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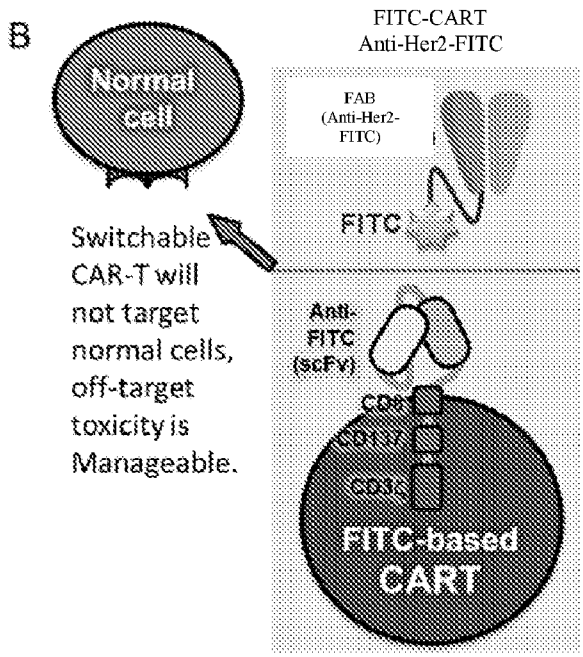
[Continued on next page]

(54) Title: CHIMERIC RECEPTOR T CELL SWITCHES FOR HER2

FIG. 1



(57) Abstract: Disclosed herein are switches for regulating the activity of a chimeric antigen receptor effector cells (CAR-ECs). The switches generally comprise a chimeric antigen receptor-interacting domain (CAR-ID) and a target interacting domain TID. The switch may further comprise a linker. Further disclosed herein are methods of using the switches for the treatment of one or more conditions or diseases in a subject in need thereof.

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CHIMERIC RECEPTOR T CELL SWITCHES FOR HER2

CROSS-REFERENCE

[0001] This application claims priority to U.S. Provisional Application No. 62/148,070, filed, April 15, 2015, and U.S. Provisional Application No. 62/253,465, November 10, 2015, each of which application is incorporated by reference herein in its entirety.

STATEMENT REGARDING SEQUENCE LISTING

[0002] The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is CIBR_010_01WO_ST25.txt. The text file is 30 KB, was created on April 15, 2016, and is being submitted electronically via EFS-Web.

BACKGROUND OF THE INVENTION

[0003] Immunotherapies are becoming attractive alternatives to chemotherapies, including immunotherapies that use adoptive transfer of genetically modified T cells to "reteach" the immune system to recognize and eliminate malignant tumor cells. Genetically modified T cells express chimeric antigen receptors (CARs), which generally consist of an intracellular signaling domain, a CD3-zeta (ζ) transmembrane domain, and an extracellular single-chain variable fragment (scFv) derived from a monoclonal antibody which gives the receptor specificity for a tumor-associated antigen on a target malignant cell. Upon binding the tumor-associated antigen via the chimeric antigen receptor, the chimeric antigen receptor expressing T cell (CAR T-cell) mounts an immune response that is cytotoxic to the malignant cell. Such therapies can circumvent chemotherapy resistance and are active against relapsed/refractory disease, resulting in sustained remission for some chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), and Acute myeloid leukemia (AML) patients. However, these therapies require further investigation and optimization, as they can cause undesirable effects such as toxic cytopenias and chronic hypogammaglobulinemia for hematological targets, fatal off-target cytolysis for solid tumor targets, persistent B cell

aplasia with the use of anti-CD19 antibody expressing CAR T-cells, and, in some cases, death.

SUMMARY OF THE INVENTION

[0004] Disclosed herein are CAR switches comprising: (i) a CAR interacting domain and (ii) a target interacting domain (TID); wherein the TID comprises an anti-Her2 antibody fragment Fab (clone 4D5), or a fragment thereof, which Fab comprises an unnatural amino acid located at each of Glycine 212 of a light chain of the anti-Her2 antibody Fab (LG212X; SEQ ID NO: 2) and Lysine 221 of a heavy chain of the anti-Her2 antibody Fab (HK221X; SEQ ID NO: 1), and wherein each of LG212X and HK221X is attached or linked to a separate CAR interacting domain. The fragment may be a variable region of the targeting antibody. The heavy chain may have the sequence of SEQ ID NO: 1, wherein X indicates the location of an unnatural amino acid. The heavy chain may have a sequence that is at least about 50% homologous to SEQ ID NO: 1. The light chain may have the sequence of SEQ ID NOS: 2, wherein X indicates the location of an unnatural amino acid. The light chain may have a sequence that is at least about 50% homologous to SEQ ID NO: 2.

[0005] As used herein, the term "TID" means a target interaction domain that comprises an anti-Her2 antibody fragment Fab (clone 4D5), or a fragment thereof, which Fab comprises an unnatural amino acid located at each of Glycine 212 of a light chain of the anti-Her2 antibody Fab (LG212X; SEQ ID NO: 2) and Lysine 221 of a heavy chain of the anti-Her2 antibody Fab (HK221X; SEQ ID NO: 1), and wherein each of LG212X and HK221X is attached or linked to a separate CAR interacting domain.

[0006] In some embodiments, the CAR interacting domain is a small molecule. In some embodiments, the CAR interacting domain is a hapten. In some embodiments, the CAR interacting domain is selected from FITC, biotin, and dinitrophenol.

[0007] The CAR switch may further comprise a linker, wherein the linker connects the CAR interacting domain and the TID. The TID may comprise an unnatural amino acid. The CAR interacting domain and the TID may be connected or linked by the unnatural amino acid.

[0008] Disclosed herein are methods of treating a disease or condition in a subject in need thereof, comprising administering a CAR switch disclosed herein, wherein the CAR switch is administered by a method selected from intraperitoneal injection and intravenous injection.

The method may comprise administering the CAR switch and/or a CAR effector cell. The method may comprise administering the CAR switch and/or a CAR effector cell multiple times. The disease or condition may be a Her2 expressing cancer (e.g., Her2-expressing breast cancer, cervical cancer, endometrial cancer, ovarian cancer, AML, ALL, CLL, multiple myeloma, neuroblastoma, pancreatic cancer, endometrial cancer, or colon cancer). The method may comprise administering a first CAR switch comprising a first TID and a second CAR switch comprising a second TID, wherein the first TID binds a first antigen and the second TID binds a second antigen. In some embodiments, the first antigen and the second antigen are different. In some embodiments, the first and the second antigen are the same. The first and/or second antigen may be Her2.

[0009] Disclosed herein are CAR-effector cell platforms, comprising: (i) a CAR-effector cell that expresses a CAR and (ii) a CAR switch comprising: (a) a CAR interacting domain and (b) a target interacting domain TID; wherein the TID comprises an anti-Her2 antibody fragment Fab (clone 4D5), or a fragment thereof, which Fab comprises an unnatural amino acid located at each of Glycine 212 of a light chain of the anti-Her2 antibody Fab (LG212X; SEQ ID NO: 2) and Lysine 221 of a heavy chain of the anti-Her2 antibody Fab (HK221X; SEQ ID NO: 1), and wherein each of LG212X and HK221X is attached or linked to a separate CAR interacting domain. The heavy chain may have the sequence of SEQ ID NO: 1, wherein X indicates the location of an unnatural amino acid. The heavy chain may have a sequence that is at least about 50% homologous to SEQ ID NO: 1. The light chain may have the sequence of SEQ ID NOS: 2, wherein X indicates the location of an unnatural amino acid. The light chain may have a sequence that is at least about 50% homologous to SEQ ID NO: 2.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] **FIG. 1A** illustrates a general overview of chimeric antigen receptor-T cell (CAR T-cell) and CAR T-cell switch therapy with switches disclosed herein. Lymphocytes are isolated from a subject and an expression vector encoding a chimeric antigen receptor is subsequently introduced to the lymphocytes to produce chimeric antigen receptor expressing cells. Resulting engineered lymphocytes are administered to the subject, along with a CAR T-cell switch.

[0011] **FIG. 1B** illustrates a CAR T-cell switch, comprising FITC that is bound by the chimeric antigen receptor of the CAR T-cell and a targeting antibody that is selective for a

target cell. Binding of the CAR T-cell switch to the CAR T-cell induces an immune response that would be cytotoxic to the malignant cell also bound to the CAR T-cell switch.

[0012] **FIG. 2** shows a general scheme to generate site-specific FITC antibody conjugates. Mutant antibodies incorporated with pAzF were conjugated with BCN-PEG4-FITC by "Click" reaction.

[0013] **FIG. 3** shows the location of LG212X/HK221X sites and relative distances between bivalent sites, which were calculated by UCSF chimera 1.10.2. The structures are derived from a reported crystal structure (Protein Data Bank ID 1N8Z).

