receptor-expressing cells, total T cells, or total peripheral blood mononuclear cells (PBMCs), each inclusive. In some embodiments, the cell therapy comprises administration of a dose of cells comprising a number of cells at least at or about 1 x 10^5 total recombinant receptor-expressing cells, total T cells, or total peripheral blood mononuclear cells (PBMCs), such at least or at least 1 x 10^6, at least at or about 1 x 10^7, at least at or about 1 x 10^8 of such cells. In some embodiments, the number is with reference to the total number of CD3+ or CD8+, in some cases also recombinant receptor-expressing (e.g. CAR+) cells. In some embodiments, the cell therapy comprises administration of a dose comprising a number of cell from or from about 1 x 10^5 to 5 x 10^8 CD3+ or CD8+ total T cells or CD3+ or CD8+ recombinant receptor-expressing cells, from or from about 5 x 10^5 to 1 x 10^7 CD3+ or CD8+ total T cells or CD3+ or CD8+ recombinant receptor-expressing cells, or from or from about 1 x 10^6 to 1 x 10^7 CD3+ or CD8+ total T cells or CD3+ or CD8+recombinant receptor-expressing cells, each inclusive. In some embodiments, the cell therapy comprises administration of a dose comprising a number of cell

from or from about 1 x 10^5 to 5 x 10^8 total CD3+/CAR+ or CD8+/CAR+ cells, from or from about 5 x 10^5 to 1 x 10^7 total CD3+/CAR+ or CD8+/CAR+ cells, or from or from about 1 x 10^6 to 1 x 10^7 total CD3+/CAR+ or CD8+/CAR+ cells, each inclusive.

[0449] In some embodiments, the T cells of the dose include CD4+ T cells, CD8+ T cells or CD4+ and CD8+ T cells.

[0450] In some embodiments, for example, where the subject is human, the CD8+ T cells of the dose, including in a dose including CD4+ and CD8+ T cells, includes between about 1 x 10^6 and 5 x 10^8 total recombinant receptor (e.g., CAR)-expressing CD8+cells, e.g., in the range of about 5 x 10^6 to 1 x 10^8 such cells, such cells 1 x 10^7, 2.5 x 10^7, 5 x 10^7, 7.5 x 10^7, 1 x 10^8, or 5 x 10^8 total such cells, or the range between any two of the foregoing values. In some embodiments, the patient is administered multiple doses, and each of the doses or the total dose can be within any of the foregoing values. In some embodiments, the dose of cells comprises the administration of from or from about 1 x 10^7 to 0.75 x 10^8 total recombinant receptor-expressing CD8+ T cells, 1 x 10^7 to 2.5 x 10^7 total recombinant receptor-expressing CD8+ T cells, from or from about 1 x 10^7 to 0.75 x 10^8 total recombinant receptor-expressing CD8+ T cells, each inclusive. In some embodiments, the dose of cells comprises the administration of or about 1 x 10^7, 2.5 x 10^7, 5 x 10^7 7.5 x 10^7, 1 x 10^8, or 5 x 10^8 total recombinant receptor-expressing CD8+ T cells.

[0451] In some embodiments, the dose of cells, e.g., recombinant receptor-expressing T cells, is administered to the subject as a single dose or is administered only one time within a period of two weeks, one month, three months, six months, 1 year or more.

[0452] In some embodiments, the cells are administered as part of a combination treatment, such as simultaneously with or sequentially with, in any order, another therapeutic intervention, such as an antibody or engineered cell or receptor or agent, such as a cytotoxic or therapeutic agent. The cells in some embodiments are co-administered with one or more additional therapeutic agents or in connection with another therapeutic intervention, either simultaneously or sequentially in any order. In some contexts, the cells are co-administered with another therapy sufficiently close in time such that the cell populations enhance the effect of one or more additional therapeutic agents, or vice versa. In some embodiments, the cells are administered prior to the one or more additional therapeutic agents. In some embodiments, the cells are administered after the one or more additional therapeutic agents. In some embodiments, the one or more additional agents include a cytokine, such as IL-2, for example, to enhance persistence. In some embodiments, the methods comprise administration of a chemotherapeutic agent.

[0453] Following administration of the cells, the biological activity of the engineered cell populations in some embodiments is measured, e.g., by any of a number of known methods. Parameters to assess include specific binding of an engineered or natural T cell or other immune cell to antigen, in vivo, e.g., by imaging, or ex vivo, e.g., by ELISA or flow cytometry. In certain embodiments, the ability of the engineered cells to destroy target cells can be measured using any suitable method known in the art, such as cytotoxicity assays described in, for example, Kochenderfer et al., J. Immunotherapy, 32(7): 689-702 (2009), and Herman et al. J. Immunological Methods, 285(1): 25-40 (2004). In certain embodiments, the biological activity of the cells is measured by assaying expression and/or secretion of one or more cytokines, such as CD107a, IFNγ, IL-2, and TNF. In some aspects the biological activity is measured by assessing clinical outcome, such as reduction in tumor burden or load.

[0454] In certain embodiments, the engineered cells are further modified in any number of ways, such that their therapeutic or prophylactic efficacy is increased. For example, the engineered recombinant receptor, such as CAR or TCR, expressed by the population can be conjugated either directly or indirectly through a linker to a targeting moiety. The practice of conjugating compounds, e.g., the CAR or TCR, to targeting moieties is known in the art. See, for instance, Wadwa et al., J. Drug Targeting 3: 1 1 1 (1995), and U.S. Patent 5,087,616.

B. Detection and Monitoring

[0455] In some embodiments, methods are provided for monitoring, such as detecting or identifying, cells administered to the subject, such as for determining or assessing the presence, number or location of such cells in the subject. In some embodiments, detection is carried out *ex vivo* from a sample from the subject. In some embodiments, detection is carried out *in vivo*.

[0456] In some embodiments, the method of monitoring is performed *ex vivo* and includes detecting cells expressing the cell surface conjugate by contacting a composition containing cells that express or are likely to express the cell surface conjugate with a binding molecule capable of recognizing the agent of the cell surface conjugate. In some aspects, a sample is obtained from the subject and contacted with a binding molecule that binds the agent of the conjugate, such as an antibody or non-antibody reagent, including any as described. For example, biological samples include, but are not limited to, body fluids, such as blood, plasma, serum, cerebrospinal fluid, synovial fluid, urine and sweat, tissue and organ samples, including processed samples derived therefrom.

[0457] In some embodiments, any of the methods as described above can be employed for detecting or identifying cells expressing the cell surface conjugate obtained from a sample from a subject. In certain embodiments, recombinant cells expressing the conjugate may be detected or tracked *ex vivo* by using antibodies that bind with specificity to the agent or by using a non-antibody reagent (e.g., Strep-Tactin® binding to the Strep-tag®). In some embodiments, the agent is a streptavidin binding peptide, such as a Strep-tag, including a Strep-tag® II or twin-Strep-tag as described. In some embodiments, the binding molecule that recognizes the agent is a reagent capable of reversibly binding to the agent, such as a streptavidin mutein, including Strep-Tactin or other streptavidin mutein that specifically binds to the agent. In some embodiments, the binding molecule that recognizes the agent is an antibody, such as an anti-Strep-tag antibody.

[0458] In some aspects, the detection of cells expressing the cell surface conjugate is followed by a step for isolating or selecting the cells bound to the binding molecule. In some embodiments, the cells can be further analyzed or assessed for one or more properties or activities, such as for cell surface phenotype based on expression of cell surface markers (e.g. activation markers), expression of the recombinant receptor (e.g. CAR), or for one or more antigen-specific activities, including cytotoxic activity, ability to secrete cytokines or ability to proliferate.

[0459] In some embodiments, the method of monitoring is performed *in vivo* by administering to the subject a binding molecule that specifically binds the agent of the conjugate. In some embodiments, the binding molecule administered to the subject is one that recognizes the agent, such as any as described herein. In some embodiments, the agent is a streptavidin binding peptide, such as a Strep-tag, including a Strep-tag® II or twin-Strep-tag as described. In some embodiments, the binding molecule is a non-antibody agent capable of reversibly binding to the agent, such as a streptavidin mutein, including Strep-Tactin or other streptavidin mutein that specifically binds to the agent. In some embodiments, the binding molecule that recognizes the agent is an antibody, such as an anti-Strep-tag antibody. In some embodiments, imaging of cells, such as cells expressing the conjugate and hence, a recombinant receptor, in real time reveals the locations of transduced cells *in vivo*.

[0460] In aspects of such methods, the binding molecule administered to a subject is soluble. In embodiments, the binding molecule is an antibody or is an antigen-binding fragment comprising a portion of an intact antibody that binds the agent (e.g. Strep-tag) to which the

intact antibody binds. In other embodiments, the binding molecule is a non-antibody reagent capable of binding to the agent of the conjugate.

[0461] In the case of non-antibody reagents, such as a streptavidin mutein, including Strep-Tactin or other streptavidin mutein, the binding molecule is not bound to a solid support, i.e. it is present in soluble form or is soluble. In principle, the same reagent can be used as in the case of a reagent that is immobilized on a support, such as a solid support or stationary phase, such as described above. For example, any of the exemplary of reagents described above can be used without immobilizing or attaching such reagent to a support, e.g. not attaching solid support or stationary phase. In some cases, the reagent is an oligomer or polymer of individual molecules or an oligomer or polymer of a complex of subunits that make up the individual molecule (e.g. oligomers or polymers of a dimeric, trimeric or tetrameric protein). In some embodiments, the reagent can, for example, be a streptavidin mutein oligomer, a calmodulin oligomer, a compound (oligomer) that provides least two chelating groups K, wherein the at least two chelating groups are capable of binding to a transition metal ion, thereby rendering the reagent capable of binding to an oligohistidine affinity tag, multimeric glutathione-S-transferase, or a biotinylated carrier protein.

[0462] In some embodiments, the binding molecule, such as a non-antibody reagent (e.g. a streptavidin or mutein, such as tetrameric streptavidin muteins), is characterized by the absence of a solid support (surface) attached to the reagent. For example, in some embodiments, the reagent does not comprise or is not attached (directly or indirectly) to a particle, bead, nanoparticle, microsphere or other solid support. In some embodiments, the reagent is not rigid, inflexible or stiff or does not comprise or is not attached to a rigid, inflexible, or stiff surface. In some embodiments, the reagent is flexible or substantially flexible. In some cases, the reagent is able to adjust or adapt to the form of the surface of the cells. In some embodiments, the reagent does not or does not comprise a shape that is spherical or substantially spherical.

[0463] In some embodiments, substantially all, i.e. more than 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more of the binding molecule, such as a non-antibody reagent (e.g. a streptavidin or mutein, such as tetrameric streptavidin muteins), is composed of or contains organic material. For example, in some embodiments, more than 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more of the reagent is, is composed of or contains lipids, carbohydrates, proteins, peptides or mixtures thereof. In some embodiments, the binding molecule, such as a non-antibody reagent (e.g. a streptavidin or mutein, such as tetrameric streptavidin muteins), is composed of or contains an essential absence of inorganic

material, an inorganic core, e.g. metal, e.g. iron, synthetic or inorganic polymers, such as styrene polymers, e.g. polystyrene, latex, silica or magnetic cores. For example, in some embodiments, the relative percentage of inorganic material of the reagent or that is comprised as part of the reagent is less than 20%, 15%, 10%, 5% or less.

[0464] In some embodiments, the majority (i.e. more than 50%), such as more than 60%, 70%, 80%, 90%, 95%, 99% or more of the total volume of the binding molecule, such as anon-antibody reagent (e.g. a streptavidin or mutein, such as tetrameric streptavidin muteins), in aqueous solution consists of the individual protein molecules that comprise the reagent, such as oligomers or polymers of individual molecules or a complex of subunits that make up an individual molecule (e.g. tetrameric molecule). In some embodiments, the total density of the soluble reagent is less than 1.2 g/cm^3, 1.1 g/cm^3, 1.0 g/cm^3 or less.

[0465] In some embodiments, the soluble reagent, e.g. not being attached to a support or solid support (e.g. is not attached to a bead), has a relatively small size, such as generally less than or about less than 20 nM in size, such as less than or about less than 15 nM, less than or about less than 10 nM, less than or about less than 5 nM or smaller.

[0466] In some embodiments, the soluble reagent, e.g. not being attached to a support or solid support (e.g. is not attached to a bead), is biologically inert, i.e. it is non-toxic to living cells. In some embodiments, the reagent may be biodegradable, for example, it can be degraded by enzymatic activity or cleared by phagocytic cells.

[0467] In some embodiments, it is possible to react the binding molecule, such as a non-antibody reagent (e.g. a streptavidin mutein or oligomers thereof) to a carrier, such as an organic carrier. In some aspects, in addition to a reaction with a polysaccharide, it is also possible to use physiologically or pharmaceutically acceptable proteins such as serum albumin (for example human serum albumin (HSA) or bovine serum albumin (BSA)) as carrier protein. In such a case, the reagent, such as streptavidin or a streptavidin mutein (either as individual tetramer or also in the form of oligomers), can be coupled to the carrier protein via non-covalent interaction. In some such embodiments, biotinylated BSA (which is commercially available from various suppliers such as ThermoFisher Scientific, Sigma Aldrich or Vectorlabs, to name only a few) can be reacted with the reagent (e.g. streptavidin mutein). In some aspects, some of the reagent oligomers (e.g. streptavidin oligomers) can non-covalently bind via one or more binding sites Z to the biotinylated carrier protein, leaving the majority of the binding sites Z of the oligomer available for binding the agent (e.g., receptor-binding agent or selection agent) and any further

agent as described herein. Thus, by such an approach a soluble reagent with a multitude of binding sites Z can be prepared.

[0468] In other embodiments, a reagent, such as a streptavidin mutein (either as an individual tetramer or also in the form of an oligomer), can be covalently coupled to a synthetic carrier such as a polyethylene glycol (PEG) molecule. Any suitable PEG molecule can be used for this purpose, for example, and the PEG molecule and the respective reagent can be soluble. Typically, PEG molecules up to a molecular weight of 1000 Da are soluble in water or culture media that may be used in the present methods. In some cases, such PEG based reagent can be prepared using commercially available activated PEG molecules (for example, PEG-NHS derivatives available from NOF North America Corporation, Irvine, California, USA, or activated PEG derivatives available from Creative PEGWorks, Chapel Hills, North Carolina, USA) with amino groups of the streptavidin mutein.

[0469] In some aspects, *in vivo* detection is carried out using a binding molecule, such antibody or non-antibody reagent (e.g. a streptavidin or mutein, such as tetrameric streptavidin muteins) that is conjugated to a moiety that provides a signal or induces a signal that is detectable *in vivo.* In some embodiments, the binding molecule is conjugated to an imaging modality. In some aspects, the imaging modality includes but is not limited to a fluorescent compound, radioisotope, bioluminescent compound, chemiluminescent compound, metal chelate, enzyme, iron-oxide nanoparticle, or other imaging agent known in the art for detection by X-ray, CT-scan, MRI-scan, PET-scan, ultrasound, flow-cytometry, near infrared imaging systems, or other imaging modalities (see, e.g., Yu et al., Theranostics 2:3, 2012).

[0470] In some embodiments, the reagent is tagged with a detectable marker, such as a bioluminescent compound, chemiluminescent compound, metal chelate, enzyme, iron-oxide nanoparticle, a nanoparticle, a fluorescent compound, a fluorescent marker, and an enzyme. Examples of detectable markers / labels include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase. Examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin. Examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorot[pi]azinylamine fluorescein, dansyl chloride or phycoerythrin. Example of a luminescent material includes luminol. Examples of bioluminescent materials include luciferase, luciferin, and aequorin. In certain embodiments of the method of diagnosis described herein, the detectable moiety is a

radionuclide. In certain embodiments, the radionuclide is selected from the group consisting of ^{47}Sc, ^{64}Cu, ^{67}Cu, ^{89}Sr, ^{86}Y, ^{87}Y, ^{90}Y, ^{105}Rh, ^{108}Ag, ^{111}In, ^{117m}Sn, ^{149}Pm, ^{153}Sm, ^{166}Ho, ^{177}Lu, ^{186}Re, ^{188}Re, ^{211}At, ^{212}Bi, ^{18}F, ^{124}I, ^{125}I, ^{131}I, ^{55}Co, ^{60}Cu, ^{61}Cu, ^{62}Cu, ^{64}Cu, ^{66}Ga, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{82}Rb, ^{86}Y, ^{87}Y, ^{90}Y, ^{111}In, ^{99}Tc, and ^{201}Tl.

[0471] In some embodiments, the *in vivo* imaging method for detecting cells can be magnetic resonance imaging (MRI), single-photon emission computed tomography (SPECT), computed tomography (CT), computed axial tomography (CAT), electron beam computed tomography (EBCT), high resolution computed tomography (HRCT), hypocycloidal tomography, positron emission tomography (PET), scintigraphy, gamma camera, a β+ detector, a γ detector, fluorescence imaging, low-light imaging, X- rays, bioluminescence imaging, and other imaging modalities.

[0472] In some embodiments, the detection and/or monitoring can be performed by detecting and/or monitoring the cell surface molecule portion of the conjugate. For example, in some embodiments, the modified cell surface molecule can be detected and/or monitored by contacting with binding molecules or targeting molecules that can bind or target the cell surface molecule, and that can be detected, e.g., contains a detectable label. In some embodiments, the cell surface conjugate can be detected using corresponding antibodies or antigen-binding fragment thereof or other cell surface molecule-targeting molecules, such as any antibodies or antigen binding fragment therein described in Table 1. In some embodiments, such antibodies or antigen-binding fragments thereof or other targeting molecules can be used in any of the detection or monitoring methods provided herein.

[0473] In some embodiments, the targeting molecule to target, detect and/or monitor the cell surface molecule portion of the cell surface conjugate can include, e.g., an antibody including, but not limited to, 3F8, abagovomab, abciximab, adecatumumab, afutuzumab, alemtuzumab, altumomab pentetate, anatumomab mafenatox, apolizumab, arcitumomab, aselizumab, atlizumab (=tocilizumab), basiliximab, bectumomab, benralizumab, besilesomab, bivatuzumab mertansine, blinatumomab, brentuximab vedotin, cantuzumab mertansine, capromab pendetide, catumaxomab, CC49, cedelizumab, celmoleukin, citatuzumab bogatox, clenoliximab, clivatuzumab tetraxetan, CNTO-95, conatumumab, dacetuzumab, daclizumab, daratumumab, detumomab, ecromeximab, edrecolomab, efalizumab, elotuzumab, enlimomab pegol, epitumomab cituxetan, epratuzumab, erlizumab, etaracizumab, fanolesomab, faralimomab, farletuzumab, galiximab, gavilimomab, gemtuzumab ozogamicin, glembatumumab vedotin, gomiliximab, ibalizumab, ibritumomab tiuxetan, igovomab, intetumumab, iratumumab,

inolimomab, inotuzumab ozogamicin, ipilimumab, keliximab, labetuzumab, lintuzumab, lexatumumab, lucatumumab, lumiliximab, mapatumumab, maslimomab, milatuzumab, minretumomab, mitumomab, muromonab-CD3, naptumomab estafenatox, natalizumab, ocrelizumab, odulimomab, ofatumumab, olaratumab, oportuzumab monatox, oregovomab, otelixizumab, pemtumomab, priliximab, PRO 140, rituximab, rovelizumab, ruplizumab, satumomab pendetide, siplizumab, sontuzumab, tadocizumab, taplitumomab paptox, teneliximab, teplizumab, TGN1412, ticilimumab (=tremelimumab), tigatuzumab, tocilizumab (=atlizumab), toralizumab, tositumomab, tremelimumab, tucotuzumab, vedolizumab, veltuzumab, visilizumab, vitaxin, volociximab, votumumab, zanolimumab, ziralimumab, zolimomab aritox. Atezolizumab, bevacizumab (Avastin ®), denosumab, dinutuximab, nivolumab, obinutuzumab, pembrolizumab, pidilizumab (CT-011), ramucirumab, siltuximab, ado-trastuzumab emtansine, CEA-scan Fab fragment, OC125 monoclonal antibody, ab75705, B72.3, MPDL3280A, MSB001078C, MEDI4736, or an antigen-binding fragment thereof, analogs or derivatives thereof , or an antigen-binding antibody fragment selected from a Fab fragment, Fab' fragment F(ab)'2 fragment, single chain Fv (scFv) or a disulfide stabilized Fv (dsFv). In some embodiments, the modified cell surface molecule comprises an epitope recognized by any of the above antibodies or an antigen-binding fragment thereof.

[0013] In some embodiments, the cell surface molecule is a PSMA or a modified form thereof. In some embodiments, the binding molecule or targeting molecule is or comprises an antibody or antigen-binding fragment thereof. In some embodiments, the binding molecule or targeting moleculeis or comprises a ligand and/or small molecule. In some embodiments, the binding molecule is or comprises a small molecule that is capable of binding the active site or substrate binding site of PSMA. In some embodiments, the binding molecule is or comprises is an antagonist, a selective antagonist, an inverse agonist, a selective inverse agonist, an agonist, a selective agonist, an inhibitor, and/or a selective inhibitor of a PSMA and/or of the modified form thereof. In some embodiments, the binding molecule is or comprises an inhibitor of PSMA. In some embodiments, the binding molecule is or comprises a small molecule, and/or a low molecular weight molecule and/or a low molecular weight inhibitor. In some embodiments, the binding molecule that is or comprises a portion that is capable of binding PSMA or modified form thereof that is a small molecule and/or detectable moiety. In some embodiments, the detectable moiety or is capable of producing a detectable signal. In some instances, the detectable moiety contains a fluorescent protein and/or a radionucleide. In some embodiments,

the binding molecule or targeting molecule is or includes an aptamer, a peptide, or a conjugate thereof.

[0474] In some embodiments, the binding molecule or targeting molecule is or includes antibody or antigen-binding fragment thereof is selected from among J591, DFO-J591, CYT-356, J415, 3/A12, 3/F11, 3/E7, D2B, 107-1A4, YPSMA-1, YPSMA-2, 3E6, 2G7, 24.4E6, GCP-02, GCP-04, GCP-05, J533, E99, 1G9, 3C6, 4.40, 026, D7-Fc, D7-CH3, 4D4, A5, or an antigen-binding fragment thereof, analogs or derivatives thereof , or an antigen-binding antibody fragment selected from a Fab fragment, Fab' fragment F(ab)'2 fragment, single chain Fv (scFv) or a disulfide stabilized Fv (dsFv). In some embodiments, the modified cell surface molecule comprises an epitope recognized by any of the above antibodies or an antigen-binding fragment thereof.

[0475] In some embodiments, the binding molecule or targeting molecule is or includes those described in, e.g., US 2002/0049712; US 2002/0147312; US 2003/0082187; US 2004/0136998; US 2005/0202020; US 2006/0088539; US 2007/0071759; US 2010/0297653; US 2011/0020273; US 2013/0225541; US 2013/0315830; US 2014/0099257; US 2014/0227180; US 2015/0168413; US 2016/0303253; US 2017/0051074; US 6572856; US 7476513; US 8470330; US 8986655; WO 2006/078892; WO 2010/135431; WO 2014/198223; WO 2015/177360; WO 2016/057917; WO 2016/130819; WO 2016/145139; WO 2016/201300; WO 2017/004144; WO 2017/023761; AU 2002/356844; AU 2006/204913; AU 2006/235421; AU 2006/262231; AU 2006/315500; AU 2010/325969; AU 2013/328619; AU 2015/205574; CA 2353267; EP 1390069; EP 1520588; EP 1581794; EP 1599228; EP 1610818; EP 2906250; Banerjee et al. (2011) Angew Chem Int Ed Engl. 50(39): 9167–9170; Maurer et al. (2016) Nature Reviews Urology 13:226-235; Rowe et al. (2016) Prostate Cancer Prostatic Dis. 19(3):223-230; Mease et al., (2013) Curr Top Med Chem. 13(8):951-962; Osborne et al., (2013) Urol Oncol. 31(2): 144–154; Philipp Wolf (2011), Prostate Specific Membrane Antigen as Biomarker and Therapeutic Target for Prostate Cancer, Prostate Cancer - Diagnostic and Therapeutic Advances, Dr. Philippe E. Spiess (Ed.), Intech, pp.81-100; Ruggiero et al., (2011) J Nucl Med. 52(10): 1608–1615; Liu et al., (1997) Cancer Research 57:3629-3634; Regino et al., (2009) Curr Radiopharm. January ; 2(1): 9–17; Kampmeier et al. (2014) EJNMMI Research 4:13; Wolf et al., (2010) The Prostate 70:562-569; Tykvart et al. (2014) The Prostate 74:1674-1690; Jin et al., (2016) EMJ Urol. 4(1):62-69 and Tino et al. (2000) Hybridoma 19(3):24957, or a fragment thereof, a conjugate thereof or a derivative thereof.

C. Suicide Killing

[0476] In some embodiments, provided are methods can be used for ablation and/or depletion of engineered cells *in vivo*, for example, mediated via antibody-dependent cell-mediated cytotoxicity (ADCC) or via specific targeting of cells with a cytotoxic agent.