[0014] **FIG. 4A-B** shows sCAR-T cytotoxicity. **FIG. 4A** shows activation by combinations of various switches and hinge length anti-FITC sCAR-T cells against Her2 1+ MDA MB435 cancer cells. T cell activation and cytokine release with 100 pM switch against MDA MB435 cells at the E:T ratio of 10:1. ***= $p<0.005$, **=$p<0.05$ and NS=$p>0.05$ were calculated using the Student's t-test. Black bars, CD8 hinge; white bars, IgG4m hinge. **FIG. 4B** shows cytotoxicity comparisons of bivalent FITC conjugate LG212X/HK221X with different anti-FITC CAR-T cells (CD8 or IgG4m hinge) against Her2 1+ MDA MB231 cancer cells. The cytolytic activity was determined by measuring the amount of LDH released into cultured medium.

[0015] **FIG. 5** shows cytotoxicity of anti-FITC (CD8 or IgG4m) sCAR-T cells with bivalent switches against MDA MB435 Her2 1+ cells at the E: T ratio of 10: 1.

DETAILED DESCRIPTION OF THE INVENTION

[0016] Current chimeric antigen receptor T cell (CAR T-cell) therapies can be unreliable due to lack of means to control CAR T-cell activity. Disclosed herein are compositions and methods for selectively activating and deactivating chimeric antigen receptor T cells, which may provide for safer and more versatile immunotherapies than those currently being tested and administered.

[0017] Disclosed herein are chimeric antigen receptor effector cell (CAR-EC) switches, wherein the CAR-EC switches have a first region that is bound by a chimeric antigen receptor on the CAR-EC and a second region that binds Her2 on target cell, bringing the target cell in proximity of the CAR-EC and stimulating an immune response from the CAR-EC that is cytotoxic to the bound target cell. In general, the CAR-EC is a T cell, and the CAR-EC is referred to as a switchable CAR-T cell (sCAR-T cell). In this way, the sCAR-EC switch may act as an "on-switch" for CAR-EC activity. Activity may be "turned off" by

reducing or ceasing administration of the switch or adding a switch component that competes with the switch. These methods and compositions disclosed herein allow for the site-specific incorporation of an unnatural amino acid at one or more desired sites of the region that binds the cell surface molecule and subsequent site-specific modification of antibody with FITC *via* click chemistry, which enables establishment of the most productive pseudo-immunological synapse between engineered T cells and target cells by precisely adjusting the sites and stoichiometry of FITC conjugation.

[0018] Major advantages of the switchable CAR platforms disclosed herein include control, safety, titratability, and universality. Switchable CARs can be turned on and off with addition and cessation or competition of the switch. In addition, CAR-EC switches can be titrated to a desired response. For example, solid tumors may be targeted by titration of therapy to achieve a suitable therapeutic index. The response may be titrated "on" to avoid cytokine release syndrome (CRS) and tumor lysis syndrome (TLS) events, providing for personalized therapy. Furthermore, administration of a switch can be terminated in case of an adverse event. The sCAR-EC can be designed to target a non-endogenous antigen which is only active in the presence of a switch that can be reduced at any time. In contrast, a canonical CAR-T cell is always "on" as long as a target exists. This always "on" can lead to T cell anergy as exemplified by functionally exhausted CD8 T cells during chronic viral infection. In contrast, a sCAR-EC can be stimulated and rested. This is more analogous to the natural stimulation of a T cell responding to an infection. Iterative stimulations in this nature, if timed corrected, may be able to better recapitulate the natural stimulation and resting cycles of T cells that would be encountered, for example, with an acute infection. This type of natural life expansion and contraction of T cells may off-set anergy (or T cell dysfunction) and promote the formation of long-lived memory cells. Long-lived memory cells are known to be advantageous in CAR-T cells. Therefore, a switchable approach to CAR-T cells may be advantageous in that it can promote more favorable T cell responses and phenotypes than canonical CAR-T cells.

[0019] Another advantage of the sCAR-EC system is that it is easier and faster to design multiple switches for each CAR-EC rather than empirically building and testing CAR hinge designs. This is because the switches are biologics which may be easier, less expensive, and faster to build multiple variants of than the CAR which requires cell engineering and cell handling. Further, a universal sCAR-EC has a significant advantage in design of the optimal immunological synapse, as CAR-EC switches make it possible for a single CAR-EC to be

redirected to multiple therapeutic targets. Redirection during therapy, by variation of switches, can combat antigen-loss escape mutants with a single CAR-EC. Treatment of heterologous tumors with multiple switches is more straightforward than with multiple CARs. Switches also enable standardized treatment protocols which may increase safety and lower up front treatment costs.

[0020] These CAR-EC switches may be used with sCAR-ECs disclosed herein, as well as existing CAR T-cells known in the art for the treatment of a disease or condition, such as cancer, wherein the target cell is a malignant cell. Such treatment may be referred to herein as switchable immunotherapy, for which an exemplary schematic overview is depicted in **FIG. 1**.

[0021] Disclosed herein are switches for use in regulating the activity of a CAR-EC. The switches comprise (i) a CAR interacting domain and (ii) a TID; wherein the TID comprises an anti-Her2 antibody fragment Fab (clone 4D5), or a fragment thereof, which Fab comprises an unnatural amino acid located at each of Glycine 212 of a light chain of the anti-Her2 antibody Fab (LG212X; SEQ ID NO: 2) and Lysine 221 of a heavy chain of the anti-Her2 antibody Fab (HK221X; SEQ ID NO: 1), and wherein each of LG212X and HK221X is attached or linked to a separate CAR interacting domain. The heavy chain may have the sequence of SEQ ID NO: 1. The heavy chain may have a sequence that is at least about 50% homologous to SEQ ID NO: 1. The light chain may have the sequence of SEQ ID NOS: 2. The light chain may have a sequence that is at least about 50% homologous to SEQ ID NO: 2. As used herein, "linked" means attached via a linker, e.g., a linker disclosed herein. "Attached" means connected, e.g., via a conjugation. The conjugation may be covalent. The conjugation may be non-covalent.

[0022] As used herein, notwithstanding anything else stated herein, the term "TID" or "target interacting domain" refers to a portion of a switch comprising an anti-Her2 antibody fragment Fab (clone 4D5), or a fragment thereof, which Fab comprises an unnatural amino acid located at each of Glycine 212 of a light chain of the anti-Her2 antibody Fab (LG212X; SEQ ID NO: 2) and Lysine 221 of a heavy chain of the anti-Her2 antibody Fab (HK221X; SEQ ID NO: 1), and wherein each of the unnatural amino acids LG212X and HK221X is attached or linked to a separate CAR interacting domain, wherein each of the CAR interacting domain must be the same molecule or substance. For example, if the CAR interacting domain is FITC, then both LG212X and HK221X must be conjugated with FITC.

As used herein, "Homology" refers to the percentage number of amino acids that are identical or constitute conservative substitutions. Homology may be determined using sequence comparison programs such as GAP (Deveraux et al., Nucleic Acids Research. 12, 387-395, 1984), which is incorporated herein by reference. In this way sequences of a similar or substantially different length to those cited herein could be compared by insertion of gaps into the alignment, such gaps being determined, for example, by the comparison algorithm used by GAP.

[0023] The switch may further comprise one or more linkers. The TID may be modified to comprise one or more unnatural amino acids. The CAR-ID may comprise a small molecule. The CAR-ID may comprise a hapten. The CAR-ID may comprise FITC. The CAR-ID and TID may be attached/connected through the unnatural amino acid.

[0024] Disclosed herein are switch platforms for use in regulating the activity of a CAR-EC. The switch platforms comprise (i) a CAR-effector cell that expresses a CAR and (ii) a CAR switch comprising:(a) a CAR interacting domain and (b) a TID; wherein the TID comprises an anti-Her2 antibody fragment Fab (clone 4D5), or a fragment thereof, which Fab comprises an unnatural amino acid located at each of Glycine 212 of a light chain of the anti-Her2 antibody Fab (LG212X; SEQ ID NO: 2) and Lysine 221 of a heavy chain of the anti-Her2 antibody Fab (HK221X; SEQ ID NO: 1), and wherein each of LG212X and HK221X is attached or linked to a separate CAR interacting domain. The heavy chain may have the sequence of SEQ ID NO: 1. The heavy chain may have a sequence that is at least about 50% homologous to SEQ ID NO: 1. The light chain may have the sequence of SEQ ID NOS: 2. The light chain may have a sequence that is at least about 50% homologous to SEQ ID NO: 2.

[0025] Disclosed herein are compositions (e.g., pharmaceutical compositions) comprising one or more switches for regulating the activity of a CAR-EC, wherein at least one of the switches comprises (a) a CAR interacting domain and (b) a TID; wherein the TID comprises an anti-Her2 antibody fragment Fab (clone 4D5), or a fragment thereof, which Fab comprises an unnatural amino acid located at each of Glycine 212 of a light chain of the anti-Her2 antibody Fab (LG212X; SEQ ID NO: 2) and Lysine 221 of a heavy chain of the anti-Her2 antibody Fab (HK221X; SEQ ID NO: 1), and wherein each of LG212X and HK221X is attached or linked to a separate CAR interacting domain.. In some embodiments, the switch comprised in the composition may comprise a heavy chain having the sequence of SEQ ID NO: 1 and a light chain having the sequence of SEQ ID NOS: 2, wherein X represents an

unnatural amino acid, and wherein the switch further comprises a CAR interacting domain connected to each the unnatural amino acids designated by X. The heavy chain may have a sequence that is at least about 50% homologous to SEQ ID NO: 1. The light chain may have a sequence that is at least about 50% homologous to SEQ ID NO: 2.