I. ADCC

[0477] In some embodiments, the cell surface conjugate may be used to induce cell suicide. For example, the cell surface molecule, e.g., modified cell surface molecules described herein, may be used as a suicide gene via antibody dependent cell mediated cytotoxicity (ADCC) pathways. ADCC refers to a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors, such as natural killer cells, neutrophils, and macrophages, recognize bound antibody on a target cell and cause lysis of the target cell. ADCC activity may be assessed using methods, such as those described in U.S. Pat. No. 5,821,337.

[0478] In some embodiments, ADCC may be mediated by a administering to a subject any antibody targeting the cell surface molecule of the conjugate. In some embodiments, exemplary modified cell surface molecules provided in Table 1 may be used as a suicide gene via activation of ADCC mediated by administration to the subject of the corresponding antibodies provided in Table 1. In some aspects, modified EGFR cell surface molecule may be used as a suicide gene via cetuximab mediated activation of ADCC. In some aspects, suicide killing mediated by cetuximab or the ADCC pathway is unaffected by selection process that utilizes the agent (Strep-Tag®) linked to the cell surface molecule. In some aspects, PSMA or modified form thereof, engineered to be expressed on the cell surface, may be used as a suicide gene via administration of a binding molecule or targeting molecule that is an anti-PSMA antibody, such as any described herein, for example, by activation of ADCC. In another embodiment, elimination of engineered T cells expressing the cell surface conjugate provided herein may be accomplished by administering an antibody specific for the agent (e.g. affinity tag) of the conjugate. Exemplary antibody agents specific for affinity tags, including those described herein, are known. In some embodiments, if a streptavidin binding peptide, such as a Strep-Tag®, is used as the agent, then an anti- Strep-Tag® antibody or anti- Strep-Tag® scFv can be used to activate the ADCC pathway. Exemplary anti-Strep-tag antibodies include commercially available StrepMAB-Classic, monoclonal antibodies StrepMAB-Immo (IBA), anti-Streptag II antibody (Genscript), or Strep-tag antibody (Qiagen).

2. Agent targeted by a Cytotoxic Molecule

[0479] In some embodiments, suicide killing is accomplished by administering to the subject a cytotoxic molecule specific for the agent (e.g. affinity tag) of the conjugate. In some embodiments, such cytotoxic molecules molecules include those in which a binding molecule specific for the agent, including an antibody or non-antibody reagent, is conjugated to a cytotoxic agent. In aspects of such methods, a cytotoxic molecule is administered to a subject when the subject is known or suspected of having or likely having or developing an adverse side effect to the administered cells, such as associated with toxicity or immunogencitiy of the engineered cells.

[0480] In some embodiments, the binding molecule is a streptavidin mutein, such as any as described including Strept-Tactin or other streptavidin mutein or is an oligomer thereof. Also provided herein are streptavidin or streptavidin muteins or oligomers of streptavidin or a streptavidin mutein, such as any described herein, linked or conjugated to a cytotoxic agent. In some aspects, the binding molecule reagent comprises a streptavidin or streptavidin mutein set forth in any of SEQ ID NOS: 3-6, 27 or 28 or a sequence of amino acids that exhibits at least at or about 85%, 86%,87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 3-6, 27 or 28 and binds the streptavidin binding peptide agent (e.g. Strep-tag). In some embodiments, the binding molecule is an antibody or antigen-binding fragment specific for the agent.

[0481] In some cases, the cytotoxic agent can be a toxin or a radiometal. Other cytotoxic agents include, but are not limited to cytotoxic components (e.g., chemotherapeutic drugs such as anti-mitotics (e.g., vindesine), antifolates, alkylating agents (e.g., temozolomide), bacterial toxins, ricin, anti-virals, radioisotopes, radiometals). Such cytotoxic agents, when targeted to specific cells, can be useful for specific killing or disabling an engineered cells, for example, when activity of a recombinant receptor is not desired.

[0482] In some embodiments the cell-toxic reagent is a bacterial toxin that belongs to a major class of bacterial toxins, termed AB toxins, which use a transporter protein (B or binding unit) that actively translocates enzymes (A unit) into cells. Examples of AB toxins include botulinum neurotoxin, anthrax toxin, diphtheria toxin, shiga toxin, shiga like toxin, exotoxin A, and cholera toxin. Due to the similar mechanism of action between all of these toxins, all these toxins are contemplated to work in the various aspects of the present invention. The A and B components of these and a variety of other toxins are well known.

[0483] Bacterial toxins frequently have two functionally distinct moieties, termed A and B. The "A" component is usually the "active" portion, and the "B" component is usually the "binding" portion. Thus, the A moiety or component contains the catalytic activity, while the B moiety or component possesses determinants needed for the cytoplasmic delivery of the A moieties into target cells. These delivery determinants include receptor binding activity, and often, but not always, membrane penetration activity. Many bacterial toxins, such as diphtheria toxin, contain both moieties within a single polypeptide. Anthrax toxin, by contrast, is a member of the so-called binary toxins, a class in which the A and B functions inhabit separate proteins. Although separate, the proteins having the A and B functions interact during the intoxication of cells. Anthrax toxin uses a single B moiety, protective antigen (PA; 83 kDa), for the delivery of two alternative A moieties, edema factor (EF; 89 kDa) and lethal factor (LF; 89 kDa) into the cytoplasm (see international patent application publication number WO2012096926 for examples of bacterial toxins).

[0484] In some aspects, the toxin is a peptide toxin, ricin A chain toxin, Abrin A chain, Diptheria Toxin (DT) A chain, Pseudomonas exotoxin, Shiga Toxin A chain, Gelonin, Momordin, Pokeweed Antiviral Protein, Saporin, Trichosanthin, or Barley Toxin. In some aspects, the toxin is a phototoxin. In some embodiments, the peptide toxin comprises a sequence of amino acids set forth in SEQ ID NO:100.

[0485] In some embodiments, administration of the cytotoxic agent does not, or does not substantially, induce killing or destruction of healthy tissue or healthy cells, of cells or tissues not containing the engineered cells and/or not expressing the antigen.

3. Dimerization-Mediated Killing

[0486] In some embodiments, suicide killing of cells expressing the cell surface conjugate is accomplished by employing a cell surface conjugate having an intracellular signaling domain capable of mediating killing of cells, such as upon dimerization. In some embodiments, the killing is mediated via caspase activity which initiates cellular destruction leading to apoptosis. In some aspects, the cell surface conjugate comprises the signaling domain of caspase-9, which is a part of the apoptotic pathway.

[0487] In some embodiments, dimerization is carried out by administering to the subject a binding molecule that specifically binds the agent of the conjugate. In some embodiments, binding of the binding molecule to the agent of the conjugate induces dimerization of caspase

subunits and induces, modulates, activates, mediates and/or promotes signaling through the signaling domain. In some embodiments, dimerization can result in caspase-9 dependent cell death of the cell.

[0488] In some embodiments, the binding molecule administered to the subject is one that recognizes the agent, such as any as described herein. In some embodiments, the agent is a streptavidin binding peptide, such as a Strep-tag, including a Strep-tag® II or twin-Strep-tag as described. In some embodiments, the binding molecule is a non-antibody agent capable of specifically, and in some cases reversibly, binding to the agent, such as a streptavidin mutein, including Strep-Tactin or other streptavidin mutein and oligomers thereof. In some embodiments, the binding molecule that recognizes the agent is an antibody, such as an anti-Strep-tag antibody.

VII. DEFINITIONS

[0489] As used herein, recitation that nucleotides or amino acid positions "correspond to" nucleotides or amino acid positions in a disclosed sequence, such as set forth in the Sequence listing, refers to nucleotides or amino acid positions identified upon alignment with the disclosed sequence to maximize identity using a standard alignment algorithm, such as the GAP algorithm. By aligning the sequences, one skilled in the art can identify corresponding residues, for example, using conserved and identical amino acid residues as guides. In general, to identify corresponding positions, the sequences of amino acids are aligned so that the highest order match is obtained (see, e.g. : Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New.Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; Carrillo et al. (1988) SIAM J Applied Math 48: 1073).

[0490] The term “vector,” as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as

"expression vectors." Among the vectors are viral vectors, such as retroviral, e.g., gammaretroviral and lentiviral vectors.

[0491] The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

[0492] As used herein, a statement that a cell or population of cells is "positive" for a particular marker refers to the detectable presence on or in the cell of a particular marker, typically a surface marker. When referring to a surface marker, the term refers to the presence of surface expression as detected by flow cytometry, for example, by staining with an antibody that specifically binds to the marker and detecting said antibody, wherein the staining is detectable by flow cytometry at a level substantially above the staining detected carrying out the same procedure with an isotype-matched control under otherwise identical conditions and/or at a level substantially similar to that for cell known to be positive for the marker, and/or at a level substantially higher than that for a cell known to be negative for the marker.

[0493] As used herein, a statement that a cell or population of cells is "negative" for a particular marker refers to the absence of substantial detectable presence on or in the cell of a particular marker, typically a surface marker. When referring to a surface marker, the term refers to the absence of surface expression as detected by flow cytometry, for example, by staining with an antibody that specifically binds to the marker and detecting said antibody, wherein the staining is not detected by flow cytometry at a level substantially above the staining detected carrying out the same procedure with an isotype-matched control under otherwise identical conditions, and/or at a level substantially lower than that for cell known to be positive for the marker, and/or at a level substantially similar as compared to that for a cell known to be negative for the marker.

[0494] As used herein, "percent (%) amino acid sequence identity" and "percent identity" when used with respect to an amino acid sequence (reference polypeptide sequence) is defined as the percentage of amino acid residues in a candidate sequence (e.g., the subject antibody or fragment) that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent

sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0495] As used herein, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. For example, "a" or "an" means "at least one" or "one or more." It is understood that aspects and variations described herein include "consisting" and/or "consisting essentially of" aspects and variations.

[0496] Throughout this disclosure, various aspects of the claimed subject matter are presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the claimed subject matter. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, where a range of values is provided, it is understood that each intervening value, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the claimed subject matter. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the claimed subject matter, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the claimed subject matter. This applies regardless of the breadth of the range.

[0497] The term "about" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X".

[0498] As used herein, a composition refers to any mixture of two or more products, substances, or compounds, including cells. It may be a solution, a suspension, liquid, powder, a paste, aqueous, non-aqueous or any combination thereof.

[0499] As used herein, a "subject" is a mammal, such as a human or other animal, and typically is human.

[0500] Unless defined otherwise, all terms of art, notations and other technical and scientific terms or terminology used herein are intended to have the same meaning as is commonly understood by one of ordinary skill in the art to which the claimed subject matter pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art.

[0501] All publications, including patent documents, scientific articles and databases, referred to in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication were individually incorporated by reference. If a definition set forth herein is contrary to or otherwise inconsistent with a definition set forth in the patents, applications, published applications and other publications that are herein incorporated by reference, the definition set forth herein prevails over the definition that is incorporated herein by reference.

[0502] The section heading used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

VIII. EXEMPLARY EMBODIMENTS

[0503] Among the provided embodiments are:

1. A cell surface conjugate, comprising:

(a) a cell surface molecule that lacks a functional intracellular signaling domain and/or is not capable of mediating intracellular signaling; and

(b) at least one agent linked to the cell surface molecule, the agent being capable of binding a streptavidin, a streptavidin analog or a streptavidin mutein.

2. The cell surface conjugate of embodiment 1, wherein the agent exhibits a binding affinity for streptavidin or a streptavidin mutein with an equilibrium dissociation constant (K_D) of from or from about 10^{-4} M to or to about 10^{-10} M.

3. A cell surface conjugate, comprising:

(a) a cell surface molecule that lacks a functional intracellular signaling domain and/or is not capable of mediating intracellular signaling; and

(b) at least one agent linked to the cell surface molecule and being capable of reversibly binding to a reagent and/or capable of being competed in the presence of a competition substance, wherein the agent is a peptide of less than 50 amino acids in length.

4. The cell surface conjugate of embodiment 3, wherein the agent exhibits a binding affinity for the reagent with an equilibrium dissociation constant (K_D) of from or from about 10^{-4} M to or to about 10^{-10} M.

5. The cell surface conjugate of embodiment 3 or embodiment 4, wherein the reagent is a streptavidin, a streptavidin analog or a streptavidin mutein.

6. A cell surface conjugate, comprising:

(a) a cell surface molecule that lacks a functional intracellular signaling domain and/or is not capable of mediating intracellular signaling; and

(b) at least one agent linked to the cell surface molecule, the agent having a binding affinity for a reagent with an equilibrium dissociation constant (K_D) of more than 10^{-7} M or an equilibrium association constant (K_A) of less than 10^7 M^{-1}.

7. The cell surface conjugate of embodiment 6, wherein the reagent is a streptavidin, a streptavidin analog or a streptavidin mutein.

8. The cell surface conjugate of any of embodiments 1-7, wherein the cell surface molecule comprises a transmembrane domain and/or is capable of being expressed on the surface of the cell.

9. The cell surface conjugate of any of embodiments 1-8, wherein the cell surface molecule is modified compared to a reference cell surface molecule, optionally wherein the reference cell surface molecule is a cell surface receptor comprising an intracellular signaling domain.

10. The cell surface conjugate of embodiment 9, wherein the modified cell surface molecule exhibits altered cellular internalization, enzymatic activity and/or ligand binding, compared to the reference cell surface molecule.

11. A cell surface conjugate, comprising:

(a) a cell surface molecule that is modified compared to a reference cell surface molecule, wherein the modified cell surface molecule exhibits altered cellular internalization, enzymatic activity and/or ligand binding, compared to the reference cell surface molecule; and

(b) at least one agent linked to the cell surface molecule, the agent being capable of binding a streptavidin, a streptavidin analog or a streptavidin mutein.

12. The cell surface conjugate of embodiment 11, wherein the cell surface molecule lacks a functional intracellular signaling domain and/or is not capable of mediating intracellular signaling.13. A cell surface conjugate, comprising:

(a) a cell surface molecule comprising a prostate-specific membrane antigen (PSMA) or a modified cell surface molecule thereof; and

(b) at least one agent linked to the cell surface molecule, the agent being capable of binding a streptavidin, a streptavidin analog or a streptavidin mutein.

14. The cell surface conjugate of embodiment 13, wherein:

the modified cell surface molecule lacks a functional intracellular signaling domain and/or is not capable of mediating intracellular signaling; and/or

the modified cell surface molecule is modified compared to a reference cell surface molecule, wherein the modified cell surface molecule exhibits altered cellular internalization, enzymatic activity and/or ligand binding, compared to the reference cell surface molecule.

15. The cell surface conjugate of any of embodiments 11-14, wherein the cell surface molecule comprises a transmembrane domain and/or is capable of being expressed on the surface of the cell.

16. The cell surface conjugate of any of embodiments 11-15, wherein the agent exhibits a binding affinity for a streptavidin, a streptavidin analog or a streptavidin mutein with an equilibrium dissociation constant (K_D) of from or from about 10^{-4} M to or to about 10^{-10} M.

17. The cell surface conjugate of any of embodiments 1-16, wherein the binding of the agent to the reagent is reversible and/or capable of being competed in the presence of a competition substance.

18. The cell surface conjugate of embodiment 17, wherein the competition substance exhibits a higher binding affinity for the reagent than the binding affinity of the agent for the reagent.

19. The cell surface conjugate of embodiment 18, wherein:

the competition substance exhibits a binding affinity for the reagent with an equilibrium dissociation constant (K_D) of between or about between 10^{-10} M and 10^{-14} M; and/or

the agent exhibits a binding affinity for the reagent with an equilibrium dissociation constant (K_D) of more than 10^{-10} M.

20. The cell surface conjugate of any of embodiments 1, 2, 5, 7-20, wherein the binding of the agent to the streptavidin, streptavidin analog or streptavidin mutein is reversible and/or capable of being competed in the presence of biotin, a biotin analog or a biologically active fragment thereof.

21. The cell surface conjugate of any of embodiments 1-20, wherein the at least one agent is linked directly to the cell surface molecule.

22. The cell surface conjugate of any of embodiments 1-20, wherein the at least one agent is linked indirectly to the cell surface molecule via at least one linker.

23. The cell surface conjugate of any of embodiments 1-22, wherein the at least one agent comprises from or from about 1 to 4 or 1 to 2 agents.

24. The cell surface conjugate of any of embodiments 1-23, wherein the at least one agent comprises only one agent.

25. The cell surface conjugate of any of embodiments 1-24, wherein the agent is linked to an extracellular portion or region of the cell surface molecule, optionally wherein the extracellular portion or region is at the N-terminus or C-terminus of the cell surface molecule.

26. The cell surface conjugate of any of embodiments 1-25, wherein the agent is linked at the N-terminus of the cell surface molecule.

27. The cell surface conjugate of any of embodiments 1-26, wherein the agent is linked at the C-terminus of the cell surface molecule.

28. A cell surface conjugate, comprising a cell surface molecule linked, at an extracellular portion or region of the cell surface molecule, to an agent, the agent being capable of binding a reagent that is or comprises streptavidin or a streptavidin mutein, optionally wherein the extracellular portion or region is at the N-terminus or C-terminus of the cell surface molecule.

29. A cell surface conjugate, comprising a cell surface molecule linked, at an extracellular portion or region of the cell surface molecule, to an agent, the agent being capable of reversibly binding to a reagent, wherein the agent is a peptide of less than 50 amino acids in length optionally wherein the extracellular portion or region is at the N-terminus or C-terminus of the cell surface molecule.

30. The cell surface conjugate of embodiment 28 or embodiment 29, wherein the agent exhibits a binding affinity with an equilibrium dissociation constant (K_D) of from or from about 10^{-4} M to or to about 10^{-10} M.

31. A cell surface conjugate, comprising a cell surface molecule linked, at an extracellular portion or region of the cell surface molecule, to an agent, wherein the agent exhibits a binding affinity for a reagent with an equilibrium dissociation constant (K_D) of more than 10^{-7} M or an equilibrium association constant (K_A) of less than 10^7 M^{-1} optionally wherein the extracellular portion or region is at the N-terminus or C-terminus of the cell surface molecule.

32. The cell surface conjugate of any of embodiments 28-31, wherein the agent is linked at the N-terminus of the cell surface molecule.

33. The cell surface conjugate of any of embodiments 28-31, wherein the agent is linked at the C-terminus of the cell surface molecule.

34. The cell surface conjugate of any of embodiments 28-33, wherein the reagent is or comprises a streptavidin, a streptavidin analog or a streptavidin mutein.

35. The cell surface conjugate of any of embodiments 28-34, wherein the binding of the agent to the reagent is reversible and/or capable of being competed in the presence of a competition substance.

36. The cell surface conjugate of embodiment 35, wherein the competition substance exhibits a higher binding affinity for the reagent than the binding affinity of the agent for the reagent.

37. The cell surface conjugate of embodiment36, wherein:

the competition substance exhibits a binding affinity for the reagent with an equilibrium dissociation constant (K_D) of between or about between 10^{-10} M and 10^{-14} M; and/or

the agent exhibits a binding affinity for the reagent with an equilibrium dissociation constant (K_D) of more than 10^{-10} M.

38. The cell surface conjugate of any of embodiments 28, 34-37, wherein the binding of the agent to the streptavidin, streptavidin analog or streptavidin mutein is reversible and/or capable of being competed in the presence of biotin or a biotin analog.

39. The cell surface conjugate of any of embodiments 28-38, wherein the agent is linked directly to the cell surface molecule.

40. The cell surface conjugate of any of embodiments 28-38, wherein the agent is linked indirectly to the cell surface molecule via at least one linker.

41. The cell surface conjugate of any of embodiments 28-40, wherein the cell surface molecule is linked to only one agent.

42. The cell surface conjugate of any of embodiments 1-41, wherein the cell surface molecule is not a chimeric antigen receptor (CAR).

43. The cell surface conjugate of any of embodiments 28-30, wherein the cell surface molecule is modified compared to a reference cell surface molecule.

44. The cell surface conjugate of embodiment 43, wherein the modified cell surface molecule lacks a functional intracellular signaling domain and/or is not capable of mediating intracellular signaling; and/or the modified cell surface molecule exhibits altered cellular

internalization, enzymatic activity and/or ligand binding, compared to the reference cell surface molecule.

45. The cell surface conjugate of embodiment 43 or embodiment 44, wherein the reference cell surface molecule is a native mammalian cell surface molecule.

46. The cell surface conjugate of any of embodiments 1-45, wherein the cell surface molecule comprises an epitope capable of being recognized by an antibody or antigen-binding fragment thereof.

47. The cell surface conjugate of any of embodiments 1-33 that is a fusion protein.

48. The cell surface conjugate of any of embodiments 1, 2, 5, 7-28 and and 34-47, wherein the streptavidin analog or mutein comprises the amino acid sequence Val^{44}-Thr^{45}-Ala^{46}-Arg^{47} or Ile^{44}-Gly^{45}-Ala^{46}-Arg^{47} at sequence positions corresponding to positions 44 to 47 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO:1.

49. The cell surface conjugate of any of embodiments 1, 2, 5, 7-28 and and 34-48, wherein the streptavidin analog or mutein comprises:

a) the sequence of amino acids set forth in any of SEQ ID NOS: 3-6, 27 and 28;

b) a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS:3-6, 27 and 28 and contains the amino acid sequence corresponding to Val^{44}-Thr^{45}-Ala^{46}-Arg^{47} or Ile^{44}-Gly^{45}-Ala^{46}-Arg^{47} and that reversibly binds to the agent; or

c) a functional fragment of a) or b) that reversibly binds to the agent.

50. The cell surface conjugate of embodiment 48 or embodiment 49, wherein the streptavidin analog or mutein further comprises an amino acid replacement or replacements at a position corresponding to 117, 120 and/or 121 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO:1.

51. The cell surface conjugate of embodiment 50, wherein:

the amino acid replacement or replacements are selected from among Glu^{117}, Asp^{117}, Arg^{117}, Ser^{120}, Ala^{120}, Gly^{120}, Trp^{121}, Tyr^{121} or Phe^{121}; or

the amino acid replacement or replacements are selected from one or more of Glu^{117}, Gly^{120} or Tyr^{121}; or

the amino acid replacements are selected from Glu^{117}, Gly^{120} or Tyr^{121}.

52. The cell surface conjugate of any of embodiments any of embodiments 1, 2, 5, 7-28 and and 34-51, wherein the streptavidin analog or mutein comprises:

a) the sequence of amino acids set forth in SEQ ID NO: 27 or 28;

b) a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NOS: 27 or 28 and contains the amino acid sequence corresponding to Val^{44}, Thr^{45}, Ala^{46}, Arg^{47}, Glu^{117}, Gly^{120} and Tyr^{121} and reversibly binds to the agent; or

c) a functional fragment of a) or b) that reversibly binds to the agent.

53. The cell surface conjugate of any of embodiments 3-5, 17-19 and 35-37, wherein the competition substance is or comprises biotin, a biotin analog or a biologically active fragment thereof.

54. The cell surface conjugate of any of embodiments 1-53, wherein the agent is an affinity tag.

55. The cell surface conjugate of any of embodiments 3, 4, 6, 8-10, 17-19, 21-27, 29-33, 35-37, 39-47 and 54, wherein the agent is or comprises a Strep tag, His tag, Flag tag, Xpress tag, Avi tag, Calmodulin tag, Polyglutamate tag, HA tag, Myc tag, Nus tag, S tag, X tag, SBP tag, Softag, V5 tag, CBP, GST, MBP, GFP, Thioredoxin tag, or any combination thereof.

56. The cell surface conjugate of any of embodiments 1-55, wherein the agent is or comprises one or more streptavidin binding peptide, which optionally is a Strep tag.

57. The cell surface conjugate of embodiment 56, wherein the streptavidin binding peptide comprises the sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8) or Trp-Arg-His-Pro-Gln-Phe-Gly-Gly (SEQ ID NO:7).

58. The cell surface conjugate of embodiment 56 or embodiment 57, wherein the agent comprises the sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_3$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 17), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 18) and Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$Gly-Gly-Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 19).

59. The cell surface conjugate of any of embodiments 9-58, wherein the reference cell surface molecule is a cell surface receptor, ligand, glycoprotein, cell adhesion molecule, antigen, integrin or cluster of differentiation (CD).

60. The cell surface conjugate of embodiment 59, wherein the reference cell surface molecule is a cell surface receptor.

61. The cell surface conjugate of any of embodiments 9-60, wherein the reference cell surface molecule is selected from EpCAM, VEGFR, integrin, optionally integrins αvβ3, α4, αIIbβ3, α4β7, α5β1, αvβ3 or αv, a member of the TNF receptor superfamily, optionally TRAIL-R1 or TRAIL-R2, a member of the epidermal growth factor receptor family, PDGF Receptor,

interferon receptor, folate receptor, GPNMB, ICAM-1, HLA-DR, CEA, CA-125, MUC1, TAG-72, IL-6 receptor, 5T4, GD2, GD3, prostate-specific membrane antigen (PSMA) or a clusters of differentiation cell surface molecule, optionally CD2, CD3, CD4, CD5, CD11, CD11a/LFA-1, CD15, CD18/ITGB2, CD19, CD20, CD22, CD23/IgE Receptor, CD25, CD28, CD30, CD33, CD38, CD40, CD41, CD44, CD51, CD52, CD62L, CD74, CD80, CD125, CD147/basigin, CD152/CTLA-4, CD154/CD40L, CD195/CCR5 and CD319/SLAMF7.