[0026] Methods of producing the switches and switch intermediates disclosed herein may advantageously provide for control of sCAR-EC cell activity, titration of off-target reactivity, abrogation of TLS, attenuation of CRS, and/or optimization of CAR-EC switch binding by affinity, valency, geometry, linker length and/or linker chemistry through site-specific conjugation of CAR-EC switch components/regions.

[0027] Disclosed herein are methods of producing a switch for regulating the activity of a CAR-EC. The method may comprise (a) obtaining or producing a modified Her2 antibody comprising the anti-Her2 antibody fragment Fab (clone 4D5), or a fragment thereof, comprising an unnatural amino acid located at Glycine 212 of a light chain of the anti-Her2 antibody Fab (LG212X; SEQ ID NO: 2) and an unnatural amino acid located at Lysine 221 of a heavy chain of the anti-Her2 antibody Fab (HK221X; SEQ ID NO: 1); and (b) attaching or linking a CAR-ID to each of the unnatural amino acids in the Her2 antibody, thereby producing the switch. In some embodiments, the linking is via a linker, e.g., any linker disclosed herein.

[0028] Further disclosed herein are methods of producing a switch for regulating the activity of a CAR-EC comprising (a) contacting a CAR-ID with a Her2 antibody; and (b) producing the switch by attaching the CAR-ID to a predetermined site on the Her2 antibody, wherein the site comprises Glycine 212 of a light chain of the anti-Her2 antibody Fab and Lysine 221 of a heavy chain of the anti-Her2 antibody Fab. .

[0029] Methods of treating a disease or condition comprising administering the CAR-EC switches, disclosed herein, may provide for a titratable response, improved safety and/or cessation of CAR-EC activity by reducing or ceasing administration of the CAR-EC switch. In contrast to other approaches of controlling CAR-EC activity, which "turn off" CAR-EC activity by competing with the target cell surface molecule for binding the CAR, the CAR-EC switches disclosed herein, generally function as CAR-EC activators or "on" switches.

[0030] Further disclosed herein are CAR-EC platforms including (A) CAR-EC switches comprising: (i) a CAR interacting domain and (ii) a target interacting domain (TID); wherein the TID comprises an anti-Her2 antibody fragment Fab (clone 4D5), or a fragment thereof, which Fab comprises an unnatural amino acid located at each of Glycine 212 of a light chain

of the anti-Her2 antibody Fab (LG212X; SEQ ID NO: 2) and Lysine 221 of a heavy chain of the anti-Her2 antibody Fab (HK221X; SEQ ID NO: 1), and wherein each of LG212X and HK221X is attached or linked to a separate CAR interacting domain. and (B) effector cells comprising universal chimeric antigen receptors (CAR) that can bind multiple CAR-EC switches, providing for sequential targeting of one or more types of target cells (e.g. treatment of heterogeneous tumors). The CAR may comprise an ultra-high affinity antibody or antibody fragment (e.g. scFv) to the switch. Methods of producing the CAR-EC switches disclosed herein may advantageously provide for control of CAR-EC cell activity, titration of off-target reactivity, abrogation of tumor lysis syndrome (TLS), attenuation of cytokine release syndrome (CRS), and/or optimization of CAR-EC switch binding by affinity, valency, geometry, length and/or chemistry through site-specific attachment of the TID and CAR-ID.

[0031] The switches disclosed herein may be used in combination with a CAR comprising an extracellular domain, a transmembrane domain an intracellular signaling domain, wherein the extracellular domain comprises: a region that interacts with a chimeric antigen receptor switch; and wherein the CAR optionally comprises a hinge domain. The chimeric antigen receptor switch may comprise a hapten. The hapten may be FITC or a derivative thereof. The region that interacts with a chimeric antigen receptor switch may be an anti-FITC antibody or antibody fragment.

[0032] Unless otherwise specified, the terms "switch" and "CAR-EC switch", as used herein, are used interchangeably and may refer to a FITC switch. The target interacting domain of the switch may comprise an antibody portion (e.g., a FAB portion). The antibody portion of the switch may comprise at least a portion of an antibody or an entire antibody. For example, the antibody portion of the switch may comprise at least a portion of a heavy chain, a portion of a light chain, a portion of a variable region, a portion of a constant region, a portion of a complementarity determining region (CDR), or a combination thereof. The antibody portion of the switch may comprise at least a portion of the Fc (fragment, crystallizable) region. The antibody portion of the switch may comprise at least a portion of the complementarity determining region (e.g., CDR1, CDR2, CDR3). The antibody portion of the switch may comprise at least a portion of the Fab (fragment, antigen-binding) region.

[0033] Before the present methods, kits and compositions are described in greater detail, it is to be understood that this invention is not limited to particular method, kit or composition described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be

limiting, since the scope of the present invention will be limited only by the appended claims. Examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

[0034] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limits of that range is also specifically disclosed. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also encompassed within the invention, 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 invention.

[0035] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, some potential and preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. It is understood that the present disclosure supersedes any disclosure of an incorporated publication to the extent there is a contradiction.

[0036] As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present invention. Any recited method can be carried out in the order of events recited or in any other order which is logically possible.

[0037] It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells and reference to "the peptide" includes reference to one or more peptides and equivalents thereof, e.g. polypeptides, known to those skilled in the art, and so forth.

[0038] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

[0039] Methods, kits and compositions are provided for producing sCAR-EC platforms and CAR-EC switches used to bring an effector cell together with a target in a subject. These methods, kits and compositions find therapeutic use in a number of diseases. For example, heterogeneous tumors and blood cell malignancies (e.g. acute myeloid leukemia and chronic lymphocytic leukemia) may be more effectively treated with a CAR-EC platform when the length, valency and orientation of the CAR-EC switch linkage as well as the CAR-EC switch cell targeting moiety is optimized. Heterogeneous tumors may be more effectively treated with multiple CAR-EC switches that target more than one tumor antigens. AML, ALL, CLL, multiple myeloma, breast cancer, neuroblastoma, pancreatic cancer, endometrial cancer, ovarian cancer, or colon cancer may be more effectively treated with a CAR-EC platform when the length, valency and orientation of the CAR-EC switch linkage as well as the CAR-EC switch cell targeting moiety is optimized.Advantages, and features of the invention will become apparent to those persons skilled in the art upon reading the details of the compositions and methods as more fully described below.

[0040] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CAR-EC Switch

[0041] Disclosed herein are CAR switches (also refered to as "chimeric antigen receptor-effector cell switches" and "CAR-EC switches", interchangeably), methods of producing such switches, and uses thereof. Generally, a switch may comprise (a) a chimeric antigen receptor-interacting domain (CAR-ID); and (b) a target interacting domain TID, .

[0042] The switch may further comprise one or more additional CAR-IDs. The TID comprises an anti-Her2 antibody fragment Fab (clone 4D5) or a target antigen binding fragment thereof. A switch may further comprise one or more linkers. **FIG. 1b** depicts an exemplary switch. A switch may comprise a CAR-ID (**e.g., FITC in FIG. 1b**) attached to an anti-Her2 antibody fragment Fab (clone 4D5) or a target antigen binding fragment thereof. A switch may comprise a CAR-ID, a linker and a TID. The linker may attach the CAR-ID to the TID (**1405**). A switch may comprise a CAR-ID, a first linker, a second linker and a TID. The first linker and second linker may be connected to each other. In addition, the first linker may be attached to the CAR-ID and the second linker may be attached to the TID, thereby resulting in attachment of the CAR-ID to the TID. The first linker and the second linker may be the same. The first linker and the second linker may be different.

[0043] The switch may comprise a TID and two or more CAR-IDs. As shown in **FIG. 56G**, a switch may comprise a TID (**1405**), a first CAR-ID (**1401**), and a second CAR-ID (**1425**). The first CAR-ID (**1401**) and the second CAR-ID (**1425**) may be attached to the TID (**1405**). As shown in **FIG. 56H**, a switch may comprise a TID (**1405**), a linker (**1410**), a first CAR-ID (**1401**), and a second CAR-ID (**1425**). The linker (**1410**) may attach the first CAR-ID (**1401**) to the TID (**1405**). The second CAR-ID (**1425**) may be attached to the TID (**1405**). As shown in **FIG. 56I**, a switch may comprise a TID (**1405**), a first linker (**1410**), a second linker (**1415**), a first CAR-ID (**1401**), and a second CAR-ID (**1425**). The first linker (**1410**) may attach the first CAR-ID (**1401**) to the TID (**1405**). The second linker (**1415**) may attach the second CAR-ID (**1425**) to the TID (**1405**). The first CAR-ID (**1401**) and the second CAR-ID (**1425**) may be the same. The first CAR-ID (**1401**) and the second CAR-ID (**1425**) may be different. The first linker (**1410**) and the second linker (**1415**) may be the same. The first linker (**1410**) and the second linker (**1415**) may be different. The switch may further comprise one or more additional CAR-IDs. The switch may further comprise one or more additional TIDs. The switch may further comprise one or more linkers.