62. The cell surface conjugate of any of embodiments 9-61, wherein the reference cell surface molecule is a member of the epidermal growth factor receptor family.

63. The cell surface conjugate of any of embodiments 9-62, wherein the reference cell surface molecule is an epidermal growth factor receptor (EGFR), an erbB-2 receptor tyrosine-protein kinase (errb2, HER2), an erbB-3 receptor tyrosine-protein kinase, an erbB-4 receptor tyrosine-protein kinase, a hepatocyte growth factor receptor (HGFR/c-MET) or an insulin-like growth factor receptor-1 (IGF-1 R).

64. The cell surface conjugate of any of embodiments 9-63, wherein the reference cell surface molecule is human.

65. The cell surface conjugate of any of embodiments 9-64, wherein the modified cell surface molecule lacks a functional intracellular signaling domain and/or is not capable of mediating intracellular signaling.

66. The cell surface conjugate of any of embodiments 9-65, wherein the modified cell surface molecule is truncated to lack all or a portion of the intracellular signaling domain or trafficking domain compared to the reference cell surface molecule.

67. The cell surface conjugate of any of embodiments 9-66, wherein the modified cell surface molecule exhibits altered cellular internalization, enzymatic activity and/or ligand binding, compared to the reference cell surface molecule.

68. The cell surface conjugate of any of embodiments 9-67, wherein the modified cell surface molecule comprises one or more extracellular domains of the reference cell surface molecule.

69. The cell surface conjugate of any of embodiments 9-68, wherein the modified cell surface molecule is capable of binding to a native ligand and/or substrate of the reference cell surface molecule.

70. The cell surface conjugate of any of embodiments 9-68, wherein the modified cell surface molecule is reduced for or does not bind the native ligand and/or substrate of the reference cell surface molecule.

71. The cell surface conjugate of embodiment 70, wherein the modified cell surface molecule comprises at least one extracellular domain of the reference cell surface molecule but lacks one or more other extracellular domains recognized by the native ligand and/or substrate of the reference cell surface molecule.

72. The cell surface conjugate of embodiment 71, wherein the at least one extracellular domain comprises an epitope recognized by an antibody or antigen-binding fragment thereof that specifically binds the reference cell surface molecule.

73. The cell surface conjugate of any of embodiments 46-72, wherein the antibody or antigen-binding fragment is selected from AMG-102, AMG-479, BIIB022OA-5D5, CP-751,871, IMC-Al2, R1507, 3F8, abagovomab, abciximab, adecatumumab, afutuzumab, alemtuzumab, altumomab pentetate, anatumomab mafenatox, apolizumab, arcitumomab, aselizumab, atlizumab (=tocilizumab), basiliximab, bectumomab, benralizumab, besilesomab, bivatuzumab mertansine, blinatumomab, brentuximab vedotin, cantuzumab mertansine, capromab pendetide, catumaxomab, CC49, cedelizumab, celmoleukin, cetuximab, cixutumumab, clenoliximab, clivatuzumab tetraxetan, CNTO-95, conatumumab, dacetuzumab, daclizumab, daratumumab, detumomab, ecromeximab, ertumaxomab, edrecolomab, efalizumab, elotuzumab, enlimomab pegol, epitumomab cituxetan, epratuzumab, erlizumab, etaracizumab, fanolesomab, faralimomab, farletuzumab, figitumumab, galiximab, gavilimomab, gemtuzumab ozogamicin, glembatumumab vedotin, gomiliximab, ibalizumab, ibritumomab tiuxetan, igovomab, intetumumab, iratumumab, inolimomab, inotuzumab ozogamicin, ipilimumab, keliximab, labetuzumab, lintuzumab, lexatumumab, lucatumumab, lumiliximab, mapatumumab, maslimomab, matuzumab, milatuzumab, minretumomab, mitumomab, muromonab-CD3, naptumomab estafenatox, natalizumab, necitumumab, ocrelizumab, odulimomab, ofatumumab, olaratumab, oportuzumab monatox, oregovomab, otelixizumab, panitumumab, pertuzumab, pemtumomab, priliximab, PRO 140, nimotuzumab, robatumumab, rituximab, rovelizumab, ruplizumab, satumomab pendetide, siplizumab, sontuzumab, tadocizumab, taplitumomab paptox, teneliximab, teplizumab, TGN1412, ticilimumab (=tremelimumab), tigatuzumab, tocilizumab (=atlizumab), toralizumab, tositumomab, trastuzumab, tremelimumab, tucotuzumab, vedolizumab, veltuzumab, visilizumab, vitaxin, volociximab, votumumab, zalutumumab, zanolimumab, ziralimumab, zolimomab aritox, Atezolizumab, bevacizumab (Avastin ®), denosumab, dinutuximab, nivolumab, obinutuzumab, pembrolizumab, pidilizumab (CT-011), ramucirumab, siltuximab, ado-trastuzumab emtansine, CEA-scan Fab fragment,

OC125 monoclonal antibody, ab75705, B72.3, MPDL3280A, MSB001078C, MEDI4736, or an antigen binding fragment thereof.

74. The cell surface conjugate of any of embodiments 9-73, wherein the reference cell surface molecule is a reference EGFR and the modified cell surface molecule is a modified EGFR.

75. The cell surface conjugate of embodiment 74 wherein the modified EGFR comprises an epitope specifically recognized by cetuximab or an antigen binding fragment thereof.

76. The cell surface conjugate of embodiment 74 or embodiment 75, wherein the modified EGFR lacks one or more of an EGFR Domain I, an EGFR Domain II, an EGFR Juxtamembrane Domain, and an EGFR Tyrosine Kinase Domain of the reference EGFR.

77. The cell surface conjugate of any of embodiments 74-76, wherein the modified EGFR lacks all of the domains EGFR Domain I, an EGFR Domain II, an EGFR Juxtamembrane Domain, and an EGFR Tyrosine Kinase Domain of the reference EGFR.

78. The cell surface conjugate of any of embodiments 74-77, wherein the modified EGFR comprises an extracellular domain that consists of or consists essentially of subdomain III and subdomain IV of the reference EGFR.

79. The cell surface conjugate of any of embodiments 74-78, wherein the modified EGFR comprises the sequence of amino acids set forth in SEQ ID NOS: 44 or 46 or a sequence of amino acids that exhibits at least at or about 85%, 90%, or 95% sequence identity to SEQ ID NOS: 44 or 46.

80. The cell surface conjugate of any of embodiments 973, wherein the reference cell surface molecule is a reference HER2 and the modified cell surface molecule is a modified HER2.

81. The cell surface conjugate of embodiment 80, wherein the modified HER2 comprises an epitope specifically recognized by trastuzumab or an antigen binding fragment thereof.

82. The cell surface conjugate of embodiment 80 or embodiment 81, wherein the modified HER2 lacks one or more of an HER2 Domain I, an HER2 Domain II, an HER2 Domain III of the reference HER2.

83. The cell surface conjugate of any of embodiments 80-82, wherein the modified HER2 lacks all of the domains HER2 Domain I, HER2 Domain II, and HER2 Domain III of the reference EGFR of the reference HER2.

84. The cell surface conjugate of any of embodiments 80-83, wherein the modified HER2 comprises an extracellular domain that consists of or consists essentially of Domain IV of the reference HER2.

85. The cell surface conjugate of any of embodiments 80-84, wherein the modified HER2 comprises the sequence of amino acids set forth in SEQ ID NO: 92 or a sequence of amino acids that exhibits at least at or about 85%, 90%, or 95% sequence identity to SEQ ID NO: 92.

86. The cell surface conjugate of any of embodiments 9-72, wherein the reference cell surface molecule is a reference PSMA and the modified cell surface molecule is a modified PSMA.

87. The cell surface conjugate of embodiment 86, wherein the reference PSMA is a wild-type PSMA, optionally wild-type human PSMA.

88. The cell surface conjugate of embodiment 87, wherein the reference PSMA is a human PSMA and/or comprises the sequence of amino acids set forth in SEQ ID NO: 94 or a sequence of amino acids encoded by the sequence of nucleotides set forth in SEQ ID NO: 96 or 97.

89. The cell surface conjugate of any of embodiments 86-88, wherein the modified PSMA comprises an extracellular portion and a transmembrane domain of the reference PSMA.

90. The cell surface conjugate of any of embodiments 86-89, wherein the modified PSMA comprises one or more amino acid modifications in the intracellular region compared to the reference PSMA.

91. The cell surface conjugate of any of embodiments 86-90, wherein the one or more amino acid modification comprises one or more amino acid substitutions, deletions and/or insertions.

92. The cell surface conjugates of any of embodiments 86-91, wherein the modified PSMA exhibits altered cellular internalization compared to the reference PSMA.

93. The cell surface conjugate of any of embodiments 86-92, wherein the modified PSMA comprises an amino acid substitution corresponding to W2G or does not comprise W2 or does not comprise any residue at position 2, with reference to positions in the sequence of amino acids set forth in SEQ ID NO:94.

94. The cell surface conjugate of any of embodiments 86-93, wherein the modified PSMA comprises a deletion or truncation of 11 N-terminal amino acids, compared to the reference PSMA.

95. The cell surface conjugate of any of embodiments 86-94, wherein the modified PSMA comprises an epitope capable of being recognized by an antibody or antigen-binding fragment thereof.

96. The cell surface conjugate of embodiment 95, wherein the antibody or antigen-binding fragment thereof is selected from among J591, DFO-J591, CYT-356, J415, 3/A12, 3/F11, 3/E7, D2B, 107-1A4, YPSMA-1, YPSMA-2, 3E6, 2G7, 24.4E6, GCP-02, GCP-04, GCP-05, J533, E99, 1G9, 3C6, 4.40, 026, D7-Fc, D7-CH3, 4D4, A5, and antigen-binding fragments thereof.

97. The cell surface conjugate of any of embodiments 1-96, wherein the cell surface conjugate is not immunogenic and/or does not induce an immune response in a subject in which it is administered.

98. A polynucleotide, comprising a nucleic acid sequence encoding the cell surface conjugate of any of embodiments 1-97.

99. The polynucleotide of embodiment 98, wherein the nucleic acid sequence further comprising a signal sequence.

100. The polynucleotide of embodiment 99, wherein the signal sequence encodes a signal peptide derived from GMCSFR alpha chain.

101. The polynucleotide of any of embodiments 98-100, wherein the nucleic acid sequence is a first nucleic acid sequence and the polynucleotide further comprises a second nucleic acid sequence encoding a recombinant receptor.

102. The polynucleotide of embodiment 101, wherein the recombinant receptor is or comprises a chimeric antigen receptor (CAR).

103. The polynucleotide of embodiment 101 or embodiment 102, wherein the first and second nucleic acid sequences are separated by an internal ribosome entry site (IRES), or a nucleotide sequence encoding a self-cleaving peptide or a peptide that causes ribosome skipping, which optionally is a T2A, a P2A, an E2A or an F2A.

104. The polynucleotide of any of embodiments 101-103, wherein the first nucleic acid sequence is upstream of the second nucleic acid sequence.

105. The polynucleotide of any of embodiments 101-103, wherein the first nucleic acid sequence is downstream of the second nucleic acid sequence.

106. A vector, comprising the polynucleotide of any of embodiments 98-105.

107. The vector of embodiment 106 that is a viral vector.

108. The vector of embodiment 106 or embodiment 107 that is a retroviral vector.

109. The vector of any of embodiments 106-108 that is a lentiviral vector or a gammaretroviral vector.

110. A method of producing an engineered cell, comprising introducing the polynucleotide of any of embodiments 96-105 or the vector of any of embodiments 106-109into a cell.

111. An engineered cell produced by the method of embodiment 110.

112. An engineered cell, comprising the polynucleotide of any of embodiments 98-105 or the vector of any of embodiments 106-109.

113. An engineered cell, comprising the cell surface conjugate of any of embodiments 1-97.

114. The engineered cell of embodiment 113, further comprising a recombinant receptor.

115. The engineered cell of embodiment 114, wherein the recombinant receptor is capable of binding to a target antigen that is associated with, specific to, and/or expressed on a cell or tissue of a disease or disorder.

116. The engineered cell of embodiment 115, wherein the disease or disorder is an infectious disease or disorder, an autoimmune disease, an inflammatory disease, or a tumor or a cancer.

117. The engineered cell of embodiment 115 or embodiment 116, wherein the target antigen is a tumor antigen.

118. The engineered cell of any of embodiments 115-117, wherein the target antigen is selected from the group consisting of αvβ6 integrin (avb6 integrin), B cell maturation antigen (BCMA), B7-H3, B7-H6, carbonic anhydrase 9 (CA9, also known as CAIX or G250), a cancer-testis antigen, cancer/testis antigen 1B (CTAG, also known as NY-ESO-1 and LAGE-2), carcinoembryonic antigen (CEA), a cyclin, cyclin A2, C-C Motif Chemokine Ligand 1 (CCL-1), CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD138, CD171, epidermal growth factor protein (EGFR), truncated epidermal growth factor protein (tEGFR), type III epidermal growth factor receptor mutation (EGFR vIII), epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), ephrinB2, ephrine receptor A2 (EPHa2), estrogen receptor, Fc receptor like 5 (FCRL5; also known as Fc receptor homolog 5 or FCRH5), fetal acetylcholine receptor (fetal AchR), a folate binding protein (FBP), folate receptor alpha, ganglioside GD2, O-acetylated GD2 (OGD2), ganglioside GD3, glycoprotein 100 (gp100), G Protein Coupled Receptor 5D (GPCR5D), Her2/neu (receptor tyrosine kinase

erb-B2), Her3 (erb-B3), Her4 (erb-B4), erbB dimers, Human high molecular weight-melanoma-associated antigen (HMW-MAA), hepatitis B surface antigen, Human leukocyte antigen A1 (HLA-A1), Human leukocyte antigen A2 (HLA-A2), IL-22 receptor alpha(IL-22Ra), IL-13 receptor alpha 2 (IL-13Ra2), kinase insert domain receptor (kdr), kappa light chain, L1 cell adhesion molecule (L1-CAM), CE7 epitope of L1-CAM, Leucine Rich Repeat Containing 8 Family Member A (LRRC8A), Lewis Y, Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, mesothelin, c-Met, murine cytomegalovirus (CMV), mucin 1 (MUC1), MUC16, natural killer group 2 member D (NKG2D) ligands, melan A (MART-1), neural cell adhesion molecule (NCAM), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME), progesterone receptor, a prostate specific antigen, prostate stem cell antigen (PSCA), prostate specific membrane antigen (PSMA), Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1), survivin, Trophoblast glycoprotein (TPBG also known as 5T4), tumor-associated glycoprotein 72 (TAG72), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor receptor 2 (VEGFR2), Wilms Tumor 1 (WT-1)

119. The engineered cell of any of embodiments 115-118, wherein the target antigen is selected from the group consisting of ROR1, HER2, L1-CAM, CD19, CD20, CD22, mesothelin, CEA, hepatitis B surface antigen, anti-folate receptor, CD23, CD24, CD30, CD33, CD38, CD44, EGFR, EGP-2, EGP-4, EPHa2, ErbB2, ErbB3, ErbB4, FBP, fetal acethycholine e receptor, GD2, GD3, HMW-MAA, IL-22R-alpha, IL-13R-alpha2, kdr, kappa light chain, Lewis Y, L1-cell adhesion molecule, MAGE-A1, mesothelin, MUC1, MUC16, PSCA, NKG2D Ligands, NY-ESO-1, MART-1, gp100, oncofetal antigen, TAG72, VEGF-R2, carcinoembryonic antigen (CEA), prostate specific antigen, PSMA, estrogen receptor, progesterone receptor, ephrinB2, CD123, CS-1, c-Met, GD-2, MAGE A3, CE7, Wilms Tumor 1 (WT-1), and cyclin A1 (CCNA1).

120. The engineered cell of any of embodiments 114-119, wherein the recombinant receptor is a functional non-TCR antigen receptor or a transgenic TCR.

121. The engineered cell of any of embodiments 114-120, wherein the recombinant receptor is a chimeric antigen receptor (CAR).

122. The engineered cell of any of embodiments 114-121, wherein the recombinant receptor comprises an extracellular portion comprising an antigen-binding domain.

123. The engineered cell of embodiment 122, wherein the antigen-binding domain is or comprises an antibody or an antibody fragment.

124. The engineered cell of embodiment 123, wherein the antibody fragment is a single chain fragment.

125. The engineered cell of embodiment 123 or embodiment 124, wherein the fragment comprises antibody variable regions joined by a flexible linker.

126. The engineered cell of any of embodiments123-125, wherein the fragment comprises an scFv.

127. The engineered cell of any of embodiments 114-126, wherein the recombinant receptor comprises an intracellular signaling region.

128. The engineered cell of embodiment 127, wherein the intracellular signaling region comprises an intracellular signaling domain.

129. The engineered cell of embodiment 128, wherein the intracellular signaling domain is or comprises a primary signaling domain, a signaling domain that is capable of inducing a primary activation signal in a T cell, a signaling domain of a T cell receptor (TCR) component, and/or a signaling domain comprising an immunoreceptor tyrosine-based activation motif (ITAM).

130. The engineered cell of embodiment 128 or embodiment 129, wherein the intracellular signaling domain is or comprises an intracellular signaling domain of a CD3 chain, optionally a CD3-zeta (CD3ζ) chain or a signaling portion thereof.

131. The engineered cell of any of embodiments 127-130, further comprising a transmembrane domain disposed between the extracellular domain and the intracellular signaling region.

132. The engineered cell of any of embodiments 127-131, wherein the intracellular signaling region furthercomprises a costimulatory signaling domain.

133. The engineered cell of embodiment 132, wherein the costimulatory signaling domain comprises an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof.

134. The engineered cell of embodiment 132 or embodiment 133, wherein the costimulatory signaling domain comprises an intracellular signaling domain of a CD28, a 4-1BB or an ICOS or a signaling portion thereof.

135. The engineered cell of any of embodiments 132-134, wherein the costimulatory signaling domain is between the transmembrane domain and the intracellular signaling domain.

136. The engineered cell of any of embodiments 111-135, wherein the cell is an immune cell.

137. The engineered cell of embodiment 136, wherein the cell is a lymphocyte.

138. The engineered cell of any of embodiments 111-137, wherein the cell is a T cell or an NK cell.

139. The engineered cell of embodiment 138, wherein the cell is a T cell that is a CD8+ T cell or a CD4+ T cell.

140. A composition comprising the engineered cells of any of embodiments 111-139.

141. The composition of embodiment 140, further comprising a pharmaceutically acceptable excipient.

142. A method of treatment comprising administering the engineered cells of any of embodiments 111-139 or the composition of embodiment 140 or embodiment 141 to a subject having a disease or disorder.

143. The method of embodiment 142, wherein the disease or disorder is a cancer, a tumor, an autoimmune disease or disorder, or an infectious disease.

144. The method of embodiment 142 or embodiment 143, further comprising administering to the subject a binding molecule capable of recognizing the agent of the cell surface conjugate expressed on the engineered cell and detecting cells that express the cell surface conjugate.

145. The method of embodiment 144, wherein detection comprises in vivo imaging.

146. A method of identifying a cell expressing a cell surface conjugate, comprising contacting a composition comprising cells that express or are likely to express a cell surface conjugate of any of embodiments 1-97 or the engineered cell of any of embodiments 111-139 or the composition of embodiment 140 or embodiment 141, with a binding molecule capable of recognizing the agent of the cell surface conjugate.

147. The method of embodiment 146 that is performed in vitro, ex vivo or in vivo.

148. The method of any of embodiment 146 or embodiment 147, wherein the cell expressing the cell surface molecule is detected via in vivo imaging.

149. The method of embodiment 145 or embodiment 148, wherein the in vivo imaging method is selected from among magnetic resonance imaging (MRI), single-photon emission computed tomography (SPECT), computed tomography (CT), computed axial tomography (CAT), electron beam computed tomography (EBCT), high resolution computed tomography (HRCT), hypocycloidal tomography, positron emission tomography (PET), scintigraphy, gamma camera, a β+ detector, a γ detector, fluorescence imaging, low-light imaging, X- rays, and bioluminescence imaging.

150. The method of embodiment 145, embodiment 148, or embodiment 149, wherein the binding molecule is conjugated to a moiety that provides a signal or induces a signal that is detectable in vivo.

151. The method of embodiment 150, wherein the moiety is a radioisotope, bioluminescent compound, chemiluminescent compound, fluorescent compound, metal chelate or enzyme.

152. A method of identifying cells transduced with a cell surface conjugate, comprising:

(a) contacting a composition transduced with a polynucleotide of any of embodiments 98-105 or the vector of any of embodiments 106-109 encoding the cell surface conjugate or the engineered cell of any of embodiments 111-139 or the composition of embodiment 140 or embodiment 141 with a binding molecule capable of recognizing the agent of the cell surface conjugate; and

(b) identifying cells bound to the binding molecule.

153. A method of identifying cells transduced with a cell surface conjugate, comprising:

(a) introducing a polynucleotide of any of embodiments 98-105 or the vector of any of embodiments 106-109 encoding the cell surface conjugate into a cell;

(b) contacting a composition comprising the cell of (a) with a binding molecule capable of recognizing the agent of the cell surface conjugate; and

(c) identifying cells of the composition bound to the binding molecule.

154. A method of selecting cells transduced with a cell surface conjugate, comprising:

(a) contacting a composition transduced with a polynucleotide of any of embodiments 98-105 or the vector of any of embodiments 106-109 encoding the cell surface conjugate or the engineered cell of any of embodiments 111-139 or the composition of embodiment 140 or embodiment 141 with a binding molecule capable of recognizing the agent of the cell surface conjugate; and

(b) isolating cells bound to the binding molecule.

155. A method of selecting cells transduced with a cell surface conjugate, comprising:

(a) introducing a polynucleotide of any of embodiments 98-105 or the vector of any of embodiments 106-109 encoding the cell surface conjugate into a cell;

(b) contacting a composition comprising the cell of (a) with a binding molecule capable of recognizing the agent of the cell surface conjugate; and

(c) isolating cells of the composition bound to the binding molecule.

156. The method of embodiment 154 or embodiment 155, wherein the binding molecule is conjugated to a detectable moiety or is capable of producing a detectable signal.

157. The method of embodiment 156, wherein the detectable moiety comprises a fluorescent protein.

158. The method of any of embodiments 144-157, wherein the agent is is a streptavidin binding peptide.

159. The method of embodiment 158, wherein the streptavidin binding peptide is or comprises the sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8) or Trp-Arg-His-Pro-Gln-Phe-Gly-Gly (SEQ ID NO:7).

160. The method of embodiment 159, wherein the streptavidin binding peptide is or comprises the sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_3$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 17), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 18) and Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$Gly-Gly-Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 19).

161. The method of any of embodiments 144-160, wherein the binding molecule is a reagent capable of reversibly binding to the agent and/or capable of being competed in the presence of a competition substance.

162. The method of embodiment 161, wherein the reagent is a streptavidin, a streptavidin analog or mutein.

163. The method of embodiment 162, wherein the streptavidin analog or mutein comprises the amino acid sequence Val^{44}-Thr^{45}-Ala^{46}-Arg^{47} or Ile^{44}-Gly^{45}-Ala^{46}-Arg^{47} at sequence positions corresponding to positions 44 to 47 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO:1.

164. The method of embodiment 162 or embodiment 163, wherein the streptavidin analog or mutein comprises:

a) the sequence of amino acids set forth in any of SEQ ID NOS: 3-6, 27 and 28;

b) a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 3-6, 27 and 28 and contains the amino acid sequence corresponding to Val^{44}-Thr^{45}-Ala^{46}-Arg^{47} or Ile^{44}-Gly^{45}-Ala^{46}-Arg^{47} and that reversibly binds to the agent; or

c) a functional fragment of a) or b) that reversibly binds to the agent.

165. The method of embodiment 163 or embodiment 164, wherein the streptavidin analog or mutein further comprises an amino acid replacement or replacements at a position corresponding to 117, 120 and/or 121 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO:1.

166. The method of embodiment 165, wherein:

the amino acid replacement or replacements are selected from among Glu^{117}, Asp^{117}, Arg^{117}, Ser^{120}, Ala^{120}, Gly^{120}, Trp^{121}, Tyr^{121} or Phe^{121}; or

the amino acid replacement or replacements are selected from one or more of Glu^{117}, Gly^{120} or Tyr^{121}; or

the amino acid replacements are selected from Glu^{117}, Gly^{120} or Tyr^{121}.