[0044] The CAR-ID may be attached to the TID. Attachment of the CAR-ID to the TID may occur by any method known in the art. For example, the CAR-ID may be attached to the TID by fusion, insertion, grafting, or conjugation. The CAR-ID may be fused to the TID. The

CAR-ID may be inserted into the TID. The CAR-ID may be conjugated to the TID. The CAR-ID may be linked to the TID.

[0045] A switch may comprise (a) a chimeric antigen receptor-interacting domain (CAR-ID); and (b) a target interacting domain TID. The CAR-ID may comprise fluorescein isothiocyanate (FITC). Switches that comprise a CAR-ID comprising a hapten and a TID comprising an antibody or antibody fragment may be referred to as hapten-antibody switches.

[0046] A switch may comprise a chimeric antigen receptor-interacting domain (or "CAR-ID"); and (b) a target interacting domain TID. The CAR-ID may interact with a chimeric antigen receptor (CAR) on an effector cell. The TID may interact with a surface molecule on a target. The TID may comprise an unnatural amino acid. A TID may comprise a polypeptide that is based on or derived from the anti-Her2 antibody fragment Fab (clone 4D5), or a target antigen binding fragment thereof. The antibody or antibody fragment may be modified to contain one or more unnatural amino acids. The CAR-ID may be site-specifically attached to the TID. The CAR-ID may be site-specifically attached to the unnatural amino acid in the TID. The CAR-EC switch may further comprise one or more linkers. The one or more linkers may attach the CAR-ID to the TID. The CAR-EC switch may further comprise one or more unnatural amino acids. The TID may comprise one or more unnatural amino acids. The CAR-ID may be attached to the TID via the one or more unnatural amino acids in the CAR-ID. The CAR-ID may be attached to the TID via the one or more unnatural amino acids in the TID.

Chimeric Antigen Receptor Interacting Domain (CAR-ID)

[0047] The switches disclosed herein may comprise one or more chimeric antigen receptor-interacting domains (CAR-IDs). The switches disclosed herein may comprise two or more chimeric antigen receptor-interacting domains (CAR-IDs). The two or more CAR-IDs may be the same. The two or more CAR-IDs may be different.

[0048] The CAR-ID may be a naturally-occurring molecule. The CAR-ID may be an artificial or synthetic molecule. At least a portion of a CAR-ID may be synthetic. The CAR-ID may comprise a polypeptide that is not naturally occurring. The CAR-ID may be an organic molecule. The CAR-ID may be inorganic molecule.

[0049] The CAR-ID may be a small molecule. The small molecule may be an organic compound. The small molecule may have a size on the order of about 10^{-8} m, about 10^{-9} m, about 10^{-10} m. The small molecule may have a size of less than about 10^{-7} m. The small molecule may have a size of less than about 10^{-8} m. The small molecule may have a size of less than about 10^{-9} m. The small molecule may have a size of less than about 10^{-10} m. The

small molecule may have a size of less than about 10^{-11} m. The small molecule may have a mass of less than about 5000 Da, less than about 4500 Da, less than about 4000 Da, less than about 3500 Da, less than about 3000 Da, less than about 2500 Da, less than about 2000 Da, less than about 1500 Da, less than about 1000 Da, less than about 900 D, less than about 500 Da or less than about 100 Da. In some instances, the small molecule does not comprise a polypeptide. In some instances, the small molecule does comprise two or more amino acids that are linked by an amide bond. The small molecule may be a chemical compound.

[0050] The CAR-ID may be selected from DOTA, dinitrophenol, quinone, biotin, aniline, atrazine, an aniline-derivative, o-aminobenzoic acid, p-aminobenzoic acid, m-aminobenzoic acid, hydralazine, halothane, digoxigenin, benzene arsonate, lactose, trinitrophenol, biotin, FITC, or a derivative thereof. The CAR-ID may be a quinone or a derivative thereof. The CAR-ID may be DOTA or a derivative thereof. The CAR-ID may be dinitrophenol or a derivative thereof. The CAR-ID may be biotin or a derivative thereof. The CAR-ID may comprise a hapten. The CAR-ID may induce an immune response when attached to a larger carrier molecule, such as a protein, antibody or antibody fragment. The CAR-ID may be FITC or a derivative thereof. The CAR-ID may comprise biotin. The CAR-ID may comprise dinitrophenol.

[0051] Alternatively, the CAR-ID does not comprise a hapten. The CAR-ID may be selected from a steroid, a vitamin, a vitamer, a metabolite, an antibiotic, a monosaccharide, a disaccharide, a lipid, a fatty acid, a nucleic acid, an alkaloid, a glycoside, a phenzine, a polyketide, a terpene, and a tetrapyrrole, and portions thereof, and combinations thereof. The CAR-ID may be a penicillin drug or a derivative thereof.

[0052] The CAR-ID may be linked and/or conjugated to the target interacting domain. The target interacting domain is the anti-Her2 antibody fragment Fab (clone 4D5), or a target antigen binding fragment thereof and the CAR-ID may be linked and/or conjugated to an unnatural amino acid of the targeting antibody or antibody fragment, wherein the unnatural amino acid is present in the light chain, present in the heavy chain, or wherein there is an unnatural amino acid present in both the light chain and the heavy chain, to which the CAR-ID is linked and/or conjugated to the TID. The TID may comprise a light chain and/or heavy chain selected from SEQ ID NOS: 1-2 and the unnatural amino acids may be located at respective sites light chain glycine 212 and heavy chain lysine 221.

Target Interacting Domain TID

[0053] The switches disclosed herein may comprise one target interacting domain (TIDs), wherein the TID comprises a polypeptide that is based on or derived from the anti-Her2 antibody fragment Fab (clone 4D5), or a target antigen binding fragment thereof, such polypeptide comprising one or more unnatural amino acids replacing glycine 212 of a light chain of the anti-Her2 antibody or fragment thereof, lysine 221 of a heavy chain of the anti-Her2 antibody or fragment thereof, or replacing both of glycine 212 of a light chain and lysine 221 of a heavy chain of an anti-Her2 antibody or fragment thereof.

[0054] The targeting moiety may bind to Her2 expressed on the surface of a target cell.

[0055] The targeting polypeptide may be a targeting antibody or antibody fragment. The targeting antibody or antibody fragment may be an immunoglobulin (Ig). The immunoglobulin may selected from an IgG, an IgA, an IgD, an IgE, an IgM, a fragment thereof or a modification thereof. The immunoglobulin may be IgG. The IgG may be IgG1. The IgG may be IgG2. The IgG may have one or more Fc mutations for modulating endogenous T cell FcR binding to the CAR-EC switch. The IgG may have one or more Fc mutations for removing the Fc binding capacity to the FcR of FcR-positive cells. Removal of the Fc binding capacity may reduce the opportunity for crosslinking of the CAR-EC to FcR positive cells, wherein crosslinking of the CAR-EC to FcR positive cells would activate the CAR-EC in the absence of the target cell. As such, modulating the endogenous T cell FcR binding to the CAR-EC switch may reduce an ineffective or undesirable immune response. The one or more Fc mutations may remove a glycosylation site. The one or more Fc mutations may be selected from E233P, L234V, L235A, delG236, A327G, A330S, P331S, N297Q and any combination thereof. The one or more Fc mutations may be in IgG1. The one or more Fc mutations in the IgG1 may be L234A, L235A, or both. Alternatively, or additionally, the one or more Fc mutations in the IgG1 may be L234A, L235E, or both. Alternatively, or additionally, the one or more Fc mutations in the IgG1 may be N297A. Alternatively, or additionally, the one or more mutations may be in IgG2. The one or more Fc mutations in the IgG2 may be V234A, V237A, or both.

[0056] The targeting antibody or antibody fragment may be an Fc null immunoglobulin or a fragment thereof.

[0057] As used herein, the term "antibody fragment" refers to any form of an antibody other than the full-length form. Antibody fragments herein include antibodies that are smaller components that exist within full-length antibodies, and antibodies that have been engineered.

Antibody fragments include, but are not limited to, Fv, Fc, Fab, and (Fab')2, single chain Fv (scFv), diabodies, triabodies, tetrabodies, bifunctional hybrid antibodies, CDRl, CDR2, CDR3, combinations of CDRs, variable regions, framework regions, constant regions, heavy chains, light chains, alternative scaffold non-antibody molecules, and bispecific antibodies. Unless specifically noted otherwise, statements and claims that use the term "antibody" or "antibodies" may specifically include "antibody fragment" and "antibody fragments."

[0058] The targeting antibody fragment may be human, fully human, humanized, human engineered, non-human, and/or chimeric antibody. The non-human antibody may be humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Chimeric antibodies may refer to antibodies created through the joining of two or more antibody genes which originally encoded for separate antibodies. A chimeric antibody may comprise at least one amino acid from a first antibody and at least one amino acid from a second antibody, wherein the first and second antibodies are different. At least a portion of the antibody or antibody fragment may be from a bovine species, a human species, or a murine species. At least a portion of the antibody or antibody fragment may be from a rat, a goat, a guinea pig or a rabbit. At least a portion of the antibody or antibody fragment may be from a human. At least a portion of the antibody or antibody fragment antibody may be from cynomolgus monkey.

[0059] The targeting antibody or antibody fragment may be based on or derived from an antibody or antibody fragment from a mammal, bird, fish, amphibian, or reptile. Mammals include, but are not limited to, carnivores, rodents, elephants, marsupials, rabbits, bats, primates, seals, anteaters, cetaceans, odd-toed ungulates and even-toed ungulates. The mammal may be a human, non-human primate, mouse, sheep, cat, dog, cow, horse, goat, or pig.

[0060] The targeting antibody or an antibody fragment may target a Her2 antigen or a fragment thereof.

[0061] The TID may comprise an anti-Her2 antibody or fragment thereof. The light chain of the anti-Her2 antibody or fragment thereof may comprise SEQ ID NO: 1 or a homologous amino acid sequence. The homologous amino acid sequence may be about 99%, about 98%, about 97%, about 96%, about 95%, about 92%, about 90%, about 85%, about 80%, about 75%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, about 15%, about 10%, about 5% or about 2% homologous to SEQ ID NO: 1. The heavy chain of the anti-Her2 antibody or fragment

thereof may comprise SEQ ID NO: 2 or a homologous amino acid sequence. The amino acid sequence may be about 99%, about 98%, about 97%, about 96%, about 95%, about 92%, about 90%, about 85%, about 80%, about 75%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, about 15%, about 10%, about 5% or about 2% homologous to SEQ ID NO: 2.

[0062] The CAR-EC switches disclosed herein may comprise one or more unnatural amino acids. The one or more CAR-IDs may comprise one or more unnatural amino acids. The TID may comprise one or more unnatural amino acids. The one or more linkers may comprise one or more unnatural amino acids. Attachment (or "connection," interchangeably) of the CAR-ID to the TID may occur via the one or more unnatural amino acids. The one or more linkers may link the one or more CAR-IDs to the TID site-specifically through the one or more unnatural amino acids.

[0063] The targeting antibodies or antibody fragments disclosed herein may comprise 2, 3, 4, 5, 6, 7, 8, 9, 10 or more unnatural amino acids. The unnatural amino acid may react with the linker to create a chemical bond.

[0064] The one or more unnatural amino acids may be inserted between two naturally occurring amino acids in the TID. The one or more unnatural amino acids may replace one or more naturally occurring amino acids in the TID. The one or more unnatural amino acids may be incorporated at the N terminus of the TID. The one or more unnatural amino acids may be incorporated at the C terminus of the TID. The unnatural amino acid may be incorporated distal to the region of the TID that interacts with a molecule on or from a target. The unnatural amino acid may be incorporated near the region of the TID that interacts with a molecule on or from a target. The unnatural amino acid may be incorporated in the region of the TID that interacts with a molecule on or from a target.

[0065] The one or more unnatural amino acids may replace one or more amino acids in the TID. The one or more unnatural amino acids may replace any natural amino acid in the TID.

[0066] The one or more unnatural amino acids may be incorporated in a light chain of the immunoglobulin from which the TID is based or derived. The one or more unnatural amino acids may be incorporated in a heavy chain of the immunoglobulin from which the TID is based or derived. The one or more unnatural amino acids may be incorporated in a heavy chain and a light chain of the immunoglobulin from which the TID is based or derived. The one or more unnatural amino acids may replace an amino acid in the light chain of the

immunoglobulin from which the TID is based or derived. The one or more unnatural amino acids may replace an amino acid in a heavy chain of the immunoglobulin from which the TID is based or derived. The one or more unnatural amino acids may replace an amino acid in a heavy chain and a light chain of the immunoglobulin from which the TID is based or derived.

[0067] The one or more unnatural amino acids may replace a glycine of a light chain of the immunoglobulin from which the TID is based or derived. The one or more unnatural amino acids may replace an arginine of a light chain of the immunoglobulin from which the TID is based or derived. The one or more unnatural amino acids may replace a serine of a light chain of the immunoglobulin from which the TID is based or derived. The one or more unnatural amino acids may replace a threonine of a light chain of the immunoglobulin from which the TID is based or derived. The one or more unnatural amino acids may replace an alanine of a light chain of the immunoglobulin from which the TID is based or derived. The one or more unnatural amino acids may replace an alanine of a heavy chain of the immunoglobulin from which the TID is based or derived. The one or more unnatural amino acids may replace a serine of a heavy chain of the immunoglobulin from which the TID is based or derived. The one or more unnatural amino acids may replace a lysine of a heavy chain of the immunoglobulin from which the TID is based or derived. The one or more unnatural amino acids may replace a proline of a heavy chain of the immunoglobulin from which the TID is based or derived.

[0068] The one or more unnatural amino acids may replace an amino acid of the TID, wherein the TID is an anti-Her2 antibody or fragment thereof. The one or more unnatural amino acids may replace glycine 212 of a light chain of the anti-Her2 antibody or fragment thereof. The one or more unnatural amino acids may replace lysine 221 of a heavy chain of the anti-Her2 antibody or fragment thereof. The antibody or antibody fragment may be an anti-Her2 antibody or fragment thereof, wherein the one or more unnatural amino acids may replace one or more amino acids of a light chain of the anti-Her2 antibody or fragment thereof. The light chain of the anti-Her2 antibody or fragment thereof may comprise SEQ ID NO: 1. The one or more unnatural amino acids may replace one or more amino acids of SEQ ID NO: 1. The one or more amino acids of SEQ ID NO: 1 comprises lysine 212.. The one or more unnatural amino acids may replace one or more amino acids of a heavy chain of the anti-Her2 antibody or fragment thereof. The heavy chain of the anti-Her2 antibody or fragment thereof may comprise SEQ ID NO: 2. The one or more unnatural amino acids may

replace one or more amino acids of SEQ ID NO: 2. The one or more amino acids of SEQ ID NO: 2 comprises lysine 221.

[0069] The one or more unnatural amino acids may be encoded by a codon that does not code for one of the twenty natural amino acids. The one or more unnatural amino acids may be encoded by a nonsense codon (stop codon). The stop codon may be an amber codon. The amber codon may comprise a UAG sequence. The stop codon may be an ochre codon. The ochre codon may comprise a UAA sequence. The stop codon may be an opal or umber codon. The opal or umber codon may comprise a UGA sequence. The one or more unnatural amino acids may be encoded by a four-base codon.

[0070] The one or more unnatural amino acids may be *p*-acetylphenylalanine (pAcF or pAcPhe). The one or more unnatural amino acids may be selenocysteine. The one or more unnatural amino acids may be *p*-fluorophenylalanine (pFPhe). The one or more unnatural amino acids may be selected from the group comprising *p*-azidophenylalanine (pAzF),p-azidomethylphenylalanine(pAzCH$_2$F), *p*-benzoylphenylalanine (pBpF), *p*-propargyloxyphenylalanine (pPrF), *p*-iodophenylalanine (pIF), *p*-cyanophenylalanine (pCNF), *p*-carboxylmethylphenylalanine (pCmF), 3-(2-naphthyl)alanine (NapA), *p*-boronophenylalanine (pBoF), *o*-nitrophenylalanine (oNiF), (8-hydroxyquinolin-3-yl)alanine (HQA), selenocysteine, and (2,2'-bipyridin-5-yl)alanine (BipyA).). The one or more unnatural amino acids may be 4-(6-methyl-*s*-tetrazin-3-yl)aminopheynlalanine.

[0071] The one or more unnatural amino acids may be β-amino acids (β3 and β2), homo-amino acids, proline and pyruvic acid derivatives, 3-substituted alanine derivatives, glycine derivatives, ring-substituted phenylalanine and tyrosine derivatives, linear core amino acids, diamino acids, D-amino acids, N-methyl amino acids, or a combination thereof.

[0072] Additional examples of unnatural amino acids include, but are not limited to, 1) various substituted tyrosine and phenylalanine analogues such as *O*-methyl-L-tyrosine, *p*-amino-L-phenylalanine, 3-nitro-L-tyrosine, *p*-nitro-L-phenylalanine, *m*-methoxy-L-phenylalanine and *p*-isopropyl-L-phenylalanine; 2) amino acids with aryl azide and benzophenone groups that may be photo-cross-linked; 3) amino acids that have unique chemical reactivity including acetyl-L-phenylalanine and *m*-acetyl-L-phenylalanine, *O*-allyl-L-tyrosine, *O*-(2-propynyl)-L-tyrosine, *p*-ethylthiocarbonyl-L-phenylalanine and *p*-(3-oxobutanoyl)-L-phenylalanine; 4) heavy-atom-containing amino acids for phasing in X-ray crystallography including *p*-iodo and *p*-bromo-L-phenylalanine; 5) the redox-active amino acid dihydroxy-L-phenylalanine; 6) glycosylated amino acids including b-N-

acetylglucosamine-*O*-serine and a-N-acetylgalactosamine-*O*-threonine; 7) fluorescent amino acids with naphthyl, dansyl, and 7-aminocoumarin side chains; 8) photocleavable and photoisomerizable amino acids with azobenzene and nitrobenzyl Cys, Ser, and Tyr side chains; 9) the phosphotyrosine mimetic *p*-carboxymethyl-L-phenylalanine; 10) the glutamine homologue homoglutamine; and 11) 2-aminooctanoic acid. The unnatural amino acid may be modified to incorporate a chemical group. The unnatural amino acid may be modified to incorporate a ketone group.