167. The method of any of embodiments 162-166, wherein the streptavidin analog or mutein comprises:

a) the sequence of amino acids set forth in SEQ ID NO: 27 or 28;

b) a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NOS:27 or 28 and contains the amino acid sequence corresponding to Val^{44}, Thr^{45}, Ala^{46}, Arg^{47}, Glu^{117}, Gly^{120} and Tyr^{121} and reversibly binds to the agent; or

c) a functional fragment of a) or b) that reversibly binds to the agent.

168. The method of any of embodiments 161-167, further comprising disrupting the reversible binding of the binding molecule to the agent.

169. The method of embodiment 168, wherein said disruption comprises contacting the cells with a composition comprising a competition substance capable of reversing the bond between the binding molecule and agent.

170. The method of embodiment 169, wherein the competition substance is a free binding partner and/or is a competition agent.

171. The method of embodiment 169 or embodiment 170, wherein the competition substance is or comprises biotin, a biotin analog or a biologically active fragment thereof.

172. The method of any of embodiments 144-171, wherein the binding molecule is an antibody or antigen binding fragment that specifically binds the agent.

173. The method of embodiment 172, wherein the binding molecule is an anti-StrepTag antibody.

174. A molecule, comprising a streptavidin or a streptavidin analog or mutein conjugated to a cytotoxic agent.

175. The molecule of embodiment 174, comprising a streptavidin analog or mutein.

176. The molecule of embodiment 174 or embodiment 175, wherein the streptavidin or streptavidin analog or mutein binds to a streptavidin binding peptide.

177. The molecule of embodiment 176, wherein the streptavidin binding peptide is or comprises the sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8) or Trp-Arg-His-Pro-Gln-Phe-Gly-Gly (SEQ ID NO:7).

178. The molecule of embodiment 176 or embodiment 177, wherein the streptavidin binding peptide is or comprises the sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_3$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys ((SEQ ID NO: 17), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 18) and Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$Gly-Gly-Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 19).

179. The molecule of any of embodiments 176-178, wherein the streptavidin or streptavidin analog or mutein exhibits a binding affinity for the streptavidin binding peptide with an equilibrium dissociation constant (K_D) of from or from about 10^{-4} M to or to about 10^{-10} M.

180. The molecule of any of embodiments 174-179, wherein the streptavidin analog or mutein comprises the amino acid sequence Val^{44}-Thr^{45}-Ala^{46}-Arg^{47} or Ile^{44}-Gly^{45}-Ala^{46}-Arg^{47} at sequence positions corresponding to positions 44 to 47 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO:1.

181. The molecule of any of embodiments 174-180, wherein the streptavidin analog or mutein comprises:

a) the sequence of amino acids set forth in any of SEQ ID NOS: 3-6, 27 and 28;

b) a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 3-6, 27 and 28 and contains the amino acid sequence corresponding to Val^{44}-Thr^{45}-Ala^{46}-Arg^{47} or Ile^{44}-Gly^{45}-Ala^{46}-Arg^{47} and that reversibly binds to the agent; or

c) a functional fragment of a) or b) that binds to the streptavidin binding peptide.

182. The molecule of embodiment 180 or embodiment 181, wherein the streptavidin analog or mutein further comprises an amino acid replacement or replacements at a position corresponding to 117, 120 and/or 121 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO:1.

183. The molecule of embodiment 182, wherein:

the amino acid replacement or replacements are selected from among Glu^{117}, Asp^{117}, Arg^{117}, Ser^{120}, Ala^{120}, Gly^{120}, Trp^{121}, Tyr^{121} or Phe^{121}; or

the amino acid replacement or replacements are selected from one or more of Glu^{117}, Gly^{120} or Tyr^{121}; or

the amino acid replacements are selected from Glu^{117}, Gly^{120} or Tyr^{121}.

184. The molecule of any of embodiments 174-183, wherein the streptavidin analog or mutein comprises:

a) the sequence of amino acids set forth in SEQ ID NO: 27 or 28;

b) a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NOS:27 or 28 and contains the amino acid sequence corresponding to Val^{44}, Thr^{45}, Ala^{46}, Arg^{47}, Glu^{117}, Gly^{120} and Tyr^{121} and reversibly binds to the agent; or

c) a functional fragment of a) or b) that reversibly binds to the streptavidin binding peptide.

185. The molecule of any of embodiments 174-184, wherein the cytotoxic agent is a toxin.

186. The molecule of embodiment 185, wherein the toxin is a peptide toxin, ricin A chain toxin, Abrin A chain, Diptheria Toxin (DT) A chain, Pseudomonas exotoxin, Shiga Toxin A chain, Gelonin, Momordin, Pokeweed Antiviral Protein, Saporin, Trichosanthin, or Barley Toxin.

187. The molecule of embodiment 185, wherein the the toxin is a phototoxin.

188. A method of killing cells, comprising administering the molecule of any of embodiments 174-187 to a subject previously administered the cells of any of embodiments 111-139 or the composition of embodiment 140 or embodiment 141.

189. The method of embodiment 188, wherein the molecule is administered at a time at which the subject is exhibiting a toxic outcome associated with the administered cells or at a time at which the subject is exhibiting a detectable and/or cell-mediated immune response to the administered cells.

190. The method of embodiment 189, wherein the toxic outcome is associated with neurotoxicity or cytokine release syndrome (CRS).

[0504] The present invention is not to be limited in scope by the embodiments disclosed herein, which are intended as single illustrations of individual aspects of the invention, and any

that are functionally equivalent are within the scope of the invention. Various modifications to the compositions and methods of the invention, in addition to those described herein, will become apparent to those skilled in the art from the foregoing description and teachings, and are similarly intended to fall within the scope of the invention. Such modifications or other embodiments can be practiced without departing from the true scope and spirit of the invention.

SEQUENCES

SEQ ID	Sequence	Description
1	DPSKDSKAQVSAAEAGITGTWYNQLGSTFIVTAGADGALTGTYESAVGNAES RYVLTGRYDSAPATDGSGTALGWTVAWKNNYRNAHSATTWSGQYVGGAEARI NTQWLLTSGTTEANAWKSTLVGHDTFTKVKPSAASIDAAKKAGVNNGNPLDA VQQ	Streptavidin Species: Streptomyces avidinii UniProt No. P22629
2	EAGITGTWYNQLGSTFIVTAGADGALTGTYESAVGNAESRYVLTGRYDSAPA TDGSGTALGWTVAWKNNYRNAHSATTWSGQYVGGAEARINTQWLLTSGTTEA NAWKSTLVGHDTFTKVKPSAAS	Minimal streptavidin Species: Streptomyces avidinii
3	DPSKDSKAQVSAAEAGITGTWYNQLGSTFIVTAGADGALTGTY**VTAR**GNAES RYVLTGRYDSAPATDGSGTALGWTVAWKNNYRNAHSATTWSGQYVGGAEARI NTQWLLTSGTTEANAWKSTLVGHDTFTKVKPSAASIDAAKKAGVNNGNPLDA VQQ	Mutein Streptavidin Val44-Thr45-Ala46-Arg47 Species: Streptomyces avidinii
4	EAGITGTWYNQLGSTFIVTAGADGALTGTY**VTAR**GNAESRYVLTGRYDSAPA TDGSGTALGWTVAWKNNYRNAHSATTWSGQYVGGAEARINTQWLLTSGTTEA NAWKSTLVGHDTFTKVKPSAAS	Mutein Streptavidin Val44-Thr45-Ala46-Arg47 Species: Streptomyces avidinii
5	DPSKDSKAQVSAAEAGITGTWYNQLGSTFIVTAGADGALTGTY**IGAR**GNAES RYVLTGRYDSAPATDGSGTALGWTVAWKNNYRNAHSATTWSGQYVGGAEARI NTQWLLTSGTTEANAWKSTLVGHDTFTKVKPSAASIDAAKKAGVNNGNPLDA VQQ	Mutein Streptavidin Ile44-Gly45-Ala-46-Arg47 Species: Streptomyces avidinii
6	EAGITGTWYNQLGSTFIVTAGADGALTGTY**IGAR**GNAESRYVLTGRYDSAPA TDGSGTALGWTVAWKNNYRNAHSATTWSGQYVGGAEARINTQWLLTSGTTEA NAWKSTLVGHDTFTKVKPSAAS	Mutein Streptavidin Ile44-Gly45-Ala-46-Arg47 Species: Streptomyces avidinii
7	Trp-Arg-His-Pro-Gln-Phe-Gly-Gly	Streptavidin binding peptide, Strep-tag®
8	WSHPQFEK	Strep-tag® II
9	His-Pro-Baa	Streptavidin Binding peptide Baa is selected from glutamine, asparagine and methionine
10	His-Pro-Gln-Phe	Streptavidin-binding peptide
11	Oaa-Xaa-His-Pro-Gln-Phe-Yaa-Zaa	Streptavidin-binding peptide

		Oaa is Trp, Lys or Arg; Xaa is any amino acid; Yaa is Gly or Glu Zaa is Gly, Lys or Arg
12	-Trp-Xaa-His-Pro-Gln-Phe-Yaa-Zaa-	Streptavidin-binding peptide Xaa is any amino acid; Yaa is Gly or Glu Zaa is Gly, Lys or Arg
13	Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(Xaa)n-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-	Sequential modules of streptavidin-binding peptide Xaa is any amino acid; n is either 8 or 12
14	Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)n-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys	Sequential modules of streptavidin-binding peptide n is 2 or 3
15	SAWSHPQFEKGGGSGGGSGGGSWSHPQFEK	Twin-Strep-tag
16	SAWSHPQFEKGGGSGGGSGGSAWSHPQFEK	Twin-Strep-tag
17	WSHPQFEKGGGSGGGSGGGSWSHPQFEK	Twin-Strep-tag
18	WSHPQFEKGGGSGGGSWSHPQFEK	Twin-Strep-tag
19	WSHPQFEKGGGSGGGSGGSAWSHPQFEK	Twin-Strep-tag
20	Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala	HA-tag
21	Tyr-Thr-Asp-Ile-Glu-Met-Asn-Arg-Leu-Gly-Lys	VSV-G-tag
22	Gln-Pro-Glu-Leu-Ala-Pro-Glu-Asp-Pro-Glu-Asp	HSV-tag
23	Ala-Ser-Met-Thr-Gly-Gly-Gln-Gln-Met-Gly	T7 epitope
24	Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu	HSV epitope
25	Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu	Myc epitope
26	Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Leu-Gly-Leu-Asp-Ser-Thr	V5-tag
27	EAGITGTWYNQLGSTFIVTAGADGALTGTYVTARGNAESRYVLTGRYDSAPATDGSGTALGWTVAWKNNYRNAHSATTWSGQYVGGAEARINTQWLLTSGTTEENAGYSTLVGHDTFTKVKPSAAS	Mutein Streptavidin Val44-Thr45-Ala46-Arg47 and Glu117, Gly120, Try121 (mutein m1-9) Species: Streptomyces avidinii
28	DPSKDSKAQVSAAEAGITGTWYNQLGSTFIVTAGADGALTGTYVTARGNAESRYVLTGRYDSAPATDGSGTALGWTVAWKNNYRNAHSATTWSGQYVGGAEARI	Mutein Streptavidin Val44-Thr45-Ala46-

	NTQWLLTSGTTEENAGYSTLVGHDTFTKVKPSAAS	Arg47 and Glu117, Gly120, Try121 (mutein m1-9) Species: Streptomyces avidinii
29	AMQVQLKQSGPGLVQPSQSLSITCTVSGFSLTTFGVHWVRQSPGKGLEWLGV IWASGITDYNVPFMSRLSITKDNSKSQVFFKLNSLQPDDTAIYYCAKNDPGT GFAYWGQGTLVTVSAGSTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCGSAWSHPQFEKGGGSGGGSGGSAWSHPQFEK	Variable Heavy chain of Fab fragment m13B8.2
30	AMDIQMTQSPASLSASVGETVTFTCRASEMIYSYLAWYQQKQGKSPQLLVHD AKTLAEGVPSRFSGGGSGTQFSLKINTLQPEDFGTYYCQAHYGNPPTFGGGT KLEIKRGIAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL QSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT KSFNRGECGS	Variable Light chain of Fab Fragment m13B8.2
31	Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser	Variable Heavy chain of anti-CD3 antibody OKT3
32	Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala His Phe Arg Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Gly Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Asn	Variable Light chain of anti-CD3 antibody OKT3
33	Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser Val Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Glu Tyr Ile Ile His Trp Ile Lys Leu Arg Ser Gly Gln Gly Leu Glu Trp Ile Gly Trp Phe Tyr Pro Gly Ser Asn Asp Ile Gln Tyr Asn Ala Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Val Tyr Met Glu Leu Thr Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg Arg Asp Asp Phe Ser Gly Tyr Asp Ala Leu Pro Tyr Trp Gly Gln Gly Thr Met Val Thr Val	Variable Heavy chain of anti-CD28 antibody CD28.3
34	Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Val Ser Val Gly Glu Thr Val Thr Ile Thr Cys Arg Thr Asn Glu Asn Ile Tyr Ser Asn Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Leu Ile Tyr Ala Ala Thr His Leu Val Glu Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Thr Ser Leu Gln Ser Glu Asp Phe Gly Asn Tyr Tyr Cys Gln His Phe Trp Gly Thr Pro Cys Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg	Variable Light chain of anti-CD28 antibody CD28.3
35	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL HMQALPPR	CD3 zeta Homo sapiens
36	RVKFSRSAEPPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL	CD3 zeta Homo sapiens

	HMQALPPR	
37	RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL HMQALPPR	CD3 zeta Homo sapiens
38	ESKYGPPCPPCP	spacer (IgG4hinge) (aa) Homo sapiens
39	GAATCTAAGTACGGACCGCCCTGCCCCCCTTGCCCT	spacer (IgG4hinge) (nt) Homo sapiens
40	ESKYGPPCPPCPGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGK	Hinge-CH3 spacer Homo sapiens
41	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCS VMHEALHNHYTQKSLSLSLGK	Hinge-CH2-CH3 spacer Homo sapiens
42	RWPESPKAQASSVPTAQPQAEGSLAKATTAPATTRNTGRGGEEKKKEKEKEE QEERETKTPECPSHTQPLGVYLLTPAVQDLWLRDKATFTCFVVGSDLKDAHL TWEVAGKVPTGGVEEGLLERHSNGSQSQHSRLTLPRSLWNAGTSVTCTLNHP SLPPQRLMALREPAAQAPVKLSLNLLASSDPPEAASWLLCEVSGFSPPNILL MWLEDQREVNTSGFAPARPPPQPGSTTFWAWSVLRVPAPPSPQPATYTCVVS HEDSRTLLNASRSLEVSYVTDH	IgD-hinge-Fc Homo sapiens
43	LEGGGEGRGSLLTCGDVEENPGPR	T2A artificial
44	MLLLVTSLLLCELPHPAFLLIPRKVCNGIGIGEFKDSLSINATNIKHFKNCT SISGDLHILPVAFRGDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRT DLHAFENLEIIRGRTKQHGQFSLAVVSLNITSLGLRSLKEISDGDVIISGNK NLCYANTINWKKLFGTSGQKTKIISNRGENSCKATGQVCHALCSPEGCWGPE PRDCVSCRNVSRGRECVDKCNLLEGEPREFVENSECIQCHPECLPQAMNITC TGRGPDNCIQCAHYIDGPHCVKTCPAGVMGENNTLVWKYADAGHVCHLCHPN CTYGCTGPGLEGCPTNGPKIPSIATGMVGALLLLLVVALGIGLFM	tEGFR artificial
45	atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcat tcctcctgatcccacgcaaagtgtgtaacggaataggtattggtgaatttaa agactcactctccataaatgctacgaatattaaacacttcaaaaactgcacc tccatcagtggcgatctccacatcctgccggtggcatttaggggtgactcct tcacacatactcctcctctggatccacaggaactggatattctgaaaaccgt aaaggaaatcacagggtttttgctgattcaggcttggcctgaaaacaggacg gacctccatgcctttgagaacctagaaatcatacgcggcaggaccaagcaac atggtcagttttctcttgcagtcgtcagcctgaacataacatccttgggatt acgctccctcaaggagataagtgatggagatgtgataatttcaggaaacaaa aatttgtgctatgcaaatacaataaactggaaaaaactgtttgggacctccg gtcagaaaaccaaaattataagcaacagaggtgaaaacagctgcaaggccac aggccaggtctgccatgccttgtgctcccccgagggctgctggggcccggag cccagggactgcgtctcttgccggaatgtcagccgaggcagggaatgcgtgg acaagtgcaaccttctggagggtgagccaagggagtttgtggagaactctga gtgcatacagtgccacccagagtgcctgcctcaggccatgaacatcacctgc acaggacggggaccagacaactgtatccagtgtgcccactacattgacggcc cccactgcgtcaagacctgcccggcaggagtcatgggagaaaacaacaccct ggtctggaagtacgcagacgccggccatgtgtgccacctgtgccatccaaac tgcacctacggatgcactgggccaggtcttgaaggctgtccaacgaatgggc ctaagatcccgtccatcgccactgggatggtgggggccctcctcttgctgct ggtggtggccctggggatcggcctcttcatg	tEGFR artificial
46	RKVCNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHILPVAFRGDSFTHTP PLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGQFS LAVVSLNITSLGLRSLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQKTK IISNRGENSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNL	tEGFR artificial

	LEGEPREFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVK TCPAGVMGENNTLVWKYADAGHVCHLCHPNCTYGCTGPGLEGCPTNGPKIPS IATGMVGALLLLLVVALGIGLFM	
47	atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcat tcctcctgatccca	GMCSFR alpha chain signal sequence Homo sapiens
48	MLLLVTSLLLCELPHPAFLLIP	GMCSFR alpha chain signal sequence Homo sapiens UniProt No. P15509
49	LEEKKVCQGTSNKLTQLGTFEDHFLSLQRMFNNCEVVLGNLEITYVQRNYDL SFLKTIQEVAGYVLIALNTVERIPLENLQIIRGNMYYENSYALAVLSNYDAN KTGLKELPMRNLQEILHGAVRFSNNPALCNVESIQWRDIVSSDFLSNMSMDF QNHLGSCQKCDPSCPNGSCWGAGEENCQKLTKIICAQQCSGRCRGKSPSDCC HNQCAAGCTGPRESDCLVCRKFRDEATCKDTCPPLMLYNPTTYQMDVNPEGK YSFGATCVKKCPRNYVVTDHGSCVRACGADSYEMEEDGVRKCKKCEGPCRKV CNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHILPVAFRGDSFTHTPPLD PQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGQFSLAV VSLNITSLGLRSLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQKTKIIS NRGENSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLEG EPREFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCP AGVMGENNTLVWKYADAGHVCHLCHPNCTYGCTGPGLEGCPTNGPKIPSIAT GMVGALLLLLVVALGIGLFMRRRHIVRKRTLRRLLQERELVEPLTPSGEAPN QALLRILKETEFKKIKVLGSGAFGTVYKGLWIPEGEKVKIPVAIKELREATS PKANKEILDEAYVMASVDNPHVCRLLGICLTSTVQLITQLMPFGCLLDYVRE HKDNIGSQYLLNWCVQIAKGMNYLEDRRLVHRDLAARNVLVKTPQHVKITDF GLAKLLGAEEKEYHAEGGKVPIKWMALESILHRIYTHQSDVWSYGVTVWELM TFGSKPYDGIPASEISSILEKGERLPQPPICTIDVYMIMVKCWMIDADSRPK FRELIIEFSKMARDPQRYLVIQGDERMHLPSPTDSNFYRALMDEEDMDDVVD ADEYLIPQQGFFSSPSTSRTPLLSSLSATSNNSTVACIDRNGLQSCPIKEDS FLQRYSSDPTGALTEDSIDDTFLPVPEYINQSVPKRPAGSVQNPVYHNQPLN PAPSRDPHYQDPHSTAVGNPEYLNTVQPTCVNSTFDSPAHWAQKGSHQISLD NPDYQQDFFPKEAKPNGIFKGSTAENAEYLRVAPQSSEFIGA	HER1/ErbB1/EGFR Full Length (mature) Transmembrane domain: amino acids 622-644 Cytoplasmic domain: amino acids 645-1186 Homo sapiens UniProt No. P00533
50	TQVCTGTDMKLRLPASPETHLDMLRHLYQGCQVVQGNLELTYLPTNASLSFL QDIQEVQGYVLIAHNQVRQVPLQRLRIVRGTQLFEDNYALAVLDNGDPLNNT TPVTGASPGGLRELQLRSLTEILKGGVLIQRNPQLCYQDTILWKDIFHKNNQ LALTLIDTNRSRACHPCSPMCKGSRCWGESSEDCQSLTRTVCAGGCARCKGP LPTDCCHEQCAAGCTGPKHSDCLACLHFNHSGICELHCPALVTYNTDTFESM PNPEGRYTFGASCVTACPYNYLSTDVGSCTLVCPLHNQEVTAEDGTQRCEKC SKPCARVCYGLGMEHLREVRAVTSANIQEFAGCKKIFGSLAFLPESFDGDPA SNTAPLQPEQLQVFETLEEITGYLYISAWPDSLPDLSVFQNLQVIRGRILHN GAYSLTLQGLGISWLGLRSLRELGSGLALIHHNTHLCFVHTVPWDQLFRNPH QALLHTANRPEDECVGEGLACHQLCARGHCWGPGPTQCVNCSQFLRGQECVE ECRVLQGLPREYVNARHCLPCHPECQPQNGSVTCFGPEADQCVACAHYKDPP FCVARCPSGVKPDLSYMPIWKFPDEEGACQPCPINCTHSCVDLDDKGCPAEQ RASPLTSIISAVVGILLVVVLGVVFGILIKRRQQKIRKYTMRRLLQETELVE PLTPSGAMPNQAQMRILKETELRKVKVLGSGAFGTVYKGIWIPDGENVKIPV AIKVLRENTSPKANKEILDEAYVMAGVGSPYVSRLLGICLTSTVQLVTQLMP YGCLLDHVRENRGRLGSQDLLNWCMQIAKGMSYLEDVRLVHRDLAARNVLVK SPNHVKITDFGLARLLDIDETEYHADGGKVPIKWMALESILRRRFTHQSDVW SYGVTVWELMTFGAKPYDGIPAREIPDLLEKGERLPQPPICTIDVYMIMVKC WMIDSECRPRFRELVSEFSRMARDPQRFVVIQNEDLGPASPLDSTFYRSLLE DDDMGDLVDAEEYLVPQQGFFCPDPAPGAGGMVHHRHRSSSTRSGGGDLTLG LEPSEEEAPRSPLAPSEGAGSDVFDGDLGMGAAKGLQSLPTHDPSPLQRYSE DPTVPLPSETDGYVAPLTCSPQPEYVNQPDVRPQPPSPREGPLPAARPAGAT	HER2/neu/ErbB2 Full Length (mature) Transmembrane domain: amino acids 631-653 Cytoplasmic domain: amino acids 654-1233 Homo sapiens UniProt No. P04626