[0073] The one or more unnatural amino acids may comprise at least one oxime, carbonyl, dicarbonyl, hydroxylamine group or a combination thereof. The one or more unnatural amino acids may comprise at least one carbonyl, dicarbonyl, alkoxy-amine, hydrazine, acyclic alkene, acyclic alkyne, cyclooctyne, aryl/alkyl azide, norbornene, cyclopropene, trans-cyclooctene, or tetrazine functional group or a combination thereof.

[0074] The one or more unnatural amino acids may be incorporated into the TID by methods known in the art. Cell-based or cell-free systems may be used to alter the genetic sequence of the TID, thereby producing the TID with one or more unnatural amino acids. Auxotrophic strains may be used in place of engineered tRNA and synthetase. The one or more unnatural amino acids may be produced through selective reaction of one or more natural amino acids. The selective reaction may be mediated by one or more enzymes. In one non-limiting example, the selective reaction of one or more cysteines with formylglycine generating enzyme (FGE) may produce one or more formylglycines (see Rabuka *et al.*, Nature Protocols 7:1052-1067 (2012), which is incorporated by reference in its entirety).

[0075] The one or more unnatural amino acids may take part in a chemical reaction to form a linker. The chemical reaction to form the linker may be a bioorthogonal reaction. The chemical reaction to form the linker may be click chemistry.

[0076] Additional unnatural amino acids are disclosed in Liu et al. (*Annu Rev Biochem*, 79:413-44, 2010), Wang et al. (*Angew Chem Int Ed*, 44:34-66, 2005) and PCT application numbers PCT/US2012/039472, PCT/US2012/039468, PCT/US2007/088009, PCT/US2009/058668, PCT/US2007/089142, PCT/US2007/088011, PCT/US2007/001485, PCT/US2006/049397, PCT/US2006/047822 and PCT/US2006/044682, all of which are incorporated by reference in their entireties.

Linkers

[0077] The switches disclosed herein may comprise one or more linkers. The switches disclosed herein may comprise two or more linkers. The switches disclosed herein may comprise three or more linkers. The switches disclosed herein may comprise four or more linkers. The switches disclosed herein may comprise 5, 6, 7, 8, 9, 10 or more linkers. The two or more linkers may be the same. At least two of the three or more linkers may be the same. The two or more linkers may be different. At least two of the three or more linkers may be different. The linker may be a bifunctional linker. The linker may be a heterobifunctional linker. The linker may be a homobifunctional linker. The linker may further comprise one or more polyethylene glycol subunits. The linker may comprise at least four PEG subunits. The linker may comprise at least 10 PEG subunits. The linker may comprise at least 20 PEG subunits. The linker may comprise at least 30 PEG subunits. The linker may comprise an azide at one end. The linker may comprise an aminooxy at one end. The linker may be an azide-PEG-aminooxy linker. The linker may comprise cyclooctyne at one end. The linker may be a PEG-cyclooctyne linker. The linker may comprise triazole. The triazole may be a 1,2,3-triazole or a 1,2,4-triazole. The linker may be a NHS-ester linker. The linker may be a TriA linker. The linker may be attached to the CAR-ID by oxime ligation.

[0078] Nonlimiting exemplary linkers including heterobifunctional linkers and methods of constructing linkers can be found in WO2014/153002, which is incorporated by reference in its entirety.

[0079] The linker may be attached to a chimeric antigen receptor-interacting domain (CAR-ID). The linker may be attached to a target interacting domain TID. The linker may attach a CAR-ID to a TID. The one or more linkers may attach the one or more CAR-IDs to the TIDs. The one or more linkers may attach the one or more CAR-IDs to the TIDs in a site-specific manner. Attachment in a site-specific manner may comprise attaching the one or more CAR-IDs to a predetermined site on the TIDs. Alternatively, or additionally, attachment in a site-specific manner may comprise attaching the one or more CAR-IDs to an unnatural amino acid in the one or more TIDs. The TID may be attached to 1, 2, 3, 4, 5 or more CAR-IDs in a site-specific manner. Attachment in a site-specific manner may comprise attaching the TID to a predetermined site on the one or more CAR-IDs. The TID may be attached to 1, 2, 3, 4, 5 or more CAR-IDs in a site-independent manner. Attachment in a site-independent manner may comprise attaching theTID to a random site on the one or more CAR-IDs.

[0080] The one or more linkers may be coupled to the CAR-ID, the TID, or a combination thereof.

[0081] The TID may comprise one or more amino acids. The one or more amino acids may comprise a natural amino acid. The linker may couple with one or more natural amino acids on the TID. The one or more amino acids may comprise one or more unnatural amino acids. The linker may couple with one or more unnatural amino acids on the TP. The linker may couple with an amino acid which is the product of site-specific mutagenesis. The linker may couple with a cysteine which is the product of site-specific mutagenesis. The linker (e.g., substituted maleimide) may couple with a cysteine which is the product of site-specific mutagenesis, as well as a native cysteine residue. Two linkers, each with complementary reactive functional groups, may couple with one another.

[0082] The one or more linkers may be a cleavable linker. The one or more linkers may be a non-cleavable linker. The one or more linkers may be a flexible linker. The one or more linkers may be an inflexible linker. The linker may be a bifunctional linker. A bifunctional linker may comprise a first functional group on one end and a second functional group on the second end. The bifunctional linker may be heterobifunctional linker. A heterobifunctional linker may comprise a first functional group on one end and a second functional group on the second end, wherein the first functional group and the second functional group are different. The bifunctional linker may be a homobifunctional linker. A homobifunctional linker may comprise a first functional group on one end and a second functional group on the second end, wherein the first functional group and the second functional group are the same.

[0083] The linker may comprise a chemical bond. The linker may comprise a functional group. The linker may comprise a polymer. The polymer may be a polyethylene glycol. The linker may comprise an amino acid.

[0084] The linker may comprise one or more functional groups. The linker may comprise two or more functional groups. The linker may comprise three or more functional groups. The linker may comprise four or more functional groups. The linker may comprise 5, 6, 7, 8, 9, 10 or more functional groups. The linker may be a bifunctional ethylene glycol linker.

[0085] The linker may comprise ethylene glycol. The linker may comprise about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19 or about 20 or more ethylene glycol subunits. The linker may comprise 4 or more ethylene glycol subunits. The linker may comprise 8 or more ethylene glycol subunits. The linker may comprise 10 or more ethylene glycol subunits. The linker may comprise 12 or more ethylene glycol subunits. The linker may comprise 15 or more ethylene glycol subunits. The linker may comprise 20 or

more ethylene glycol subunits. The linker may comprise 25 or more ethylene glycol subunits. The linker may comprise 30 or more ethylene glycol subunits. The linker may comprise 35 or more ethylene glycol subunits.

[0086] The linker may comprise polyethylene glycol (PEG). The linker may comprise about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19 or about 20 or more polyethylene glycol (PEG) subunits. The linker may comprise 4 or more polyethylene glycol (PEG) subunits. The linker may comprise 8 or more polyethylene glycol (PEG) subunits. The linker may comprise 10 or more polyethylene glycol (PEG) subunits. The linker may comprise 12 or more polyethylene glycol (PEG) subunits. The linker may comprise 15 or more polyethylene glycol (PEG) subunits. The linker may comprise 20 or more polyethylene glycol (PEG) subunits. The linker may comprise 25 or more polyethylene glycol (PEG) subunits. The linker may comprise 30 or more polyethylene glycol (PEG) subunits. The linker may comprise 35 or more polyethylene glycol (PEG) subunits.

[0087] The linker may comprise a triazole. The triazole may be a 1,2,3-triazole. The triazole may be a 1,2,4-triazole.

[0088] The linker may comprise an aryl or a heteroaryl. The linker may comprise an aryl. The aryl may be phenyl. The phenyl may be disubstituted. The disubstituted phenyl may be 1,4-disubstituted phenyl. The disubstituted phenyl may be 1,3-disubstituted phenyl. The phenyl may be trisubstituted. The phenyl may be tetrasubstituted. Two of the substituents of the substituted phenyl may be NO_2. In some instances, the linker does not comprise a benzyl substituent.