	LERPKTLSPGKNGVVKDVFAFGGAVENPEYLTPQGGAAPQPHPPPAFSPAFD NLYYWDQDPPERGAPPSTFKGTPTAENPEYLGLDVPV	
51	SEVGNSQAVCPGTLNGLSVTGDAENQYQTLYKLYERCEVVMGNLEIVLTGHN ADLSFLQWIREVTGYVLVAMNEFSTLPLPNLRVVRGTQVYDGKFAIFVMLNY NTNSSHALRQLRLTQLTEILSGGVYIEKNDKLCHMDTIDWRDIVRDRDAEIV VKDNGRSCPPCHEVCKGRCWGPGSEDCQTLTKTICAPQCNGHCFGPNPNQCC HDECAGGCSGPQDTDCFACRHFNDSGACVPRCPQPLVYNKLTFQLEPNPHTK YQYGGVCVASCPHNFVVDQTSCVRACPPDKMEVDKNGLKMCEPCGGLCPKAC EGTGSGSRFQTVDSSNIDGFVNCTKILGNLDFLITGLNGDPWHKIPALDPEK LNVFRTVREITGYLNIQSWPPHMHNFSVFSNLTTIGGRSLYNRGFSLLIMKN LNVTSLGFRSLKEISAGRIYISANRQLCYHHSLNWTKVLRGPTEERLDIKHN RPRRDCVAEGKVCDPLCSSGGCWGPGPGQCLSCRNYSRGGVCVTHCNFLNGE PREFAHEAECFSCHPECQPMEGTATCNGSGSDTCAQCAHFRDGPHCVSSCPH GVLGAKGPIYKYPDVQNECRPCHENCTQGCKGPELQDCLGQTLVLIGKTHLT MALTVIAGLVVIFMMLGGTFLYWRGRRIQNKRAMRRYLERGESIEPLDPSEK ANKVLARIFKETELRKLKVLGSGVFGTVHKGVWIPEGESIKIPVCIKVIEDK SGRQSFQAVTDHMLAIGSLDHAHIVRLLGLCPGSSLQLVTQYLPLGSLLDHV RQHRGALGPQLLLNWGVQIAKGMYYLEEHGMVHRNLAARNVLLKSPSQVQVA DFGVADLLPPDDKQLLYSEAKTPIKWMALESIHFGKYTHQSDVWSYGVTVWE LMTFGAEPYAGLRLAEVPDLLEKGERLAQPQICTIDVYMVMVKCWMIDENIR PTFKELANEFTRMARDPPRYLVIKRESGPGIAPGPEPHGLTNKKLEEVELEP ELDLDLDLEAEEDNLATTTLGSALSLPVGTLNRPRGSQSLLSPSSGYMPMNQ GNLGESCQESAVSGSSERCPRPVSLHPMPRGCLASESSEGHVTGSEAELQEK VSMCRSRSRSRSPRPRGDSAYHSQRHSLLTPVTPLSPPGLEEEDVNGYVMPD THLKGTPSSREGTLSSVGLSSVLGTEEEDEDEEYEYMNRRRRHSPPHPPRPS SLEELGYEYMDVGSDLSASLGSTQSCPLHPVPIMPTAGTTPDEDYEYMNRQR DGGGPGGDYAAMGACPASEQGYEEMRAFQGPGHQAPHVHYARLKTLRSLEAT DSAFDNPDYWHSRLFPKANAQRT	HER3/ErbB3 Full Length (mature) Transmembrane domain: amino acids 625-645 Cytoplasmic domain: amino acids 646-1323 Homo sapiens UniProt No. P21860
52	QSVCAGTENKLSSLSDLEQQYRALRKYYENCEVVMGNLEITSIEHNRDLSFL RSVREVTGYVLVALNQFRYLPLENLRIIRGTKLYEDRYALAIFLNYRKDGNF GLQELGLKNLTEILNGGVYVDQNKFLCYADTIHWQDIVRNPWPSNLTLVSTN GSSGCGRCHKSCTGRCWGPTENHCQTLTRTVCAEQCDGRCYGPYVSDCCHRE CAGGCSGPKDTDCFACMNFNDSGACVTQCPQTFVYNPTTFQLEHNFNAKYTY GAFCVKKCPHNFVVDSSSCVRACPSSKMEVEENGIKMCKPCTDICPKACDGI GTGSLMSAQTVDSSNIDKFINCTKINGNLIFLVTGIHGDPYNAIEAIDPEKL NVFRTVREITGFLNIQSWPPNMTDFSVFSNLVTIGGRVLYSGLSLLILKQQG ITSLQFQSLKEISAGNIYITDNSNLCYYHTINWTTLFSTINQRIVIRDNRKA ENCTAEGMVCNHLCSSDGCWGPGPDQCLSCRRFSRGRICIESCNLYDGEFRE FENGSICVECDPQCEKMEDGLLTCHGPGPDNCTKCSHFKDGPNCVEKCPDGL QGANSFIFKYADPDRECHPCHPNCTQGCNGPTSHDCIYYPWTGHSTLPQHAR TPLIAAGVIGGLFILVIVGLTFAVYVRRKSIKKKRALRRFLETELVEPLTPS GTAPNQAQLRILKETELKRVKVLGSGAFGTVYKGIWVPEGETVKIPVAIKIL NETTGPKANVEFMDEALIMASMDHPHLVRLLGVCLSPTIQLVTQLMPHGCLL EYVHEHKDNIGSQLLLNWCVQIAKGMMYLEERRLVHRDLAARNVLVKSPNHV KITDFGLARLLEGDEKEYNADGGKMPIKWMALECIHYRKFTHQSDVWSYGVT IWELMTFGGKPYDGIPTREIPDLLEKGERLPQPPICTIDVYMVMVKCWMIDA DSRPKFKELAAEFSRMARDPQRYLVIQGDDRMKLPSPNDSKFFQNLLDEEDL EDMMDAEEYLVPQAFNIPPPIYTSRARIDSNRSEIGHSPPPAYTPMSGNQFV YRDGGFAAEQGVSVPYRAPTSTIPEAPVAQGATAEIFDDSCCNGTLRKPVAP HVQEDSSTQRYSADPTVFAPERSPRGELDEEGYMTPMRDKPKQEYLNPVEEN PFVSRRKNGDLQALDNPEYHNASNGPPKAEDEYVNEPLYLNTFANTLGKAEY LKNNILSMPEKAKKAFDNPDYWNHSLPPRSTLQHPDYLQEYSTKYFYKQNGR IRPIVAENPEYLSEFSLKPGTVLPPPPYRHRNTVV	HER4/ErbB4 Full Length (mature) Transmembrane domain: amino acids 627-650 Cytoplasmic domain: amino acids 651-1283 Homo sapiens UniProt No. Q15303
53	ECKEALAKSEMNVNMKYQLPNFTAETPIQNVILHEHHIFLGATNYIYVLNEE DLQKVAEYKTGPVLEHPDCFPCQDCSSKANLSGGVWKDNINMALVVDTYYDD QLISCGSVNRGTCQRHVFPHNHTADIQSEVHCIFSPQIEEPSQCPDCVVSAL GAKVLSSVKDRFINFFVGNTINSSYFPDHPLHSISVRRLKETKDGFMFLTDQ SYIDVLPEFRDSYPIKYVHAFESNNFIYFLTVQRETLDAQTFHTRIIRFCSI NSGLHSYMEMPLECILTEKRKKRSTKKEVFNILQAAYVSKPGAQLARQIGAS	HGFR/c-Met Full Length (mature) Transmembrane domain: amino acids 909-931

	LNDDILFGVFAQSKPDSAEPMDRSAMCAFPIKYVNDFFNKIVNKNNVRCLQH FYGPNHEHCFNRTLLRNSSGCEARRDEYRTEFTTALQRVDLFMGQFSEVLLT SISTFIKGDLTIANLGTSEGRFMQVVVSRSGPSTPHVNFLLDSHPVSPEVIV EHTLNQNGYTLVITGKKITKIPLNGLGCRHFQSCSQCLSAPPFVQCGWCHDK CVRSEECLSGTWTQQICLPAIYKVFPNSAPLEGGTRLTICGWDFGFRRNNKF DLKKTRVLLGNESCTLTLSESTMNTLKCTVGPAMNKHFNMSIIISNGHGTTQ YSTFSYVDPVITSISPKYGPMAGGTLLTLTGNYLNSGNSRHISIGGKTCTLK SVSNSILECYTPAQTISTEFAVKLKIDLANRETSIFSYREDPIVYEIHPTKS FISGGSTITGVGKNLNSVSVPRMVINVHEAGRNFTVACQHRSNSEIICCTTP SLQQLNLQLPLKTKAFFMLDGILSKYFDLIYVHNPVFKPFEKPVMISMGNEN VLEIKGNDIDPEAVKGEVLKVGNKSCENIHLHSEAVLCTVPNDLLKLNSELN IEWKQAISSTVLGKVIVQPDQNFTGLIAGVVSISTALLLLLGFFLWLKKRKQ IKDLGSELVRYDARVHTPHLDRLVSARSVSPTTEMVSNESVDYRATFPEDQF PNSSQNGSCRQVQYPLTDMSPILTSGDSDISSPLLQNTVHIDLSALNPELVQ AVQHVVIGPSSLIVHFNEVIGRGHFGCVYHGTLLDNDGKKIHCAVKSLNRIT DIGEVSQFLTEGIIMKDFSHPNVLSLLGICLRSEGSPLVVLPYMKHGDLRNF IRNETHNPTVKDLIGFGLQVAKGMKYLASKKFVHRDLAARNCMLDEKFTVKV ADFGLARDMYDKEYYSVHNKTGAKLPVKWMALESLQTQKFTTKSDVWSFGVL LWELMTRGAPPYPDVNTFDITVYLLQGRRLLQPEYCPDPLYEVMLKCWHPKA EMRPSFSELVSRISAIFSTFIGEHYVHVNATYVNVKCVAPYPSLLSSEDNAD DEVDTRPASFWETS	Cytoplasmic domain: amino acids 932-1366 Homo sapiens UniProt No. P08581
54	MKSGSGGGSPTSLWGLLFLSAALSLWPTSGEICGPGIDIRNDYQQLKRLENC TVIEGYLHILLISKAEDYRSYRFPKLTVITEYLLLFRVAGLESLGDLFPNLT VIRGWKLFYNYALVIFEMTNLKDIGLYNLRNITRGAIRIEKNADLCYLSTVD WSLILDAVSNNYIVGNKPPKECGDLCPGTMEEKPMCEKTTINNEYNYRCWTT NRCQKMCPSTCGKRACTENNECCHPECLGSCSAPDNDTACVACRHYYYAGVC VPACPPNTYRFEGWRCVDRDFCANILSAESSDSEGFVIHDGECMQECPSGFI RNGSQSMYCIPCEGPCPKVCEEEKKTKTIDSVTSAQMLQGCTIFKGNLLINI RRGNNIASELENFMGLIEVVTGYVKIRHSHALVSLSFLKNLRLILGEEQLEG NYSFYVLDNQNLQQLWDWDHRNLTIKAGKMYFAFNPKLCVSEIYRMEEVTGT KGRQSKGDINTRNNGERASCESDVLHFTSTTTSKNRIIITWHRYRPPDYRDL ISFTVYYKEAPFKNVTEYDGQDACGSNSWNMVDVDLPPNKDVEPGILLHGLK PWTQYAVYVKAVTLTMVENDHIRGAKSEILYIRTNASVPSIPLDVLSASNSS SQLIVKWNPPSLPNGNLSYYIVRWQRQPQDGYLYRHNYCSKDKIPIRKYADG TIDIEEVTENPKTEVCGGEKGPCCACPKTEAEKQAEKEEAEYRKVFENFLHN SIFVPRPERKRRDVMQVANTTMSSRSRNTTAADTYNITDPEELETEYPFFES RVDNKERTVISNLRPFTLYRIDIHSCNHEAEKLGCSASNFVFARTMPAEGAD DIPGPVTWEPRPENSIFLKWPEPENPNGLILMYEIKYGSQVEDQRECVSRQE YRKYGGAKLNRLNPGNYTARIQATSLSGNGSWTDPVFFYVQAKTGYENFIHL IIALPVAVLLIVGGLVIMLYVFHRKRNNSRLGNGVLYASVNPEYFSAADVYV PDEWEVAREKITMSRELGQGSFGMVYEGVAKGVVKDEPETRVAIKTVNEAAS MRERIEFLNEASVMKEFNCHHVVRLLGVVSQGQPTLVIMELMTRGDLKSYLR SLRPEMENNPVLAPPSLSKMIQMAGEIADGMAYLNANKFVHRDLAARNCMVA EDFTVKIGDFGMTRDIYETDYYRKGGKGLLPVRWMSPESLKDGVFTTYSDVW SFGVVLWEIATLAEQPYQGLSNEQVLRFVMEGGLLDKPDNCPDMLFELMRMC WQYNPKMRPSFLEIISSIKEEMEPGFREVSFYYSEENKLPEPEELDLEPENM ESVPLDPSASSSSLPLPDRHSGHKAENGPGPGVLVLRASFDERQPYAHMNGG RKNERALPLPQSSTC	IGF-1 R full length (mature) Transmembrane domain: amino acids 906-929 Cytoplasmic domain: amino acids 930-1337 Homo sapiens UniProt No. P08069
55	GSTSGSGKPGSGEGSTKG	Linker artificial
56	GGGGSGGGGS	Linker Artificial
57	cgcaaagtgtgtaacggaataggtattggtgaatttaaagactcactctcca taaatgctacgaatattaaacacttcaaaaactgcacctccatcagtggcga tctccacatcctgccggtggcatttaggggtgactccttcacacatactcct cctctggatccacaggaactggatattctgaaaaccgtaaaggaaatcacag ggtttttgctgattcaggcttggcctgaaaacaggacggacctccatgcctt	tEGFR artificial

	tgagaacctagaaatcatacgcggcaggaccaagcaacatggtcagttttct cttgcagtcgtcagcctgaacataacatccttgggattacgctccctcaagg agataagtgatggagatgtgataatttcaggaaacaaaaatttgtgctatgc aaatacaataaactggaaaaaactgtttgggacctccggtcagaaaaccaaa attataagcaacagaggtgaaaacagctgcaaggccacaggccaggtctgcc atgccttgtgctcccccgagggctgctggggcccggagcccagggactgcgt ctcttgccggaatgtcagccgaggcagggaatgcgtggacaagtgcaacctt ctggagggtgagccaagggagtttgtggagaactctgagtgcatacagtgcc acccagagtgcctgcctcaggccatgaacatcacctgcacaggacggggacc agacaactgtatccagtgtgcccactacattgacggcccccactgcgtcaag acctgcccggcaggagtcatgggagaaaacaacaccctggtctggaagtacg cagacgccggccatgtgtgccacctgtgccatccaaactgcacctacggatg cactgggccaggtcttgaaggctgtccaacgaatgggcctaagatcccgtcc atcgccactgggatggtgggggccctcctcttgctgctggtggtggccctgg ggatcggcctcttcatg	
58	SAWSHPQFEK	Streptavidin binding peptide, Strep-tag® II artificial
59	GGGSGGGS	Linker artificial
60	GGGGS	Linker artificial
61	GGGS	Linker artificial
62	GGGGSGGGGSGGGGS	Linker artificial
63	His-Asn-His-Arg-His-Lys-His-Gly-Gly-Gly-Cys	MAT tag artificial
64	MRPSGTAGAALLALLAALCPASRALEEKKVCQGTSNKLTQLGTFEDHFLSLQ RMFNNCEVVLGNLEITYVQRNYDLSFLKTIQEVAGYVLIALNTVERIPLENL QIIRGNMYYENSYALAVLSNYDANKTGLKELPMRNLQEILHGAVRFSNNPAL CNVESIQWRDIVSSDFLSNMSMDFQNHLGSCQKCDPSCPNGSCWGAGEENCQ KLTKIICAQQCSGRCRGKSPSDCCHNQCAAGCTGPRESDCLVCRKFRDEATC KDTCPPLMLYNPTTYQMDVNPEGKYSFGATCVKKCPRNYVVTDHGSCVRACG ADSYEMEEDGVRKCKKCEGPCRKVCNGIGIGEFKDSLSINATNIKHFKNCTS ISGDLHILPVAFRGDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTD LHAFENLEIIRGRTKQHGQFSLAVVSLNITSLGLRSLKEISDGDVIISGNKN LCYANTINWKKLFGTSGQKTKIISNRGENSCKATGQVCHALCSPEGCWGPEP RDCVSCRNVSRGRECVDKCNLLEGEPREFVENSECIQCHPECLPQAMNITCT GRGPDNCIQCAHYIDGPHCVKTCPAGVMGENNTLVWKYADAGHVCHLCHPNC TYGCTGPGLEGCPTNGPKIPSIATGMVGALLLLLVVALGIGLFMRRRHIVRK RTLRRLLQERELVEPLTPSGEAPNQALLRILKETEFKKIKVLGSGAFGTVYK GLWIPEGEKVKIPVAIKELREATSPKANKEILDEAYVMASVDNPHVCRLLGI CLTSTVQLITQLMPFGCLLDYVREHKDNIGSQYLLNWCVQIAKGMNYLEDRR LVHRDLAARNVLVKTPQHVKITDFGLAKLLGAEEKEYHAEGGKVPIKWMALE SILHRIYTHQSDVWSYGVTVWELMTFGSKPYDGIPASEISSILEKGERLPQP PICTIDVYMIMVKCWMIDADSRPKFRELIIEFSKMARDPQRYLVIQGDERMH LPSPTDSNFYRALMDEEDMDDVVDADEYLIPQQGFFSSPSTSRTPLLSSLSA TSNNSTVACIDRNGLQSCPIKEDSFLQRYSSDPTGALTEDSIDDTFLPVPEY INQSVPKRPAGSVQNPVYHNQPLNPAPSRDPHYQDPHSTAVGNPEYLNTVQP TCVNSTFDSPAHWAQKGSHQISLDNPDYQQDFFPKEAKPNGIFKGSTAENAE	HER1/ErbB1/EGFR Full Length (precursor) Signal peptide: amino acids 1-24 Extracellular domain: amino acids 25-645 Transmembrane domain: amino acids 646-668 Cytoplasmic domain: amino acids 669-1210 Homo sapiens UniProt No.

	YLRVAPQSSEFIGA	P00533
65	MELAALCRWGLLLALLPPGAASTQVCTGTDMKLRLPASPETHLDMLRHLYQG CQVVQGNLELTYLPTNASLSFLQDIQEVQGYVLIAHNQVRQVPLQRLRIVRG TQLFEDNYALAVLDNGDPLNNTTPVTGASPGGLRELQLRSLTEILKGGVLIQ RNPQLCYQDTILWKDIFHKNNQLALTLIDTNRSRACHPCSPMCKGSRCWGES SEDCQSLTRTVCAGGCARCKGPLPTDCCHEQCAAGCTGPKHSDCLACLHFNH SGICELHCPALVTYNTDTFESMPNPEGRYTFGASCVTACPYNYLSTDVGSCT LVCPLHNQEVTAEDGTQRCEKCSKPCARVCYGLGMEHLREVRAVTSANIQEF AGCKKIFGSLAFLPESFDGDPASNTAPLQPEQLQVFETLEEITGYLYISAWP DSLPDLSVFQNLQVIRGRILHNGAYSLTLQGLGISWLGLRSLRELGSGLALI HHNTHLCFVHTVPWDQLFRNPHQALLHTANRPEDECVGEGLACHQLCARGHC WGPGPTQCVNCSQFLRGQECVEECRVLQGLPREYVNARHCLPCHPECQPQNG SVTCFGPEADQCVACAHYKDPPFCVARCPSGVKPDLSYMPIWKFPDEEGACQ PCPINCTHSCVDLDDKGCPAEQRASPLTSIISAVVGILLVVVLGVVFGILIK RRQQKIRKYTMRRLLQETELVEPLTPSGAMPNQAQMRILKETELRKVKVLGS GAFGTVYKGIWIPDGENVKIPVAIKVLRENTSPKANKEILDEAYVMAGVGSP YVSRLLGICLTSTVQLVTQLMPYGCLLDHVRENRGRLGSQDLLNWCMQIAKG MSYLEDVRLVHRDLAARNVLVKSPNHVKITDFGLARLLDIDETEYHADGGKV PIKWMALESILRRRFTHQSDVWSYGVTVWELMTFGAKPYDGIPAREIPDLLE KGERLPQPPICTIDVYMIMVKCWMIDSECRPRFRELVSEFSRMARDPQRFVV IQNEDLGPASPLDSTFYRSLLEDDDMGDLVDAEEYLVPQQGFFCPDPAPGAG GMVHHRHRSSSTRSGGGDLTLGLEPSEEEAPRSPLAPSEGAGSDVFDGDLGM GAAKGLQSLPTHDPSPLQRYSEDPTVPLPSETDGYVAPLTCSPQPEYVNQPD VRPQPPSPREGPLPAARPAGATLERPKTLSPGKNGVVKDVFAFGGAVENPEY LTPQGGAAPQPHPPPAFSPAFDNLYYWDQDPPERGAPPSTFKGTPTAENPEY LGLDVPV	HER2/neu/ErbB2 Full Length (precursor) Signal peptide: amino acids 1-22 Extracellular domain: amino acids 23-652 Transmembrane domain: amino acids 653-675 Cytoplasmic domain: amino acids 676-1255 Homo sapiens UniProt No. P04626
66	MRANDALQVLGLLFSLARGSEVGNSQAVCPGTLNGLSVTGDAENQYQTLYKL YERCEVVMGNLEIVLTGHNADLSFLQWIREVTGYVLVAMNEFSTLPLPNLRV VRGTQVYDGKFAIFVMLNYNTNSSHALRQLRLTQLTEILSGGVYIEKNDKLC HMDTIDWRDIVRDRDAEIVVKDNGRSCPPCHEVCKGRCWGPGSEDCQTLTKT ICAPQCNGHCFGPNPNQCCHDECAGGCSGPQDTDCFACRHFNDSGACVPRCP QPLVYNKLTFQLEPNPHTKYQYGGVCVASCPHNFVVDQTSCVRACPPDKMEV DKNGLKMCEPCGGLCPKACEGTGSGSRFQTVDSSNIDGFVNCTKILGNLDFL ITGLNGDPWHKIPALDPEKLNVFRTVREITGYLNIQSWPPHMHNFSVFSNLT TIGGRSLYNRGFSLLIMKNLNVTSLGFRSLKEISAGRIYISANRQLCYHHSL NWTKVLRGPTEERLDIKHNRPRRDCVAEGKVCDPLCSSGGCWGPGPGQCLSC RNYSRGGVCVTHCNFLNGEPREFAHEAECFSCHPECQPMEGTATCNGSGSDT CAQCAHFRDGPHCVSSCPHGVLGAKGPIYKYPDVQNECRPCHENCTQGCKGP ELQDCLGQTLVLIGKTHLTMALTVIAGLVVIFMMLGGTFLYWRGRRIQNKRA MRRYLERGESIEPLDPSEKANKVLARIFKETELRKLKVLGSGVFGTVHKGVW IPEGESIKIPVCIKVIEDKSGRQSFQAVTDHMLAIGSLDHAHIVRLLGLCPG SSLQLVTQYLPLGSLLDHVRQHRGALGPQLLLNWGVQIAKGMYYLEEHGMVH RNLAARNVLLKSPSQVQVADFGVADLLPPDDKQLLYSEAKTPIKWMALESIH FGKYTHQSDVWSYGVTVWELMTFGAEPYAGLRLAEVPDLLEKGERLAQPQIC TIDVYMVMVKCWMIDENIRPTFKELANEFTRMARDPPRYLVIKRESGPGIAP GPEPHGLTNKKLEEVELEPELDLDLDLEAEEDNLATTTLGSALSLPVGTLNR PRGSQSLLSPSSGYMPMNQGNLGESCQESAVSGSSERCPRPVSLHPMPRGCL ASESSEGHVTGSEAELQEKVSMCRSRSRSRSPRPRGDSAYHSQRHSLLTPVT PLSPPGLEEEDVNGYVMPDTHLKGTPSSREGTLSSVGLSSVLGTEEEDEDEE YEYMNRRRRHSPPHPPRPSSLEELGYEYMDVGSDLSASLGSTQSCPLHPVPI MPTAGTTPDEDYEYMNRQRDGGGPGGDYAAMGACPASEQGYEEMRAFQGPGH QAPHVHYARLKTLRSLEATDSAFDNPDYWHSRLFPKANAQRT	HER3/ErbB3 Full Length (precursor) Signal peptide: amino acids 1-19 Extracellular domain: amino acids 20-643 Transmembrane domain: amino acids 644-664 Cytoplasmic domain: amino acids 665-1342 Homo sapiens UniProt No. P21860
67	MKPATGLWVWVSLLVAAGTVQPSDSQSVCAGTENKLSSLSDLEQQYRALRKY YENCEVVMGNLEITSIEHNRDLSFLRSVREVTGYVLVALNQFRYLPLENLRI IRGTKLYEDRYALAIFLNYRKDGNFGLQELGLKNLTEILNGGVYVDQNKFLC YADTIHWQDIVRNPWPSNLTLVSTNGSSGCGRCHKSCTGRCWGPTENHCQTL TRTVCAEQCDGRCYGPYVSDCCHRECAGGCSGPKDTDCFACMNFNDSGACVT QCPQTFVYNPTTFQLEHNFNAKYTYGAFCVKKCPHNFVVDSSSCVRACPSSK MEVEENGIKMCKPCTDICPKACDGIGTGSLMSAQTVDSSNIDKFINCTKING	HER4/ErbB4 Full Length (precursor) Signal peptide: amino acids 1-25

	NLIFLVTGIHGDPYNAIEAIDPEKLNVFRTVREITGFLNIQSWPPNMTDFSV FSNLVTIGGRVLYSGLSLLILKQQGITSLQFQSLKEISAGNIYITDNSNLCY YHTINWTTLFSTINQRIVIRDNRKAENCTAEGMVCNHLCSSDGCWGPGPDQC LSCRRFSRGRICIESCNLYDGEFREFENGSICVECDPQCEKMEDGLLTCHGP GPDNCTKCSHFKDGPNCVEKCPDGLQGANSFIFKYADPDRECHPCHPNCTQG CNGPTSHDCIYYPWTGHSTLPQHARTPLIAAGVIGGLFILVIVGLTFAVYVR RKSIKKKRALRRFLETELVEPLTPSGTAPNQAQLRILKETELKRVKVLGSGA FGTVYKGIWVPEGETVKIPVAIKILNETTGPKANVEFMDEALIMASMDHPHL VRLLGVCLSPTIQLVTQLMPHGCLLEYVHEHKDNIGSQLLLNWCVQIAKGMM YLEERRLVHRDLAARNVLVKSPNHVKITDFGLARLLEGDEKEYNADGGKMPI KWMALECIHYRKFTHQSDVWSYGVTIWELMTFGGKPYDGIPTREIPDLLEKG ERLPQPPICTIDVYMVMVKCWMIDADSRPKFKELAAEFSRMARDPQRYLVIQ GDDRMKLPSPNDSKFFQNLLDEEDLEDMMDAEEYLVPQAFNIPPPIYTSRAR IDSNRSEIGHSPPPAYTPMSGNQFVYRDGGFAAEQGVSVPYRAPTSTIPEAP VAQGATAEIFDDSCCNGTLRKPVAPHVQEDSSTQRYSADPTVFAPERSPRGE LDEEGYMTPMRDKPKQEYLNPVEENPFVSRRKNGDLQALDNPEYHNASNGPP KAEDEYVNEPLYLNTFANTLGKAEYLKNNILSMPEKAKKAFDNPDYWNHSLP PRSTLQHPDYLQEYSTKYFYKQNGRIRPIVAENPEYLSEFSLKPGTVLPPPP YRHRNTVV	Extracellular domain: amino acids 26-651 Transmembrane domain: amino acids 652-675 Cytoplasmic domain: amino acids 676-1308 Homo sapiens UniProt No. Q15303
68	MKAPAVLAPGILVLLFTLVQRSNGECKEALAKSEMNVNMKYQLPNFTAETPI QNVILHEHHIFLGATNYIYVLNEEDLQKVAEYKTGPVLEHPDCFPCQDCSSK ANLSGGVWKDNINMALVVDTYYDDQLISCGSVNRGTCQRHVFPHNHTADIQS EVHCIFSPQIEEPSQCPDCVVSALGAKVLSSVKDRFINFFVGNTINSSYFPD HPLHSISVRRLKETKDGFMFLTDQSYIDVLPEFRDSYPIKYVHAFESNNFIY FLTVQRETLDAQTFHTRIIRFCSINSGLHSYMEMPLECILTEKRKKRSTKKE VFNILQAAYVSKPGAQLARQIGASLNDDILFGVFAQSKPDSAEPMDRSAMCA FPIKYVNDFFNKIVNKNNVRCLQHFYGPNHEHCFNRTLLRNSSGCEARRDEY RTEFTTALQRVDLFMGQFSEVLLTSISTFIKGDLTIANLGTSEGRFMQVVVS RSGPSTPHVNFLLDSHPVSPEVIVEHTLNQNGYTLVITGKKITKIPLNGLGC RHFQSCSQCLSAPPFVQCGWCHDKCVRSEECLSGTWTQQICLPAIYKVFPNS APLEGGTRLTICGWDFGFRRNNKFDLKKTRVLLGNESCTLTLSESTMNTLKC TVGPAMNKHFNMSIIISNGHGTTQYSTFSYVDPVITSISPKYGPMAGGTLLT LTGNYLNSGNSRHISIGGKTCTLKSVSNSILECYTPAQTISTEFAVKLKIDL ANRETSIFSYREDPIVYEIHPTKSFISGGSTITGVGKNLNSVSVPRMVINVH EAGRNFTVACQHRSNSEIICCTTPSLQQLNLQLPLKTKAFFMLDGILSKYFD LIYVHNPVFKPFEKPVMISMGNENVLEIKGNDIDPEAVKGEVLKVGNKSCEN IHLHSEAVLCTVPNDLLKLNSELNIEWKQAISSTVLGKVIVQPDQNFTGLIA GVVSISTALLLLLGFFLWLKKRKQIKDLGSELVRYDARVHTPHLDRLVSARS VSPTTEMVSNESVDYRATFPEDQFPNSSQNGSCRQVQYPLTDMSPILTSGDS DISSPLLQNTVHIDLSALNPELVQAVQHVVIGPSSLIVHFNEVIGRGHFGCV YHGTLLDNDGKKIHCAVKSLNRITDIGEVSQFLTEGIIMKDFSHPNVLSLLG ICLRSEGSPLVVLPYMKHGDLRNFIRNETHNPTVKDLIGFGLQVAKGMKYLA SKKFVHRDLAARNCMLDEKFTVKVADFGLARDMYDKEYYSVHNKTGAKLPVK WMALESLQTQKFTTKSDVWSFGVLLWELMTRGAPPYPDVNTFDITVYLLQGR RLLQPEYCPDPLYEVMLKCWHPKAEMRPSFSELVSRISAIFSTFIGEHYVHV NATYVNVKCVAPYPSLLSSEDNADDEVDTRPASFWETS	HGFR/c-Met Full Length (precursor) Signal peptide: amino acids 1-24 Extracellular domain: amino acids 25-932 Transmembrane domain: amino acids 933-955 Cytoplasmic domain: amino acids 956-1390 Homo sapiens UniProt No. P08581
69	MKSGSGGGSPTSLWGLLFLSAALSLWPTSGEICGPGIDIRNDYQQLKRLENC TVIEGYLHILLISKAEDYRSYRFPKLTVITEYLLLFRVAGLESLGDLFPNLT VIRGWKLFYNYALVIFEMTNLKDIGLYNLRNITRGAIRIEKNADLCYLSTVD WSLILDAVSNNYIVGNKPPKECGDLCPGTMEEKPMCEKTTINNEYNYRCWTT NRCQKMCPSTCGKRACTENNECCHPECLGSCSAPDNDTACVACRHYYYAGVC VPACPPNTYRFEGWRCVDRDFCANILSAESSDSEGFVIHDGECMQECPSGFI RNGSQSMYCIPCEGPCPKVCEEEKKTKTIDSVTSAQMLQGCTIFKGNLLINI RRGNNIASELENFMGLIEVVTGYVKIRHSHALVSLSFLKNLRLILGEEQLEG NYSFYVLDNQNLQQLWDWDHRNLTIKAGKMYFAFNPKLCVSEIYRMEEVTGT KGRQSKGDINTRNNGERASCESDVLHFTSTTTSKNRIIITWHRYRPPDYRDL ISFTVYYKEAPFKNVTEYDGQDACGSNSWNMVDVDLPPNKDVEPGILLHGLK PWTQYAVYVKAVTLTMVENDHIRGAKSEILYIRTNASVPSIPLDVLSASNSS SQLIVKWNPPSLPNGNLSYYIVRWQRQPQDGYLYRHNYCSKDKIPIRKYADG	IGF-1 R full length (precursor) Signal peptide: amino acids 1-30 Extracellular domain: amino acids 741-935 Transmembrane domain: amino acids 936-959

	TIDIEEVTENPKTEVCGGEKGPCCACPKTEAEKQAEKEEAEYRKVFENFLHN SIFVPRPERKRRDVMQVANTTMSSRSRNTTAADTYNITDPEELETEYPFFES RVDNKERTVISNLRPFTLYRIDIHSCNHEAEKLGCSASNFVFARTMPAEGAD DIPGPVTWEPRPENSIFLKWPEPENPNGLILMYEIKYGSQVEDQRECVSRQE YRKYGGAKLNRLNPGNYTARIQATSLSGNGSWTDPVFFYVQAKTGYENFIHL IIALPVAVLLIVGGLVIMLYVFHRKRNNSRLGNGVLYASVNPEYFSAADVYV PDEWEVAREKITMSRELGQGSFGMVYEGVAKGVVKDEPETRVAIKTVNEAAS MRERIEFLNEASVMKEFNCHHVVRLLGVVSQGQPTLVIMELMTRGDLKSYLR SLRPEMENNPVLAPPSLSKMIQMAGEIADGMAYLNANKFVHRDLAARNCMVA EDFTVKIGDFGMTRDIYETDYYRKGGKGLLPVRWMSPESLKDGVFTTYSDVW SFGVVLWEIATLAEQPYQGLSNEQVLRFVMEGGLLDKPDNCPDMLFELMRMC WQYNPKMRPSFLEIISSIKEEMEPGFREVSFYYSEENKLPEPEELDLEPENM ESVPLDPSASSSSLPLPDRHSGHKAENGPGPGVLVLRASFDERQPYAHMNGG RKNERALPLPQSSTC	Cytoplasmic domain: amino acids 960-1367 Homo sapiens UniProt No. P08069
70	ESKYGPPCPPCP	spacer (IgG4hinge)
71	GAATCTAAGTACGGACCGCCCTGCCCCCCTTGCCCT	spacer (IgG4hinge)
72	ESKYGPPCPPCPGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGK	Hinge-CH3 spacer
73	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCS VMHEALHNHYTQKSLSLSLGK	Hinge-CH2-CH3 spacer
74	RWPESPKAQASSVPTAQPQAEGSLAKATTAPATTRNTGRGGEEKKKEKEKEE QEERETKTPECPSHTQPLGVYLLTPAVQDLWLRDKATFTCFVVGSDLKDAHL TWEVAGKVPTGGVEEGLLERHSNGSQSQHSRLTLPRSLWNAGTSVTCTLNHP SLPPQRLMALREPAAQAPVKLSLNLLASSDPPEAASWLLCEVSGFSPPNILL MWLEDQREVNTSGFAPARPPPQPGSTTFWAWSVLRVPAPPSPQPATYTCVVS HEDSRTLLNASRSLEVSYVTDH	IgD-hinge-Fc
75	MALPVTALLLPLALLLHA	CD8 alpha signal peptide
76	EGRGSLLTCGDVEENPGP	T2A
77	FWVLVVVGGVLACYSLLVTVAFIIFWV	CD28 (amino acids 153-179 of Accession No. P10747)
78	IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKP FWVLVVVGGVLACYSLLVTVAFIIFWV	CD28 (amino acids 114-179 of Accession No. P10747)
79	RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS	CD28 (amino acids 180-220 of P10747)
80	RSKRSRGGHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS	CD28 (LL to GG)
81	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL	4-1BB (amino acids 214-255 of Q07011.1)
82	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK NPQEGLYN ELQKDKMAEA YSEIGMKGER RRGKGHDGLY QGLSTATKDTYDALHMQALP PR	CD3 zeta
83	RVKFSRSAEPPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK NPQEGLYN ELQKDKMAEA YSEIGMKGER RRGKGHDGLY QGLSTATKDTYDALHMQALP PR	CD3 zeta
84	RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK NPQEGLYN ELQKDKMAEA YSEIGMKGER RRGKGHDGLY QGLSTATKDTYDALHMQALP PR	CD3 zeta
85	GSGATNFSLLKQAGDVEENPGP	P2A
86	ATNFSLLKQAGDVEENPGP	P2A
87	QCTNYALLKLAGDVESNPGP	E2A

88	VKQTLNFDLLKLAGDVESNPGP	F2A
89	PGGG-(SGGGG)5-P- wherein P is proline, G is glycine and S is serine	linker
90	GSADDAKKDAAKKDGKS	linker
91	TGCCACCCTGAGTGTCAGCCCCAGAATGGCTCAGTGACCTGTTTTGGACCGG AGGCTGACCAGTGTGTGGCCTGTGCCCACTATAAGGACCCTCCCTTCTGCGT GGCCCGCTGCCCCAGCGGTGTGAAACCTGACCTCTCCTACATGCCCATCTGG AAGTTTCCAGATGAGGAGGGCGCATGCCAGCCTTGCCCCATCAACTGCACCC ACTCCTGTGTGGACCTGGATGACAAGGGCTGCCCCGCCGAGCAGAGAGCCAG CCCTCTGACGGGTGGAGGAAGCGGAGGTGGCAGCTCCATCATCTCTGCGGTG GTTGGCATTCTGCTGGTCGTGGTCTTGGGGGTGGTCTTTGGGATCCTCATC	Modified HER2t (nt) artificial
92	CHPECQPQNGSVTCFGPEADQCVACAHYKDPPFCVARCPSGVKPDLSYMPIW KFPDEEGACQPCPINCTHSCVDLDDKGCPAEQRASPLTGGGSGGGSSIISAV VGILLVVVLGVVFGILI	Modified HER2t (aa) artificial
93	ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCAT TCCTCCTGATCCCATGCCACCCTGAGTGTCAGCCCCAGAATGGCTCAGTGAC CTGTTTTGGACCGGAGGCTGACCAGTGTGTGGCCTGTGCCCACTATAAGGAC CCTCCCTTCTGCGTGGCCCGCTGCCCCAGCGGTGTGAAACCTGACCTCTCCT ACATGCCCATCTGGAAGTTTCCAGATGAGGAGGGCGCATGCCAGCCTTGCCC CATCAACTGCACCCACTCCTGTGTGGACCTGGATGACAAGGGCTGCCCCGCC GAGCAGAGAGCCAGCCCTCTGACGGGTGGAGGAAGCGGAGGTGGCAGCTCCA TCATCTCTGCGGTGGTTGGCATTCTGCTGGTCGTGGTCTTGGGGGTGGTCTT TGGGATCCTCATC	Modified HERt2 with signal sequence (nt) Artificial
94	MWNLLHETDSAVATARRPRWLCAGALVLAGGFFLLGFLFGWFIKSSNEATNI TPKHNMKAFLDELKAENIKKFLYNFTQIPHLAGTEQNFQLAKQIQSQWKEFG LDSVELAHYDVLLSYPNKTHPNYISIINEDGNEIFNTSLFEPPPPGYENVSD IVPPFSAFSPQGMPEGDLVYVNYARTEDFFKLERDMKINCSGKIVIARYGKV FRGNKVKNAQLAGAKGVILYSDPADYFAPGVKSYPDGWNLPGGGVQRGNILN LNGAGDPLTPGYPANEYAYRRGIAEAVGLPSIPVHPIGYYDAQKLLEKMGGS APPDSSWRGSLKVPYNVGPGFTGNFSTQKVKMHIHSTNEVTRIYNVIGTLRG AVEPDRYVILGGHRDSWVFGGIDPQSGAAVVHEIVRSFGTLKKEGWRPRRTI LFASWDAEEFGLLGSTEWAEENSRLLQERGVAYINADSSIEGNYTLRVDCTP LMYSLVHNLTKELKSPDEGFEGKSLYESWTKKSPSPEFSGMPRISKLGSGND FEVFFQRLGIASGRARYTKNWETNKFSGYPLYHSVYETYELVEKFYDPMFKY HLTVAQVRGGMVFELANSIVLPFDCRDYAVVLRKYADKIYSISMKHPQEMKT YSVSFDSLFSAVKNFTEIASKFSERLQDFDKSNPIVLRMMNDQLMFLERAFI DPLGLPDRPFYRHVIYAPSSHNKYAGESFPGIYDALFDIESKVDPSKAWGEV KRQIYVAAFTVQAAAETLSEVA	PSMA WT (full length)
95	MGNLLHETDSAVATARRPRWLCAGALVLAGGFFLLGFLFGWFIKSSNEATNI TPKHNMKAFLDELKAENIKKFLYNFTQIPHLAGTEQNFQLAKQIQSQWKEFG LDSVELAHYDVLLSYPNKTHPNYISIINEDGNEIFNTSLFEPPPPGYENVSD IVPPFSAFSPQGMPEGDLVYVNYARTEDFFKLERDMKINCSGKIVIARYGKV FRGNKVKNAQLAGAKGVILYSDPADYFAPGVKSYPDGWNLPGGGVQRGNILN LNGAGDPLTPGYPANEYAYRRGIAEAVGLPSIPVHPIGYYDAQKLLEKMGGS APPDSSWRGSLKVPYNVGPGFTGNFSTQKVKMHIHSTNEVTRIYNVIGTLRG AVEPDRYVILGGHRDSWVFGGIDPQSGAAVVHEIVRSFGTLKKEGWRPRRTI LFASWDAEEFGLLGSTEWAEENSRLLQERGVAYINADSSIEGNYTLRVDCTP LMYSLVHNLTKELKSPDEGFEGKSLYESWTKKSPSPEFSGMPRISKLGSGND FEVFFQRLGIASGRARYTKNWETNKFSGYPLYHSVYETYELVEKFYDPMFKY HLTVAQVRGGMVFELANSIVLPFDCRDYAVVLRKYADKIYSISMKHPQEMKT YSVSFDSLFSAVKNFTEIASKFSERLQDFDKSNPIVLRMMNDQLMFLERAFI DPLGLPDRPFYRHVIYAPSSHNKYAGESFPGIYDALFDIESKVDPSKAWGEV KRQIYVAAFTVQAAAETLSEVA	PSMA W2G (full length)
96	atgtggaatctccttcacgaaaccgactcggctgtggccaccgcgcgccgcc cgcgctggctgtgcgctggggcgctggtgctggcgggtggcttctttctcct cggcttcctcttcgggtggtttataaaatcctccaatgaagctactaacatt actccaaagcataatatgaaagcatttttggatgaattgaaagctgagaaca tcaagaagttcttatataatttacacagataccacatttagcaggaacaga acaaaactttcagcttgcaaagcaaattcaatcccagtggaaagaatttggc	PSMA WT (nt)

	ctggattctgttgagctagcacattatgatgtcctgttgtcctacccaaata agactcatcccaactacatctcaataattaatgaagatggaaatgagatttt caacacatcattatttgaaccacctcctccaggatatgaaaatgtttcggat attgtaccacctttcagtgctttctctcctcaaggaatgccagagggcgatc tagtgtatgttaactatgcacgaactgaagacttctttaaattggaacggga catgaaaatcaattgctctgggaaaattgtaattgccagatatgggaaagtt ttcagaggaaataaggttaaaaatgcccagctggcaggggccaaaggagtca ttctctactccgaccctgctgactactttgctcctggggtgaagtcctatcc agatggttggaatcttcctggaggtggtgtccagcgtggaaatatcctaaat ctgaatggtgcaggagaccctctcacaccaggttacccagcaaatgaatatg cttataggcgtggaattgcagaggctgttggtcttccaagtattcctgttca tccaattggatactatgatgcacagaagctcctagaaaaaatgggtggctca gcaccaccagatagcagctggagaggaagtctcaaagtgccctacaatgttg gacctggctttactggaaacttttctacacaaaaagtcaagatgcacatcca ctctaccaatgaagtgacaagaatttacaatgtgataggtactctcagagga gcagtggaaccagacagatatgtcattctgggaggtcaccgggactcatggg tgtttggtggtattgaccctcagagtggagcagctgttgttcatgaaattgt gaggagctttggaacactgaaaaaggaagggtggagacctagaagaacaatt ttgtttgcaagctgggatgcagaagaatttggtcttcttggttctactgagt gggcagaggagaattcaagactccttcaagagcgtggcgtggcttatattaa tgctgactcatctatagaaggaaactacactctgagagttgattgtacaccg ctgatgtacagcttggtacacaacctaacaaaagagctgaaaagccctgatg aaggctttgaaggcaaatctctttatgaaagttggactaaaaaaagtccttc cccagagttcagtggcatgcccaggataagcaaattgggatctggaaatgat tttgaggtgttcttccaacgacttggaattgcttcaggcagagcacggtata ctaaaaattgggaaacaaacaaattcagcggctatccactgtatcacagtgt ctatgaaacatatgagttggtggaaaagttttatgatccaatgtttaaatat cacctcactgtggcccaggttcgaggagggatggtgtttgagctagccaatt ccatagtgctccctttttgattgtcgagattatgctgtagttttaagaaagta tgctgacaaaatctacagtatttctatgaaacatccacaggaaatgaagaca tacagtgtatcatttgattcacttttttctgcagtaaagaattttacagaaa ttgcttccaagttcagtgagagactccaggactttgacaaaagcaacccaat agtattaagaatgatgaatgatcaactcatgtttctggaaagagcatttatt gatccattagggttaccagacaggccttttataggcatgtcatctatgctc caagcagccacaacaagtatgcaggggagtcattcccaggaatttatgatgc tctgtttgatattgaaagcaaagtggacccttccaaggcctggggagaagtg aagagacagatttatgttgcagccttcacagtgcaggcagctgcagagactt tgagtgaagtagcc	
97	ATGTGGAATCTCCTTCATGAAACAGACTCTGCTGTGGCCACAGCCAGAAGAC CCAGATGGCTGTGTGCTGGGGCCCTGGTGCTGGCTGGTGGCTTCTTTCTCCT GGGCTTCCTCTTTGGGTGGTTTATAAAATCCTCCAATGAAGCTACTAACATT ACTCCAAAGCATAATATGAAAGCATTTTTGGATGAATTGAAAGCTGAGAACA TCAAGAAGTTCTTATATAATTTTACACAGATACCACATTTAGCAGGAACAGA ACAAAACTTTCAGCTTGCAAAGCAAATTCAATCCCAGTGGAAAGAATTTGGC CTGGATTCTGTTGAGCTAGCACATTATGATGTCCTGTTGTCCTACCCAAATA AGACTCATCCCAACTACATCTCAATAATTAATGAAGATGGAAATGAGATTTT CAACACATCATTATTTGAACCACCTCCTCCAGGATATGAAAATGTTTCTGAT ATTGTACCACCTTTCAGTGCTTTCTCTCCTCAAGGAATGCCAGAGGGAGATC TAGTGTATGTTAACTATGCAAGAACTGAAGACTTCTTTAAATTGGAAAGGGA CATGAAAATCAATTGCTCTGGGAAAATTGTAATTGCCAGATATGGGAAAGTT TTCAGAGGAAATAAGGTTAAAAATGCCCAGCTGGCAGGGGCCAAAGGAGTCA TTCTCTACTCTGACCCTGCTGACTACTTTGCTCCTGGGGTGAAGTCCTATCC AGATGGTTGGAATCTTCCTGGAGGTGGTGTCCAGAGAGGAAATATCCTAAAT CTGAATGGTGCAGGAGACCCTCTCACACCAGGTTACCCAGCAAATGAATATG CTTATAGGAGAGGAATTGCAGAGGCTGTTGGTCTTCCAAGTATTCCTGTTCA TCCAATTGGATACTATGATGCACAGAAGCTCCTAGAAAAAATGGGTGGCTCA GCACCACCAGATAGCAGCTGGAGAGGAAGTCTCAAAGTGCCCTACAATGTTG GACCTGGCTTTACTGGAAACTTTTCTACACAAAAAGTCAAGATGCACATCCA CTCTACCAATGAAGTGACAAGAATTTACAATGTGATAGGTACTCTCAGAGGA	CpG-free PSMA

	GCAGTGGAACCAGACAGATATGTCATTCTGGGAGGTCACAGGGACTCATGGG TGTTTGGTGGTATTGACCCTCAGAGTGGAGCAGCTGTTGTTCATGAAATTGT GAGGAGCTTTGGAACACTGAAAAAGGAAGGGTGGAGACCTAGAAGAACAATT TTGTTTGCAAGCTGGGATGCAGAAGAATTTGGTCTTCTTGGTTCTACTGAGT GGGCAGAGGAGAATTCAAGACTCCTTCAAGAGAGGGGAGTGGCTTATATTAA TGCTGACTCATCTATAGAAGGAAACTACACTCTGAGAGTTGATTGTACACCC CTGATGTACAGCTTGGTACACAACCTAACAAAAGAGCTGAAAAGCCCTGATG AAGGCTTTGAAGGCAAATCTCTTTATGAAAGTTGGACTAAAAAAAGTCCTTC CCCAGAGTTCAGTGGCATGCCCAGGATAAGCAAATTGGGATCTGGAAATGAT TTTGAGGTGTTCTTCCAAAGACTTGGAATTGCTTCAGGCAGAGCAAGGTATA CTAAAAATTGGGAAACAAACAAATTCAGTGGCTATCCACTGTATCACAGTGT CTATGAAACATATGAGTTGGTGGAAAAGTTTTATGATCCAATGTTTAAATAT CACCTCACTGTGGCCCAGGTTAGAGGAGGGATGGTGTTTGAGCTAGCCAATT CCATAGTGCTCCCTTTTGATTGTAGAGATTATGCTGTAGTTTTAAGAAAGTA TGCTGACAAAATCTACAGTATTTCTATGAAACATCCACAGGAAATGAAGACA TACAGTGTATCATTTGATTCACTTTTTTCTGCAGTAAAGAATTTTACAGAAA TTGCTTCCAAGTTCAGTGAGAGACTCCAGGACTTTGACAAAAGCAACCCAAT AGTATTAAGAATGATGAATGATCAACTCATGTTTCTGGAAAGAGCATTTATT GATCCATTAGGGTTACCAGACAGGCCTTTTTATAGGCATGTCATCTATGCTC CAAGCAGCCACAACAAGTATGCAGGGGAGTCATTCCCAGGAATTTATGATGC TCTGTTTGATATTGAAAGCAAAGTGGACCCTTCCAAGGCCTGGGGAGAAGTG AAGAGACAGATTTATGTTGCAGCCTTCACAGTGCAGGCAGCTGCAGAGACTT TGAGTGAAGTAGCCTAA	
98	PLGLWA	cleavable linker
99	GFLG	linker
100	KLAKLAKKLAKLAK	peptide toxin

CLAIMS

WHAT IS CLAIMED:

1. A cell surface conjugate, comprising:

(a) a cell surface molecule that lacks a functional intracellular signaling domain and/or is not capable of mediating intracellular signaling; and

(b) at least one agent linked to the cell surface molecule, the agent being capable of binding a streptavidin, a streptavidin analog or a streptavidin mutein.

2. The cell surface conjugate of claim 1, wherein the agent exhibits a binding affinity for streptavidin or a streptavidin mutein with an equilibrium dissociation constant (K_D) of from or from about 10^{-4} M to or to about 10^{-10} M.