[0089] The linker may comprise one or more polyethylene glycol (PEG) units. The linker may comprise multiple polyethylene glycol (PEG) units. The linker may comprise 2 or more polyethylene glycol (PEG) units. The linker may comprise 3 or more polyethylene glycol (PEG) units. The linker may comprise 4 or more polyethylene glycol (PEG) units. The linker may comprise 5 or more polyethylene glycol (PEG) units. The linker may comprise 6 or more polyethylene glycol (PEG) units. The linker may comprise 7 or more polyethylene glycol (PEG) units. The linker may comprise 8 or more polyethylene glycol (PEG) units. The linker may comprise 9 or more polyethylene glycol (PEG) units. The linker may comprise 10 or more polyethylene glycol (PEG) units. The linker may comprise 11 or more polyethylene glycol (PEG) units. The linker may comprise 12 or more polyethylene glycol (PEG) units.

The linker may comprise 13 or more polyethylene glycol (PEG) units. The linker may comprise 14 or more polyethylene glycol (PEG) units.

[0090] The linker may comprise an amide on one end. The linker may comprise an amide on one end and an amine on the other end. The linker may comprise an amide on one end and a triazole on the other end.

[0091] The one or more linkers may comprise a 1,4-dicarboxylic moiety. The one or more linkers may comprise a 1,3-dinitro substituted phenyl moiety.

[0092] The one or more linkers may comprise one or more reactive functional groups. The reactive functional group may react with a complementary reactive functional group on a coupling partner. The reaction of the reactive functional group on the linker to a complementary reactive functional group on a coupling partner may occur prior to incorporation of the linker into the CAR-EC switch.

[0093] The linker may comprise at least one reactive functional group selected from alkoxy-amine, hydrazine, aryl/alkyl azide, alkyne, alkene, tetrazine, dichlorotriazine, tresylate, succinimidyl carbonate, benzotriazole carbonate, nitrophenyl carbonate, trichlorophenyl carbonate, carbonylimidazole, succinimidyl succinate, maleimide, vinylsulfone, haloacetamide, and disulfide. The alkene may be selected from norbornene, trans-cyclooctene, and cyclopropene. The linker may comprise at least one alkoxy amine. The linker may comprise at least one azide. The linker may comprise at least one cyclooctyne. The linker may comprise at least one tetrazine.

[0094] The one or more linkers may comprise an alkoxy-amine (or aminooxy) group, azide group and/or cyclooctyne group at one or more termini. The one or more linkers may comprise an alkoxy-amine at one terminus and an azide group at the other terminus. The one or more linkers may comprise an alkoxy-amine at one terminus and a cyclooctyne group at the other terminus. The alkoxy-amine may form a stable oxime with a ketone group on an amino acid. The alkoxy-amine may form a stable oxime with a ketone group on an unnatural amino acid. The ketone group may be on a *p*-acetyl phenylalanine (pAcF).

[0095] One or more linkers may be formed by reaction of reactive functional group on the CAR-ID with a complementary reactive functional group of a linker that is attached to the TID. One or more linkers may be formed by reaction of an amino acid or another reactive functional group on the TID with a complementary reactive functional group of a linker that is attached to the CAR-ID. One or more linkers may be formed by reaction of a linker that is attached to the CAR-ID with another linker that is attached to the TID.

[0096] The linker may be the product of a bioorthogonal reaction. For example, amino acids that contain ketone, azide, alkyne, alkene, and tetrazine side chains can be genetically encoded in response to nonsense and frameshift codons. These side chains can act as chemical handles for bioorthogonal conjugation reactions (Kim *et al.*, Curr Opin Chem Bio 17:412-419 (2013), which is incorporated by reference in its entirety). The linker may comprise an oxime, a tetrazole, a Diels Alder adduct, a hetero Diels Alder adduct, an aromatic substitution reaction product, a nucleophilic substitution reaction product, an ester, an amide, a carbamate, an ether, a thioether, or a Michael reaction product. The linker may be a cycloaddition product, a metathesis reaction product, a metal-mediated cross-coupling reaction product, a radical polymerization product, an oxidative coupling product, an acyl-transfer reaction product, or a photo click reaction product. The cycloaddition may be a Huisgen-cycloaddition. The cycloaddition may be a copper-free [3+2] Huisgen-cycloaddition. The cycloaddition may be a Diels-Alder reaction. The cycloaddition may be a hetero Diels-Alder reaction. The linker may be the product of an enzyme-mediated reaction. The linker may be a product of a transglutaminase-mediated reaction, non-limiting examples of which are described in Lin *et al.*, J. Am. Chem. Soc. 128:4542-4543 (2006) and WO 2013/093809. The linker may comprise a disulfide bridge that connects two cysteine residues, such as ThioBridge™ technology by PolyTherics. The linker may comprise a maleimide bridge that connects two amino acid residues. The linker may comprise a maleimide bridge that connects two cysteine residues.

[0097] Two or more linkers may be linked. The two or more linkers may be linked through one or more copper-free reactions. The two or more linkers may be linked through one or more cycloadditions. The two or more linkers may be linked through one or more Huisgen-cycloadditions. The two or more linkers may be linked through one or more copper-free [3+2] Huisgen-cycloadditions. The two or more linkers may be linked through one or more copper-containing reactions. The two or more linkers may be linked through one or more Diels Alder reactions. The two or more linkers may be linked through one or more hetero Diels Alder reactions.

[0098] CAR-EC switches may be optimized by adjusting linker length. CAR-EC switches may comprise linkers of different lengths. Linkers may be relatively short. Linkers may be relatively long. The one or more linkers may be between about 1 angstroms (Å) to about 120 angstroms (Å) in length. The one or more linkers may be between about 5 angstroms (Å) to about 105 angstroms (Å) in length. The one or more linkers may be

between about 10 angstroms (Å) to about 100 angstroms (Å) in length. The one or more linkers may be between about 10 angstroms (Å) to about 90 angstroms (Å) in length. The one or more linkers may be between about 10 angstroms (Å) to about 80 angstroms (Å) in length. The one or more linkers may be between about 10 angstroms (Å) to about 70 angstroms (Å) in length. The one or more linkers may be between about 15 angstroms (Å) to about 45 angstroms (Å) in length. The one or more linkers may be equal to or greater than about 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 27, 30 or more angstroms in length. The one or more linkers may be equal to or greater than about 10 angstroms in length. The one or more linkers may be equal to or greater than about 15 angstroms in length. The one or more linkers may be equal to or greater than about 20 angstroms in length. The one or more linkers may be equal to or less than about 110, 100, 90, 85, 80, 75, 70, 65, 60, 55, 50, 45, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30 or fewer angstroms in length. The one or more linkers may be equal to or less than about 100 angstroms in length. The one or more linkers may be equal to or less than about 80 angstroms in length. The one or more linkers may be equal to or less than about 60 angstroms in length. The one or more linkers may be equal to or less than about 40 angstroms in length.

[0099] The total length of the linkers may be between about 1 angstroms (Å) to about 120 angstroms (Å). The total length of the linkers may be between about 5 angstroms (Å) to about 105 angstroms (Å). The total length of the linkers may be between about 10 angstroms (Å) to about 100 angstroms (Å). The total length of the linkers may be between about 10 angstroms (Å) to about 90 angstroms (Å). The total length of the linkers may be between about 10 angstroms (Å) to about 80 angstroms (Å). The total length of the linkers may be between about 10 angstroms (Å) to about 70 angstroms (Å). The total length of the linkers may be between about 15 angstroms (Å) to about 45 angstroms (Å). The total length of the linkers may be equal to or greater than about 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 27, 30 or more angstroms. The total length of the linkers may be equal to or greater than about 10 angstroms. The total length of the linkers may be equal to or greater than about 15 angstroms. The total length of the linkers may be equal to or greater than about 20 angstroms. The total length of the linkers may be equal to or less than about 110, 100, 90, 85, 80, 75, 70, 65, 60, 55, 50, 45, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30 or fewer angstroms. The total length of the linkers may be equal to or less than about 100 angstroms. The total length of the linkers may be equal to or less than about 80 angstroms. The total length of the linkers may be equal to or less than about 60 angstroms. The total length of the linkers may

be equal to or less than about 40 angstroms. The total length of the linkers may be equal to or less than about 25 Å. The distance between the CAR-ID and the TID may be about 30 Å.

[0100] Disclosed herein are compositions comprising a plurality of switches, wherein a switch of the plurality of switches comprises (a) a CAR-ID; (b) a TID; and (c) a linker, wherein at least about 60% of the switches of the plurality of switches are structurally homogeneous. At least about 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68% or 69% of the switches of the plurality of switches may be structurally homogeneous. At least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78% or 79% of the switches of the plurality of switches may be structurally homogeneous. At least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88% or 89% of the switches of the plurality of switches may be structurally homogeneous. At least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% of the switches of the plurality of switches may be structurally homogeneous. Structurally homogeneous CAR-EC switches may be provided for by site-specifically linking the CAR-ID and the TID. The linker may be linked to a CAR-ID site-specifically. The linker may be linked to a TID site-specifically. A first site of the linker may be linked to a CAR-ID site-specifically and a second site of the linker may be linked to a TID site-specifically.