3. A cell surface conjugate, comprising:

(a) a cell surface molecule that lacks a functional intracellular signaling domain and/or is not capable of mediating intracellular signaling; and

(b) at least one agent linked to the cell surface molecule and being capable of reversibly binding to a reagent and/or capable of being competed in the presence of a competition substance, wherein the agent is a peptide of less than 50 amino acids in length.

4. The cell surface conjugate of claim 3, wherein the agent exhibits a binding affinity for the reagent with an equilibrium dissociation constant (K_D) of from or from about 10^{-4} M to or to about 10^{-10} M.

5. The cell surface conjugate of claim 3 or claim 4, wherein the reagent is a streptavidin, a streptavidin analog or a streptavidin mutein.

6. A cell surface conjugate, comprising:

(a) a cell surface molecule that lacks a functional intracellular signaling domain and/or is not capable of mediating intracellular signaling; and

(b) at least one agent linked to the cell surface molecule, the agent having a binding affinity for a reagent with an equilibrium dissociation constant (K_D) of more than 10^{-7} M or an equilibrium association constant (K_A) of less than 10^7 M^{-1}.

7. The cell surface conjugate of claim 6, wherein the reagent is a streptavidin, a streptavidin analog or a streptavidin mutein.

8. The cell surface conjugate of any of claims 1-7, wherein the cell surface molecule comprises a transmembrane domain and/or is capable of being expressed on the surface of the cell.

9. The cell surface conjugate of any of claims 1-8, wherein the cell surface molecule is modified compared to a reference cell surface molecule, optionally wherein the reference cell surface molecule is a cell surface receptor comprising an intracellular signaling domain.

10. The cell surface conjugate of claim 9, wherein the modified cell surface molecule exhibits altered cellular internalization, enzymatic activity and/or ligand binding, compared to the reference cell surface molecule.

11. A cell surface conjugate, comprising:

(a) a cell surface molecule that is modified compared to a reference cell surface molecule, wherein the modified cell surface molecule exhibits altered cellular internalization, enzymatic activity and/or ligand binding, compared to the reference cell surface molecule; and

(b) at least one agent linked to the cell surface molecule, the agent being capable of binding a streptavidin, a streptavidin analog or a streptavidin mutein.

12. The cell surface conjugate of claim 11, wherein the cell surface molecule lacks a functional intracellular signaling domain and/or is not capable of mediating intracellular signaling.13. A cell surface conjugate, comprising:

(a) a cell surface molecule comprising a prostate-specific membrane antigen (PSMA) or a modified cell surface molecule thereof; and

(b) at least one agent linked to the cell surface molecule, the agent being capable of binding a streptavidin, a streptavidin analog or a streptavidin mutein.

14. The cell surface conjugate of claim 13, wherein:

the modified cell surface molecule lacks a functional intracellular signaling domain and/or is not capable of mediating intracellular signaling; and/or

the modified cell surface molecule is modified compared to a reference cell surface molecule, wherein the modified cell surface molecule exhibits altered cellular internalization, enzymatic activity and/or ligand binding, compared to the reference cell surface molecule.

15. The cell surface conjugate of any of claims 11-14, wherein the cell surface molecule comprises a transmembrane domain and/or is capable of being expressed on the surface of the cell.

16. The cell surface conjugate of any of claims 11-15, wherein the agent exhibits a binding affinity for a streptavidin, a streptavidin analog or a streptavidin mutein with an equilibrium dissociation constant (K_D) of from or from about 10^{-4} M to or to about 10^{-10} M.

17. The cell surface conjugate of any of claims 1-16, wherein the binding of the agent to the reagent is reversible and/or capable of being competed in the presence of a competition substance.

18. The cell surface conjugate of claim 17, wherein the competition substance exhibits a higher binding affinity for the reagent than the binding affinity of the agent for the reagent.

19. The cell surface conjugate of claim 18, wherein:

the competition substance exhibits a binding affinity for the reagent with an equilibrium dissociation constant (K_D) of between or about between 10^{-10} M and 10^{-14} M; and/or

the agent exhibits a binding affinity for the reagent with an equilibrium dissociation constant (K_D) of more than 10^{-10} M.

20. The cell surface conjugate of any of claims 1, 2, 5, 7-20, wherein the binding of the agent to the streptavidin, streptavidin analog or streptavidin mutein is reversible and/or capable of being competed in the presence of biotin, a biotin analog or a biologically active fragment thereof.

21. The cell surface conjugate of any of claims 1-20, wherein the at least one agent is linked directly to the cell surface molecule.

22. The cell surface conjugate of any of claims 1-20, wherein the at least one agent is linked indirectly to the cell surface molecule via at least one linker.

23. The cell surface conjugate of any of claims 1-22, wherein the at least one agent comprises from or from about 1 to 4 or 1 to 2 agents.

24. The cell surface conjugate of any of claims 1-23, wherein the at least one agent comprises only one agent.

25. The cell surface conjugate of any of claims 1-24, wherein the agent is linked to an extracellular portion or region of the cell surface molecule, optionally wherein the extracellular portion or region is at the N-terminus or C-terminus of the cell surface molecule.

26. The cell surface conjugate of any of claims 1-25, wherein the agent is linked at the N-terminus of the cell surface molecule.

27. The cell surface conjugate of any of claims 1-26, wherein the agent is linked at the C-terminus of the cell surface molecule.

28. A cell surface conjugate, comprising a cell surface molecule linked, at an extracellular portion or region of the cell surface molecule, to an agent, the agent being capable of binding a reagent that is or comprises streptavidin or a streptavidin mutein, optionally wherein the extracellular portion or region is at the N-terminus or C-terminus of the cell surface molecule.

29. A cell surface conjugate, comprising a cell surface molecule linked, at an extracellular portion or region of the cell surface molecule, to an agent, the agent being capable of reversibly binding to a reagent, wherein the agent is a peptide of less than 50 amino acids in length optionally wherein the extracellular portion or region is at the N-terminus or C-terminus of the cell surface molecule.

30. The cell surface conjugate of claim 28 or claim 29, wherein the agent exhibits a binding affinity with an equilibrium dissociation constant (K_D) of from or from about 10^{-4} M to or to about 10^{-10} M.

31. A cell surface conjugate, comprising a cell surface molecule linked, at an extracellular portion or region of the cell surface molecule, to an agent, wherein the agent exhibits a binding affinity for a reagent with an equilibrium dissociation constant (K_D) of more than 10^{-7} M or an equilibrium association constant (K_A) of less than 10^{7} M^{-1} optionally wherein the extracellular portion or region is at the N-terminus or C-terminus of the cell surface molecule.

32. The cell surface conjugate of any of claims 28-31, wherein the agent is linked at the N-terminus of the cell surface molecule.

33. The cell surface conjugate of any of claims 28-31, wherein the agent is linked at the C-terminus of the cell surface molecule.

34. The cell surface conjugate of any of claims 28-33, wherein the reagent is or comprises a streptavidin, a streptavidin analog or a streptavidin mutein.

35. The cell surface conjugate of any of claims 28-34, wherein the binding of the agent to the reagent is reversible and/or capable of being competed in the presence of a competition substance.

36. The cell surface conjugate of claim 35, wherein the competition substance exhibits a higher binding affinity for the reagent than the binding affinity of the agent for the reagent.

37. The cell surface conjugate of claim36, wherein:

the competition substance exhibits a binding affinity for the reagent with an equilibrium dissociation constant (K_D) of between or about between 10^{-10} M and 10^{-14} M; and/or

the agent exhibits a binding affinity for the reagent with an equilibrium dissociation constant (K_D) of more than 10^{-10} M.

38. The cell surface conjugate of any of claims 28, 34-37, wherein the binding of the agent to the streptavidin, streptavidin analog or streptavidin mutein is reversible and/or capable of being competed in the presence of biotin or a biotin analog.

39. The cell surface conjugate of any of claims 28-38, wherein the agent is linked directly to the cell surface molecule.

40. The cell surface conjugate of any of claims 28-38, wherein the agent is linked indirectly to the cell surface molecule via at least one linker.

41. The cell surface conjugate of any of claims 28-40, wherein the cell surface molecule is linked to only one agent.

42. The cell surface conjugate of any of claims 1-41, wherein the cell surface molecule is not a chimeric antigen receptor (CAR).

43. The cell surface conjugate of any of claims 28-30, wherein the cell surface molecule is modified compared to a reference cell surface molecule.

44. The cell surface conjugate of claim 43, wherein the modified cell surface molecule lacks a functional intracellular signaling domain and/or is not capable of mediating intracellular signaling; and/or the modified cell surface molecule exhibits altered cellular internalization, enzymatic activity and/or ligand binding, compared to the reference cell surface molecule.

45. The cell surface conjugate of claim 43 or claim 44, wherein the reference cell surface molecule is a native mammalian cell surface molecule.

46. The cell surface conjugate of any of claims 1-45, wherein the cell surface molecule comprises an epitope capable of being recognized by an antibody or antigen-binding fragment thereof.

47. The cell surface conjugate of any of claims 1-33 that is a fusion protein.

48. The cell surface conjugate of any of claims 1, 2, 5, 7-28 and and 34-47, wherein the streptavidin analog or mutein comprises the amino acid sequence Val^{44}-Thr^{45}-Ala^{46}-Arg^{47} or Ile^{44}-Gly^{45}-Ala^{46}-Arg^{47} at sequence positions corresponding to positions 44 to 47 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO:1.

49. The cell surface conjugate of any of claims 1, 2, 5, 7-28 and and 34-48, wherein the streptavidin analog or mutein comprises:

a) the sequence of amino acids set forth in any of SEQ ID NOS: 3-6, 27 and 28;

b) a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS:3-6, 27 and 28 and contains the amino acid sequence corresponding to Val^{44}-Thr^{45}-Ala^{46}-Arg^{47} or Ile^{44}-Gly^{45}-Ala^{46}-Arg^{47} and that reversibly binds to the agent; or

c) a functional fragment of a) or b) that reversibly binds to the agent.

50. The cell surface conjugate of claim 48 or claim 49, wherein the streptavidin analog or mutein further comprises an amino acid replacement or replacements at a position corresponding to 117, 120 and/or 121 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO:1.

51. The cell surface conjugate of claim 50, wherein:

the amino acid replacement or replacements are selected from among Glu^{117}, Asp^{117}, Arg^{117}, Ser^{120}, Ala^{120}, Gly^{120}, Trp^{121}, Tyr^{121} or Phe^{121}; or

the amino acid replacement or replacements are selected from one or more of Glu^{117}, Gly^{120} or Tyr^{121}; or

the amino acid replacements are selected from Glu^{117}, Gly^{120} or Tyr^{121}.

52. The cell surface conjugate of any of claims any of claims 1, 2, 5, 7-28 and and 34-51, wherein the streptavidin analog or mutein comprises:

a) the sequence of amino acids set forth in SEQ ID NO: 27 or 28;

b) a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NOS: 27 or 28 and contains the amino acid sequence corresponding to Val^{44}, Thr^{45}, Ala^{46}, Arg^{47}, Glu^{117}, Gly^{120} and Tyr^{121} and reversibly binds to the agent; or

c) a functional fragment of a) or b) that reversibly binds to the agent.

53. The cell surface conjugate of any of claims 3-5, 17-19 and 35-37, wherein the competition substance is or comprises biotin, a biotin analog or a biologically active fragment thereof.

54. The cell surface conjugate of any of claims 1-53, wherein the agent is an affinity tag.

55. The cell surface conjugate of any of claims 3, 4, 6, 8-10, 17-19, 21-27, 29-33, 35-37, 39-47 and 54, wherein the agent is or comprises a Strep tag, His tag, Flag tag, Xpress tag, Avi tag, Calmodulin tag, Polyglutamate tag, HA tag, Myc tag, Nus tag, S tag, X tag, SBP tag, Softag, V5 tag, CBP, GST, MBP, GFP, Thioredoxin tag, or any combination thereof.

56. The cell surface conjugate of any of claims 1-55, wherein the agent is or comprises one or more streptavidin binding peptide, which optionally is a Strep tag.

57. The cell surface conjugate of claim 56, wherein the streptavidin binding peptide comprises the sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8) or Trp-Arg-His-Pro-Gln-Phe-Gly-Gly (SEQ ID NO:7).

58. The cell surface conjugate of claim 56 or claim 57, wherein the agent comprises the sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_3$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 17), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$-Trp-Ser-His-

Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 18) and Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$Gly-Gly-Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 19).

59. The cell surface conjugate of any of claims 9-58, wherein the reference cell surface molecule is a cell surface receptor, ligand, glycoprotein, cell adhesion molecule, antigen, integrin or cluster of differentiation (CD).

60. The cell surface conjugate of claim 59, wherein the reference cell surface molecule is a cell surface receptor.

61. The cell surface conjugate of any of claims 9-60, wherein the reference cell surface molecule is selected from EpCAM, VEGFR, integrin, optionally integrins αvβ3, α4, αIIbβ3, α4β7, α5β1, αvβ3 or αv, a member of the TNF receptor superfamily, optionally TRAIL-R1 or TRAIL-R2, a member of the epidermal growth factor receptor family, PDGF Receptor, interferon receptor, folate receptor, GPNMB, ICAM-1, HLA-DR, CEA, CA-125, MUC1, TAG-72, IL-6 receptor, 5T4, GD2, GD3, prostate-specific membrane antigen (PSMA) or a clusters of differentiation cell surface molecule, optionally CD2, CD3, CD4, CD5, CD11, CD11a/LFA-1, CD15, CD18/ITGB2, CD19, CD20, CD22, CD23/IgE Receptor, CD25, CD28, CD30, CD33, CD38, CD40, CD41, CD44, CD51, CD52, CD62L, CD74, CD80, CD125, CD147/basigin, CD152/CTLA-4, CD154/CD40L, CD195/CCR5 and CD319/SLAMF7.

62. The cell surface conjugate of any of claims 9-61, wherein the reference cell surface molecule is a member of the epidermal growth factor receptor family.

63. The cell surface conjugate of any of claims 9-62, wherein the reference cell surface molecule is an epidermal growth factor receptor (EGFR), an erbB-2 receptor tyrosine-protein kinase (errb2, HER2), an erbB-3 receptor tyrosine-protein kinase, an erbB-4 receptor tyrosine-protein kinase, a hepatocyte growth factor receptor (HGFR/c-MET) or an insulin-like growth factor receptor-1 (IGF-1 R).

64. The cell surface conjugate of any of claims 9-63, wherein the reference cell surface molecule is human.

65. The cell surface conjugate of any of claims 9-64, wherein the modified cell surface molecule lacks a functional intracellular signaling domain and/or is not capable of mediating intracellular signaling.

66. The cell surface conjugate of any of claims 9-65, wherein the modified cell surface molecule is truncated to lack all or a portion of the intracellular signaling domain or trafficking domain compared to the reference cell surface molecule.

67. The cell surface conjugate of any of claims 9-66, wherein the modified cell surface molecule exhibits altered cellular internalization, enzymatic activity and/or ligand binding, compared to the reference cell surface molecule.

68. The cell surface conjugate of any of claims 9-67, wherein the modified cell surface molecule comprises one or more extracellular domains of the reference cell surface molecule.

69. The cell surface conjugate of any of claims 9-68, wherein the modified cell surface molecule is capable of binding to a native ligand and/or substrate of the reference cell surface molecule.

70. The cell surface conjugate of any of claims 9-68, wherein the modified cell surface molecule is reduced for or does not bind the native ligand and/or substrate of the reference cell surface molecule.

71. The cell surface conjugate of claim 70, wherein the modified cell surface molecule comprises at least one extracellular domain of the reference cell surface molecule but lacks one or more other extracellular domains recognized by the native ligand and/or substrate of the reference cell surface molecule.

72. The cell surface conjugate of claim 71, wherein the at least one extracellular domain comprises an epitope recognized by an antibody or antigen-binding fragment thereof that specifically binds the reference cell surface molecule.

73. The cell surface conjugate of any of claims 46-72, wherein the antibody or antigen-binding fragment is selected from AMG-102, AMG-479, BIIB022OA-5D5, CP-751,871, IMC-Al2, R1507, 3F8, abagovomab, abciximab, adecatumumab, afutuzumab, alemtuzumab, altumomab pentetate, anatumomab mafenatox, apolizumab, arcitumomab, aselizumab, atlizumab (=tocilizumab), basiliximab, bectumomab, benralizumab, besilesomab, bivatuzumab mertansine, blinatumomab, brentuximab vedotin, cantuzumab mertansine, capromab pendetide, catumaxomab, CC49, cedelizumab, celmoleukin, cetuximab, cixutumumab, clenoliximab, clivatuzumab tetraxetan, CNTO-95, conatumumab, dacetuzumab, daclizumab, daratumumab, detumomab, ecromeximab, ertumaxomab, edrecolomab, efalizumab, elotuzumab, enlimomab pegol, epitumomab cituxetan, epratuzumab, erlizumab, etaracizumab, fanolesomab, faralimomab, farletuzumab, figitumumab, galiximab, gavilimomab, gemtuzumab ozogamicin, glembatumumab vedotin, gomiliximab, ibalizumab, ibritumomab tiuxetan, igovomab, intetumumab, iratumumab, inolimomab, inotuzumab ozogamicin, ipilimumab, keliximab, labetuzumab, lintuzumab, lexatumumab, lucatumumab, lumiliximab, mapatumumab, maslimomab, matuzumab, milatuzumab, minretumomab, mitumomab, muromonab-CD3, naptumomab estafenatox, natalizumab, necitumumab, ocrelizumab, odulimomab, ofatumumab, olaratumab, oportuzumab monatox, oregovomab, otelixizumab, panitumumab, pertuzumab, pemtumomab, priliximab, PRO 140, nimotuzumab, robatumumab, rituximab, rovelizumab, ruplizumab, satumomab pendetide, siplizumab, sontuzumab, tadocizumab, taplitumomab paptox, teneliximab, teplizumab, TGN1412, ticilimumab (=tremelimumab), tigatuzumab, tocilizumab (=atlizumab), toralizumab, tositumomab, trastuzumab, tremelimumab, tucotuzumab, vedolizumab, veltuzumab, visilizumab, vitaxin, volociximab, votumumab, zalutumumab, zanolimumab, ziralimumab, zolimomab aritox, Atezolizumab, bevacizumab (Avastin ®), denosumab, dinutuximab, nivolumab, obinutuzumab, pembrolizumab, pidilizumab (CT-011), ramucirumab, siltuximab, ado-trastuzumab emtansine, CEA-scan Fab fragment, OC125 monoclonal antibody, ab75705, B72.3, MPDL3280A, MSB001078C, MEDI4736, or an antigen binding fragment thereof.

74. The cell surface conjugate of any of claims 9-73, wherein the reference cell surface molecule is a reference EGFR and the modified cell surface molecule is a modified EGFR.

75. The cell surface conjugate of claim 74 wherein the modified EGFR comprises an epitope specifically recognized by cetuximab or an antigen binding fragment thereof.

76. The cell surface conjugate of claim 74 or claim 75, wherein the modified EGFR lacks one or more of an EGFR Domain I, an EGFR Domain II, an EGFR Juxtamembrane Domain, and an EGFR Tyrosine Kinase Domain of the reference EGFR.

77. The cell surface conjugate of any of claims 74-76, wherein the modified EGFR lacks all of the domains EGFR Domain I, an EGFR Domain II, an EGFR Juxtamembrane Domain, and an EGFR Tyrosine Kinase Domain of the reference EGFR.

78. The cell surface conjugate of any of claims 74-77, wherein the modified EGFR comprises an extracellular domain that consists of or consists essentially of subdomain III and subdomain IV of the reference EGFR.

79. The cell surface conjugate of any of claims 74-78, wherein the modified EGFR comprises the sequence of amino acids set forth in SEQ ID NOS: 44 or 46 or a sequence of amino acids that exhibits at least at or about 85%, 90%, or 95% sequence identity to SEQ ID NOS: 44 or 46.

80. The cell surface conjugate of any of claims 973, wherein the reference cell surface molecule is a reference HER2 and the modified cell surface molecule is a modified HER2.

81. The cell surface conjugate of claim 80, wherein the modified HER2 comprises an epitope specifically recognized by trastuzumab or an antigen binding fragment thereof.

82. The cell surface conjugate of claim 80 or claim 81, wherein the modified HER2 lacks one or more of an HER2 Domain I, an HER2 Domain II, an HER2 Domain III of the reference HER2.

83. The cell surface conjugate of any of claims 80-82, wherein the modified HER2 lacks all of the domains HER2 Domain I, HER2 Domain II, and HER2 Domain III of the reference EGFR of the reference HER2.

84. The cell surface conjugate of any of claims 80-83, wherein the modified HER2 comprises an extracellular domain that consists of or consists essentially of Domain IV of the reference HER2.

85. The cell surface conjugate of any of claims 80-84, wherein the modified HER2 comprises the sequence of amino acids set forth in SEQ ID NO: 92 or a sequence of amino acids that exhibits at least at or about 85%, 90%, or 95% sequence identity to SEQ ID NO: 92.

86. The cell surface conjugate of any of claims 9-72, wherein the reference cell surface molecule is a reference PSMA and the modified cell surface molecule is a modified PSMA.

87. The cell surface conjugate of claim 86, wherein the reference PSMA is a wild-type PSMA, optionally wild-type human PSMA.

88. The cell surface conjugate of claim 87, wherein the reference PSMA is a human PSMA and/or comprises the sequence of amino acids set forth in SEQ ID NO: 94 or a sequence of amino acids encoded by the sequence of nucleotides set forth in SEQ ID NO: 96 or 97.

89. The cell surface conjugate of any of claims 86-88, wherein the modified PSMA comprises an extracellular portion and a transmembrane domain of the reference PSMA.

90. The cell surface conjugate of any of claims 86-89, wherein the modified PSMA comprises one or more amino acid modifications in the intracellular region compared to the reference PSMA.

91. The cell surface conjugate of any of claims 86-90, wherein the one or more amino acid modification comprises one or more amino acid substitutions, deletions and/or insertions.

92. The cell surface conjugates of any of claims 86-91, wherein the modified PSMA exhibits altered cellular internalization compared to the reference PSMA.

93. The cell surface conjugate of any of claims 86-92, wherein the modified PSMA comprises an amino acid substitution corresponding to W2G or does not comprise W2 or does not comprise any residue at position 2, with reference to positions in the sequence of amino acids set forth in SEQ ID NO:94.

94. The cell surface conjugate of any of claims 86-93, wherein the modified PSMA comprises a deletion or truncation of 11 N-terminal amino acids, compared to the reference PSMA.

95. The cell surface conjugate of any of claims 86-94, wherein the modified PSMA comprises an epitope capable of being recognized by an antibody or antigen-binding fragment thereof.

96. The cell surface conjugate of claim 95, wherein the antibody or antigen-binding fragment thereof is selected from among J591, DFO-J591, CYT-356, J415, 3/A12, 3/F11, 3/E7, D2B, 107-1A4, YPSMA-1, YPSMA-2, 3E6, 2G7, 24.4E6, GCP-02, GCP-04, GCP-05, J533, E99, 1G9, 3C6, 4.40, 026, D7-Fc, D7-CH3, 4D4, A5, and antigen-binding fragments thereof.

97. The cell surface conjugate of any of claims 1-96, wherein the cell surface conjugate is not immunogenic and/or does not induce an immune response in a subject in which it is administered.

98. A polynucleotide, comprising a nucleic acid sequence encoding the cell surface conjugate of any of claims 1-97.

99. The polynucleotide of claim 98, wherein the nucleic acid sequence further comprising a signal sequence.

100. The polynucleotide of claim 99, wherein the signal sequence encodes a signal peptide derived from GMCSFR alpha chain.

101. The polynucleotide of any of claims 98-100, wherein the nucleic acid sequence is a first nucleic acid sequence and the polynucleotide further comprises a second nucleic acid sequence encoding a recombinant receptor.

102. The polynucleotide of claim 101, wherein the recombinant receptor is or comprises a chimeric antigen receptor (CAR).

103. The polynucleotide of claim 101 or claim 102, wherein the first and second nucleic acid sequences are separated by an internal ribosome entry site (IRES), or a nucleotide sequence encoding a self-cleaving peptide or a peptide that causes ribosome skipping, which optionally is a T2A, a P2A, an E2A or an F2A.

104. The polynucleotide of any of claims 101-103, wherein the first nucleic acid sequence is upstream of the second nucleic acid sequence.

105. The polynucleotide of any of claims 101-103, wherein the first nucleic acid sequence is downstream of the second nucleic acid sequence.

106. A vector, comprising the polynucleotide of any of claims 98-105.

107. The vector of claim 106 that is a viral vector.

108. The vector of claim 106 or claim 107 that is a retroviral vector.

109. The vector of any of claims 106-108 that is a lentiviral vector or a gammaretroviral vector.

110. A method of producing an engineered cell, comprising introducing the polynucleotide of any of claims 96-105 or the vector of any of claims 106-109into a cell.

111. An engineered cell produced by the method of claim 110.

112. An engineered cell, comprising the polynucleotide of any of claims 98-105 or the vector of any of claims 106-109.

113. An engineered cell, comprising the cell surface conjugate of any of claims 1-97.

114. The engineered cell of claim 113, further comprising a recombinant receptor.

115. The engineered cell of claim 114, wherein the recombinant receptor is capable of binding to a target antigen that is associated with, specific to, and/or expressed on a cell or tissue of a disease or disorder.

116. The engineered cell of claim 115, wherein the disease or disorder is an infectious disease or disorder, an autoimmune disease, an inflammatory disease, or a tumor or a cancer.

117. The engineered cell of claim 115 or claim 116, wherein the target antigen is a tumor antigen.

118. The engineered cell of any of claims 115-117, wherein the target antigen is selected from the group consisting of αvβ6 integrin (avb6 integrin), B cell maturation antigen (BCMA), B7-H3, B7-H6, carbonic anhydrase 9 (CA9, also known as CAIX or G250), a cancer-testis antigen, cancer/testis antigen 1B (CTAG, also known as NY-ESO-1 and LAGE-2), carcinoembryonic antigen (CEA), a cyclin, cyclin A2, C-C Motif Chemokine Ligand 1 (CCL-1), CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD138, CD171, epidermal growth factor protein (EGFR), truncated epidermal growth factor protein (tEGFR), type III epidermal growth factor receptor mutation (EGFR vIII), epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), ephrinB2, ephrine receptor A2 (EPHa2), estrogen receptor, Fc receptor like 5 (FCRL5; also known as Fc receptor homolog 5 or FCRH5), fetal acetylcholine receptor (fetal AchR), a folate binding protein (FBP), folate receptor alpha, ganglioside GD2, O-acetylated GD2 (OGD2), ganglioside GD3, glycoprotein 100 (gp100), G Protein Coupled Receptor 5D (GPCR5D), Her2/neu (receptor tyrosine kinase erb-B2), Her3 (erb-B3), Her4 (erb-B4), erbB dimers, Human high molecular weight-melanoma-

associated antigen (HMW-MAA), hepatitis B surface antigen, Human leukocyte antigen A1 (HLA-A1), Human leukocyte antigen A2 (HLA-A2), IL-22 receptor alpha(IL-22Ra), IL-13 receptor alpha 2 (IL-13Ra2), kinase insert domain receptor (kdr), kappa light chain, L1 cell adhesion molecule (L1-CAM), CE7 epitope of L1-CAM, Leucine Rich Repeat Containing 8 Family Member A (LRRC8A), Lewis Y, Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, mesothelin, c-Met, murine cytomegalovirus (CMV), mucin 1 (MUC1), MUC16, natural killer group 2 member D (NKG2D) ligands, melan A (MART-1), neural cell adhesion molecule (NCAM), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME), progesterone receptor, a prostate specific antigen, prostate stem cell antigen (PSCA), prostate specific membrane antigen (PSMA), Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1), survivin, Trophoblast glycoprotein (TPBG also known as 5T4), tumor-associated glycoprotein 72 (TAG72), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor receptor 2 (VEGFR2), Wilms Tumor 1 (WT-1)

119. The engineered cell of any of claims 115-118, wherein the target antigen is selected from the group consisting of ROR1, HER2, L1-CAM, CD19, CD20, CD22, mesothelin, CEA, hepatitis B surface antigen, anti-folate receptor, CD23, CD24, CD30, CD33, CD38, CD44, EGFR, EGP-2, EGP-4, EPHa2, ErbB2, ErbB3, ErbB4, FBP, fetal acethycholine e receptor, GD2, GD3, HMW-MAA, IL-22R-alpha, IL-13R-alpha2, kdr, kappa light chain, Lewis Y, L1-cell adhesion molecule, MAGE-A1, mesothelin, MUC1, MUC16, PSCA, NKG2D Ligands, NY-ESO-1, MART-1, gp100, oncofetal antigen, TAG72, VEGF-R2, carcinoembryonic antigen (CEA), prostate specific antigen, PSMA, estrogen receptor, progesterone receptor, ephrinB2, CD123, CS-1, c-Met, GD-2, MAGE A3, CE7, Wilms Tumor 1 (WT-1), and cyclin A1 (CCNA1).

120. The engineered cell of any of claims 114-119, wherein the recombinant receptor is a functional non-TCR antigen receptor or a transgenic TCR.

121. The engineered cell of any of claims 114-120, wherein the recombinant receptor is a chimeric antigen receptor (CAR).

122. The engineered cell of any of claims 114-121, wherein the recombinant receptor comprises an extracellular portion comprising an antigen-binding domain.

123. The engineered cell of claim 122, wherein the antigen-binding domain is or comprises an antibody or an antibody fragment.

124. The engineered cell of claim 123, wherein the antibody fragment is a single chain fragment.

125. The engineered cell of claim 123 or claim 124, wherein the fragment comprises antibody variable regions joined by a flexible linker.

126. The engineered cell of any of claims123-125, wherein the fragment comprises an scFv.

127. The engineered cell of any of claims 114-126, wherein the recombinant receptor comprises an intracellular signaling region.

128. The engineered cell of claim 127, wherein the intracellular signaling region comprises an intracellular signaling domain.

129. The engineered cell of claim 128, wherein the intracellular signaling domain is or comprises a primary signaling domain, a signaling domain that is capable of inducing a primary activation signal in a T cell, a signaling domain of a T cell receptor (TCR) component, and/or a signaling domain comprising an immunoreceptor tyrosine-based activation motif (ITAM).

130. The engineered cell of claim 128 or claim 129, wherein the intracellular signaling domain is or comprises an intracellular signaling domain of a CD3 chain, optionally a CD3-zeta (CD3ζ) chain or a signaling portion thereof.

131. The engineered cell of any of claims 127-130, further comprising a transmembrane domain disposed between the extracellular domain and the intracellular signaling region.

132. The engineered cell of any of claims 127-131, wherein the intracellular signaling region furthercomprises a costimulatory signaling domain.

133. The engineered cell of claim 132, wherein the costimulatory signaling domain comprises an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof.

134. The engineered cell of claim 132 or claim 133, wherein the costimulatory signaling domain comprises an intracellular signaling domain of a CD28, a 4-1BB or an ICOS or a signaling portion thereof.

135. The engineered cell of any of claims 132-134, wherein the costimulatory signaling domain is between the transmembrane domain and the intracellular signaling domain.

136. The engineered cell of any of claims 111-135, wherein the cell is an immune cell.

137. The engineered cell of claim 136, wherein the cell is a lymphocyte.

138. The engineered cell of any of claims 111-137, wherein the cell is a T cell or an NK cell.

139. The engineered cell of claim 138, wherein the cell is a T cell that is a CD8+ T cell or a CD4+ T cell.

140. A composition comprising the engineered cells of any of claims 111-139.

141. The composition of claim 140, further comprising a pharmaceutically acceptable excipient.

142. A method of treatment comprising administering the engineered cells of any of claims 111-139 or the composition of claim 140 or claim 141 to a subject having a disease or disorder.

143. The method of claim 142, wherein the disease or disorder is a cancer, a tumor, an autoimmune disease or disorder, or an infectious disease.

144. The method of claim 142 or claim 143, further comprising administering to the subject a binding molecule capable of recognizing the agent of the cell surface conjugate expressed on the engineered cell and detecting cells that express the cell surface conjugate.

145. The method of claim 144, wherein detection comprises in vivo imaging.

146. A method of identifying a cell expressing a cell surface conjugate, comprising contacting a composition comprising cells that express or are likely to express a cell surface conjugate of any of claims 1-97 or the engineered cell of any of claims 111-139 or the composition of claim 140 or claim 141, with a binding molecule capable of recognizing the agent of the cell surface conjugate.

147. The method of claim 146 that is performed in vitro, ex vivo or in vivo.

148. The method of any of claim 146 or claim 147, wherein the cell expressing the cell surface molecule is detected via in vivo imaging.

149. The method of claim 145 or claim 148, wherein the in vivo imaging method is selected from among magnetic resonance imaging (MRI), single-photon emission computed tomography (SPECT), computed tomography (CT), computed axial tomography (CAT), electron beam computed tomography (EBCT), high resolution computed tomography (HRCT), hypocycloidal tomography, positron emission tomography (PET), scintigraphy, gamma camera, a β+ detector, a γ detector, fluorescence imaging, low-light imaging, X- rays, and bioluminescence imaging.

150. The method of claim 145, claim 148, or claim 149, wherein the binding molecule is conjugated to a moiety that provides a signal or induces a signal that is detectable in vivo.

151. The method of claim 150, wherein the moiety is a radioisotope, bioluminescent compound, chemiluminescent compound, fluorescent compound, metal chelate or enzyme.

152. A method of identifying cells transduced with a cell surface conjugate, comprising:

(a) contacting a composition transduced with a polynucleotide of any of claims 98-105 or the vector of any of claims 106-109 encoding the cell surface conjugate or the engineered cell of any of claims 111-139 or the composition of claim 140 or claim 141 with a binding molecule capable of recognizing the agent of the cell surface conjugate; and

(b) identifying cells bound to the binding molecule.

153. A method of identifying cells transduced with a cell surface conjugate, comprising:

(a) introducing a polynucleotide of any of claims 98-105 or the vector of any of claims 106-109 encoding the cell surface conjugate into a cell;

(b) contacting a composition comprising the cell of (a) with a binding molecule capable of recognizing the agent of the cell surface conjugate; and

(c) identifying cells of the composition bound to the binding molecule.

154. A method of selecting cells transduced with a cell surface conjugate, comprising:

(a) contacting a composition transduced with a polynucleotide of any of claims 98-105 or the vector of any of claims 106-109 encoding the cell surface conjugate or the engineered cell of any of claims 111-139 or the composition of claim 140 or claim 141 with a binding molecule capable of recognizing the agent of the cell surface conjugate; and

(b) isolating cells bound to the binding molecule.

155. A method of selecting cells transduced with a cell surface conjugate, comprising:

(a) introducing a polynucleotide of any of claims 98-105 or the vector of any of claims 106-109 encoding the cell surface conjugate into a cell;

(b) contacting a composition comprising the cell of (a) with a binding molecule capable of recognizing the agent of the cell surface conjugate; and

(c) isolating cells of the composition bound to the binding molecule.

156. The method of claim 154 or claim 155, wherein the binding molecule is conjugated to a detectable moiety or is capable of producing a detectable signal.

157. The method of claim 156, wherein the detectable moiety comprises a fluorescent protein.

158. The method of any of claims 144-157, wherein the agent is is a streptavidin binding peptide.

159. The method of claim 158, wherein the streptavidin binding peptide is or comprises the sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8) or Trp-Arg-His-Pro-Gln-Phe-Gly-Gly (SEQ ID NO:7).

160. The method of claim 159, wherein the streptavidin binding peptide is or comprises the sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_3$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 17), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 18) and Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$Gly-Gly-Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 19).

161. The method of any of claims 144-160, wherein the binding molecule is a reagent capable of reversibly binding to the agent and/or capable of being competed in the presence of a competition substance.

162. The method of claim 161, wherein the reagent is a streptavidin, a streptavidin analog or mutein.

163. The method of claim 162, wherein the streptavidin analog or mutein comprises the amino acid sequence Val^{44}-Thr^{45}-Ala^{46}-Arg^{47} or Ile^{44}-Gly^{45}-Ala^{46}-Arg^{47} at sequence positions corresponding to positions 44 to 47 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO:1.

164. The method of claim 162 or claim 163, wherein the streptavidin analog or mutein comprises:

a) the sequence of amino acids set forth in any of SEQ ID NOS: 3-6, 27 and 28;

b) a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 3-6, 27 and 28 and contains the amino acid sequence corresponding to Val^{44}-Thr^{45}-Ala^{46}-Arg^{47} or Ile^{44}-Gly^{45}-Ala^{46}-Arg^{47} and that reversibly binds to the agent; or

c) a functional fragment of a) or b) that reversibly binds to the agent.

165. The method of claim 163 or claim 164, wherein the streptavidin analog or mutein further comprises an amino acid replacement or replacements at a position corresponding to 117, 120 and/or 121 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO:1.

166. The method of claim 165, wherein:

the amino acid replacement or replacements are selected from among Glu^{117}, Asp^{117}, Arg^{117}, Ser^{120}, Ala^{120}, Gly^{120}, Trp^{121}, Tyr^{121} or Phe^{121}; or

the amino acid replacement or replacements are selected from one or more of Glu^{117}, Gly^{120} or Tyr^{121}; or

the amino acid replacements are selected from Glu^{117}, Gly^{120} or Tyr^{121}.

167. The method of any of claims 162-166, wherein the streptavidin analog or mutein comprises:

a) the sequence of amino acids set forth in SEQ ID NO: 27 or 28;

b) a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NOS:27 or 28 and contains the amino acid sequence corresponding to Val^{44}, Thr^{45}, Ala^{46}, Arg^{47}, Glu^{117}, Gly^{120} and Tyr^{121} and reversibly binds to the agent; or

c) a functional fragment of a) or b) that reversibly binds to the agent.

168. The method of any of claims 161-167, further comprising disrupting the reversible binding of the binding molecule to the agent.

169. The method of claim 168, wherein said disruption comprises contacting the cells with a composition comprising a competition substance capable of reversing the bond between the binding molecule and agent.

170. The method of claim 169, wherein the competition substance is a free binding partner and/or is a competition agent.

171. The method of claim 169 or claim 170, wherein the competition substance is or comprises biotin, a biotin analog or a biologically active fragment thereof.

172. The method of any of claims 144-171, wherein the binding molecule is an antibody or antigen binding fragment that specifically binds the agent.

173. The method of claim 172, wherein the binding molecule is an anti-StrepTag antibody.

174. A molecule, comprising a streptavidin or a streptavidin analog or mutein conjugated to a cytotoxic agent.

175. The molecule of claim 174, comprising a streptavidin analog or mutein.

176. The molecule of claim 174 or claim 175, wherein the streptavidin or streptavidin analog or mutein binds to a streptavidin binding peptide.

177. The molecule of claim 176, wherein the streptavidin binding peptide is or comprises the sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8) or Trp-Arg-His-Pro-Gln-Phe-Gly-Gly (SEQ ID NO:7).

178. The molecule of claim 176 or claim 177, wherein the streptavidin binding peptide is or comprises the sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_3$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys ((SEQ ID NO: 17), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 18) and Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$Gly-Gly-Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 19).

179. The molecule of any of claims 176-178, wherein the streptavidin or streptavidin analog or mutein exhibits a binding affinity for the streptavidin binding peptide with an equilibrium dissociation constant (K_D) of from or from about 10^{-4} M to or to about 10^{-10} M.

180. The molecule of any of claims 174-179, wherein the streptavidin analog or mutein comprises the amino acid sequence Val^{44}-Thr^{45}-Ala^{46}-Arg^{47} or Ile^{44}-Gly^{45}-Ala^{46}-Arg^{47} at sequence positions corresponding to positions 44 to 47 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO:1.

181. The molecule of any of claims 174-180, wherein the streptavidin analog or mutein comprises:

a) the sequence of amino acids set forth in any of SEQ ID NOS: 3-6, 27 and 28;

b) a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 3-6, 27 and 28 and contains the amino acid sequence corresponding to Val^{44}-Thr^{45}-Ala^{46}-Arg^{47} or Ile^{44}-Gly^{45}-Ala^{46}-Arg^{47} and that reversibly binds to the agent; or

c) a functional fragment of a) or b) that binds to the streptavidin binding peptide.

182. The molecule of claim 180 or claim 181, wherein the streptavidin analog or mutein further comprises an amino acid replacement or replacements at a position corresponding to 117, 120 and/or 121 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO:1.

183. The molecule of claim 182, wherein:

the amino acid replacement or replacements are selected from among Glu^{117}, Asp^{117}, Arg^{117}, Ser^{120}, Ala^{120}, Gly^{120}, Trp^{121}, Tyr^{121} or Phe^{121}; or

the amino acid replacement or replacements are selected from one or more of Glu^{117}, Gly^{120} or Tyr^{121}; or

the amino acid replacements are selected from Glu^{117}, Gly^{120} or Tyr^{121}.

184. The molecule of any of claims 174-183, wherein the streptavidin analog or mutein comprises:

a) the sequence of amino acids set forth in SEQ ID NO: 27 or 28;

b) a sequence of amino acids that exhibits at least at or about 85%, 86%, 87%, 88%, 89%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NOS:27 or 28 and contains the amino acid sequence corresponding to Val^{44}, Thr^{45}, Ala^{46}, Arg^{47}, Glu^{117}, Gly^{120} and Tyr^{121} and reversibly binds to the agent; or

c) a functional fragment of a) or b) that reversibly binds to the streptavidin binding peptide.

185. The molecule of any of claims 174-184, wherein the cytotoxic agent is a toxin.

186. The molecule of claim 185, wherein the toxin is a peptide toxin, ricin A chain toxin, Abrin A chain, Diptheria Toxin (DT) A chain, Pseudomonas exotoxin, Shiga Toxin A chain, Gelonin, Momordin, Pokeweed Antiviral Protein, Saporin, Trichosanthin, or Barley Toxin.

187. The molecule of claim 185, wherein the the toxin is a phototoxin.

188. A method of killing cells, comprising administering the molecule of any of claims 174-187 to a subject previously administered the cells of any of claims 111-139 or the composition of claim 140 or claim 141.

189. The method of claim 188, wherein the molecule is administered at a time at which the subject is exhibiting a toxic outcome associated with the administered cells or at a time at which the subject is exhibiting a detectable and/or cell-mediated immune response to the administered cells.

190. The method of claim 189, wherein the toxic outcome is associated with neurotoxicity or cytokine release syndrome (CRS).

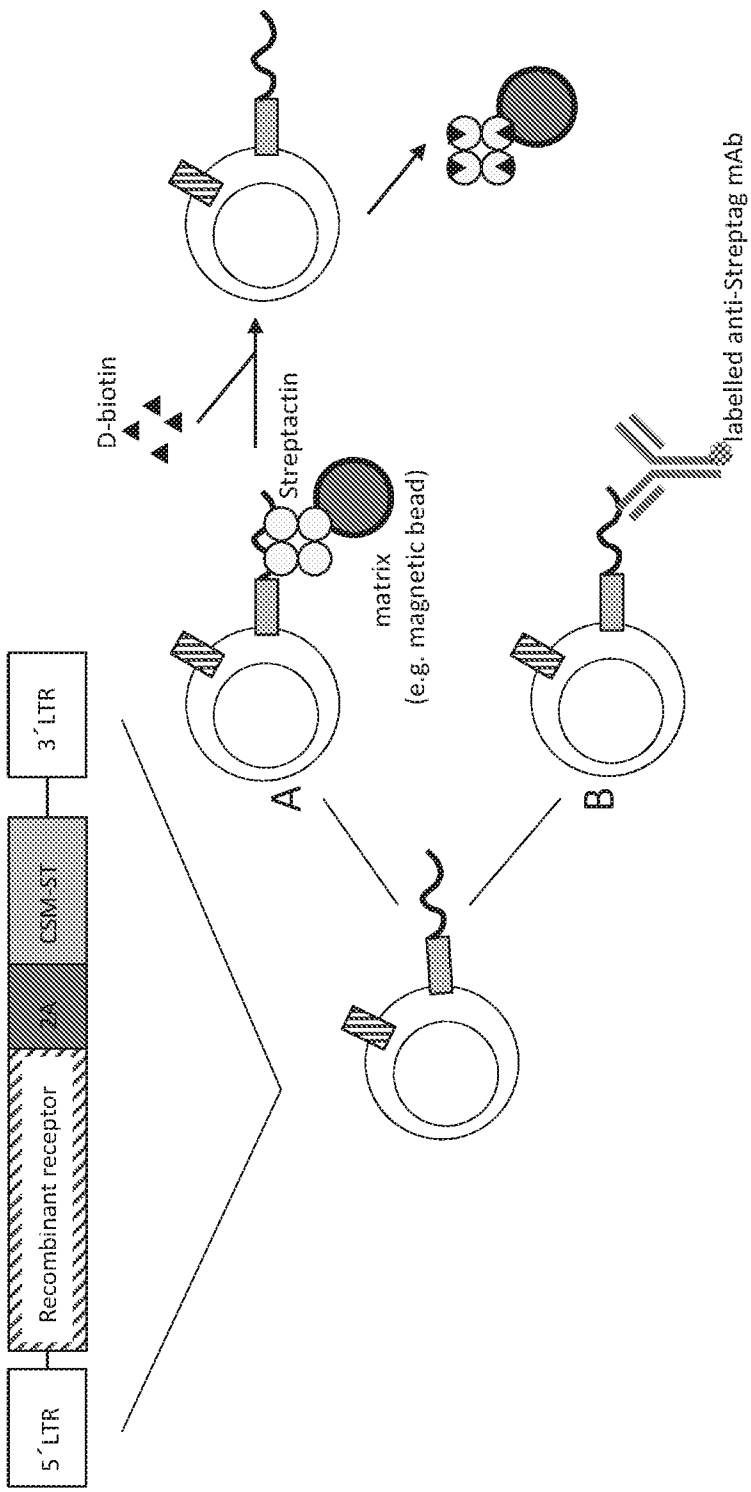
5´LTR
Recombinant receptor
CSM-ST
3´LTR
A
B
matrix
(e.g. magnetic bead)
Streptactin
D-biotin
labelled anti-Streptag mAb

FIG. 1

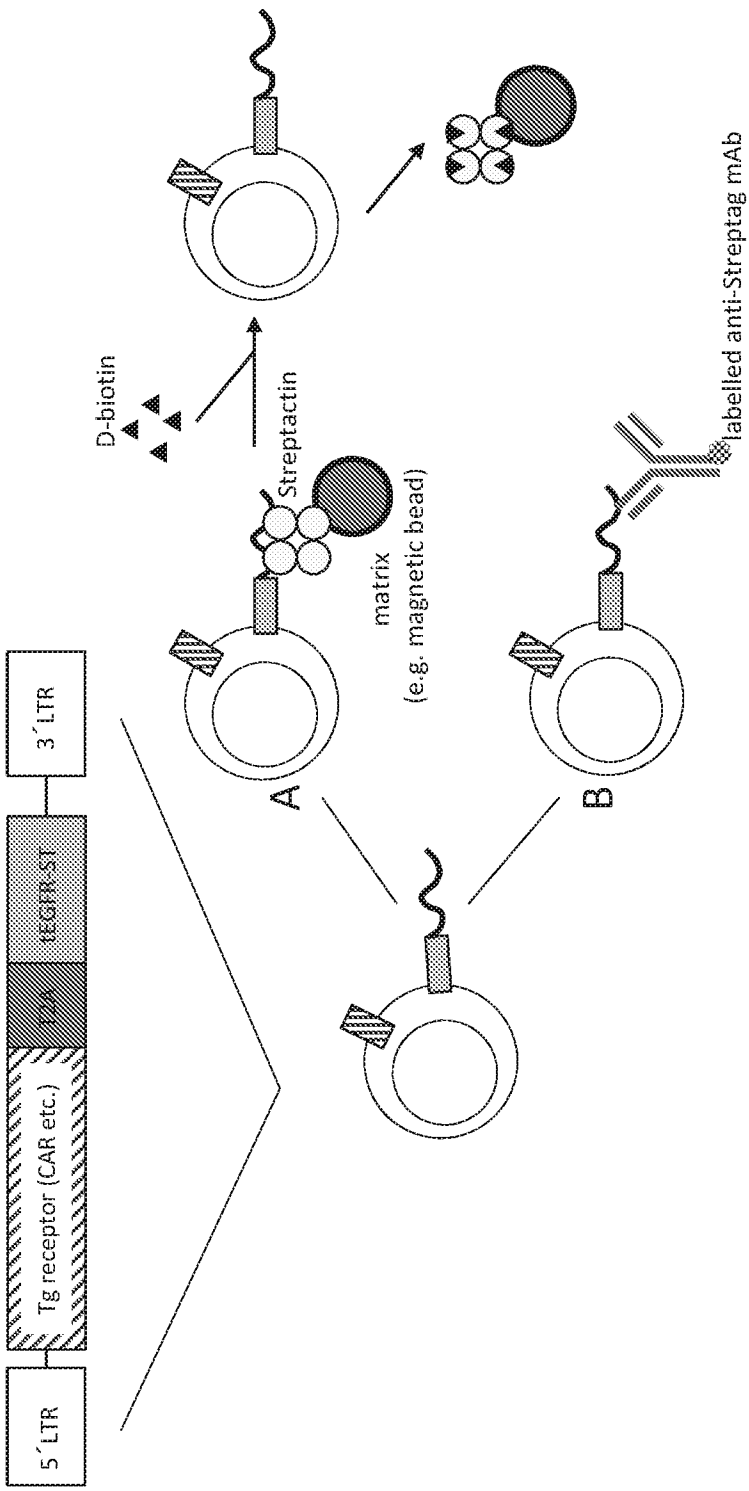
5´LTR
Tg receptor (CAR etc.)
F-A
tEGFR-ST
3´LTR
A
B
matrix
(e.g. magnetic bead)
Streptactin
D-biotin
labelled anti-Streptag mAb

FIG. 2