Targets

[0101] Disclosed herein are chimeric antigen receptor effector cell (CAR-EC) switches comprising a CAR-ID and a TID. Generally, binding of the effector cell and the target cell to the CAR-EC switch construct brings the target cell into proximity with the effector cell sufficiently close for an activity of the effector cell to have an effect on the target cell. For example, when a T cell expressing a CAR with specificitiy for the CAR-ID and a target cell are bound to a CAR-EC switch, the T cell may produce an immune response that has a cytotoxic effect on the target cell.

[0102] The CAR-EC switches may interact with a plurality of target cells. The target cell may be an infected cell. The target cell may be a pathogenically infected cell. The target cell may be a diseased cell. The target cell may be a genetically-modified cell. The target cell may not be a host cell. The target cell may come from an invading organism (e.g. yeast, worm, bacteria, fungi). Further disclosed herein are CAR-EC switches that interact with a molecule on a non-cell target. The non-cell target may be a virus or a portion thereof. The non-cell target may be a fragment of a cell. The non-cell target may be an extracellular matrix component or protein.

[0103] The target cell may be derived from a tissue. The tissue may be selected from brain, esophagus, breast, colon, lung, glia, ovary, uterus, testes, prostate, gastrointestinal tract, bladder, liver, thymus, bone and skin. The target cell may be derived from one or more endocrine glands. The endocrine gland may be a lymph gland, pituitary gland, thyroid gland, parathyroid gland, pancreas, gonad or pineal gland.

[0104] The target cell may be selected from a stem cell, a pluripotent cell, a hematopoietic stem cell or a progenitor cell. The target cell may a circulating cell. The target cell may be an immune cell.

[0105] The target cell may be a cancer stem cell. The target cell may be a cancer cell. The cancer cell may be derived from a tissue. The tissue may be selected from, by way of non-limiting example, a brain, an esophagus, a breast, a colon, a lung, a glia, an ovary, a uterus, a testicle, a prostate, a gastrointestinal tract, a bladder, a liver, a thyroid and skin. The cancer cell may be derived from bone. The cancer cell may be derived from blood. The cancer cell may be derived from a B cell, a T cell, a monocyte, a thrombocyte, a leukocyte, a neutrophil, an eosinophil, a basophil, a lymphocyte, a hematopoietic stem cell or an endothelial cell progenitor. The cancer cell may be derived from a $CD19^+$ B lymphocyte. The cancer cell may be derived from a stem cell. The cancer cell may be derived from a pluripotent cell. The cancer cell may be derived from one or more endocrine glands. The endocrine gland may be a lymph gland, pituitary gland, thyroid gland, parathyroid gland, pancreas, gonad or pineal gland.

[0106] The cancer cell may be a $CD19^+$ cell. The cancer cell may be a $CD19^+$ B lymphocyte. The cancer cell may be a $Her2^+$ cell. The Her2+ cell may be a $Her2^+$ breast cancer cell. The target cell may be a $BCMA^+$ cell. The cancer cell may be a $BCMA^+$ multiple myeloma cell. The cancer cell may be a $CS1^+$ cell. The $CS1^+$ cell may be a multiple myeloma cell. The cancer cell may be an EGFRvIII-positive cell. The cancer cell may be an EGFRvIII-positive glioblastoma cell. The cancer cell may be a $CD20^+$ cell. The cancer cell may be a $CD22^+$ cell. The cancer cell may be a $CD123^+$ cell. The cancer cell may be a $CD33^+$ cell. The cancer cell may be a CEA-positive cell.

[0107] The cell surface molecule may be a Her2 antigen. The Her2 antigen may be at least a portion of a surface antigen or a cell surface marker on a cell.

Multivalent CAR-EC Switches

[0108] Exemplified herein are CAR-EC switches comprising a chimeric antigen receptor interacting domain (CAR-ID) and a target interacting domain TID; wherein the TID comprises an anti-Her2 antibody fragment Fab (clone 4D5), or a fragment thereof, which Fab comprises an unnatural amino acid located at each of Glycine 212 of a light chain of the anti-Her2 antibody Fab (LG212X; SEQ ID NO: 2) and Lysine 221 of a heavy chain of the anti-Her2 antibody Fab (HK221X; SEQ ID NO: 1), and wherein each of LG212X and HK221X is attached or linked to a separate CAR interacting domain. However, one skilled in the art would understand that these switches could further comprise additional CAR-IDs. One or more CAR-IDs may be linked/conjugated into one or more internal sites of the TID. One or more CAR-IDs may be linked/conjugated to one or more termini of the TID. Such switches are referred to herein as a "multivalent switch." As is clear to one skilled in the art, in some embodiments, expression of a heavy and light chain portion of the TID results in the formation of a heavy/light chain molecule (e.g., LG212X/ HK221X).

[0109] Multivalent switches are advantageous in CAR-T cell activation for at least the reason that multiple CARs are recruited for every one switch (and correspondingly one antigen) (**FIG. 58**). This is expected to increase the signal transduction, CAR-T cell activation, and target cell lysis. The multivalent switch may bind to a CAR with a longer hinge region than that of canonical CARs in order for the CAR to access multiple peptides of the switch. However, the multivalent switch may, alternatively or additionally, have an optimal geometry and/or length for efficient activity with the CAR, including a canonical CARs or CARs with short hinges.

[0110] A first CAR-ID may be linked or conjugated to a first domain of the TID and a second CAR-ID may be linked or conjugated to a second domain of the TID. The first domain and the second domain may be the same. The first domain and the second domain may be different. By way of non-limiting example, the first CAR-ID may be linked to a light chain of a targeting antibody or antibody fragment and a second CAR-ID may be linked to heavy chain of the targeting antibody or antibody fragment. The first CAR-ID may be conjugated to a first terminus of the targeting polypeptide and a second CAR-ID may be conjugated to a second terminus of the targeting polypeptide. By way of non-limiting example, the first CAR-ID may be conjugated to a C terminus of a light chain of a targeting antibody or antibody fragment and a second CAR-ID may be conjugated to an N terminus of a heavy chain of the targeting antibody or antibody fragment. The first CAR-ID may be fused

to a terminus of the targeting polypeptide and a second CAR-ID may be linked/conjugated within a domain of the targeting polypeptide. The first CAR-ID and the second CAR-ID may be the same or similar, such that the CAR-EC switch may be used with a CAR-EC cell that expresses one CAR. The first CAR-ID and the second CAR-ID may be different, such that the CAR-EC switch may be used with a CAR-EC cell that expresses one or more CARs or multiple CAR-EC cells that express different CARs.

[0111] The switches disclosed herein may comprise one or more CAR-IDs. The switches disclosed herein may comprise two or more CAR-IDs. The switches disclosed herein may comprise three or more CAR-IDs. The switches disclosed herein may comprise 1, 2, 3, 4, 5, 6, 7 or more CAR-IDs. The switches disclosed herein may comprise one or more TIDs. The switches disclosed herein may comprise two or more TIDs. The switches disclosed herein may comprise three or more TIDs. The switches disclosed herein may comprise 1, 2, 3, 4, 5, 6, 7 or more TIDs. The one or more CAR-IDs may be linked and/or conjugated to the one or more TIDs via one or more linkers. Thus, the switches disclosed herein may comprise one or more linkers (e.g., L1, L2). The switches disclosed herein may comprise two or more linkers. The switches disclosed herein may comprise three or more linkers. The switches disclosed herein may comprise 1, 2, 3, 4, 5, 6, 7 or more linkers.

Chimeric Antigen Receptor (CAR)

[0112] The switches disclosed herein may interact with a chimeric antigen receptor (CAR) on a CAR-EC, thereby regulating the activities of the CAR-EC. Generally, the interaction of the CAR-ID with the CAR may result in the activation of an immune response by the cell. The CAR may comprise an extracellular domain, a transmembrane domain and an intracellular domain. The extracellular domain may interact with the CAR-ID of the CAR-EC switch. The extracellular domain may comprise at least a portion of an antibody. In some instances, the antibody is not a full-length antibody. The extracellular domain may comprise at least a portion of an immunoglobulin or fragment thereof. The immunoglobulin or fragment thereof may be selected from a group comprising IgA1, IgA2, IgD, IgM, IgE, IgG1, IgG2, IgG3, IgG4, scFv, di-scFv, bi-scFv and Fab, Fc, $F(ab')_2$, pFc', a nanobody, an affibody, a DARPin, a diabody, a camelid, an engineered T cell receptor, or a monobody. The immunoglobulin may comprise IgG4.

[0113] The antibody may have a binding affinity of about 0.01 pM, about 0.02 pM, about 0.03 pM, about 0.04 pM, 0.05 pM, about 0.06 pM, about 0.07 pM, about 0.08 pM, about 0.09

pM, about 0.1 pM, about 0.2 pM, 0.3 pM, about 0.4 pM, about 0.5 pM, about 0.6 pM, about 0.7 pM, about 0.8 pM, about 0.9 pM or about 1 pM, about 2 pM, about 3 pM, about 4 pM, about 5 pM, about 6 pM, about 7 pM, about 8 pM, about 9 pM, about 10 pM, about 0.01 nM, about 0.02 nM, about 0.03 nM, about 0.04 nM, about 0.05 nM, about 0.06 nM, about 0.07 nM, about 0.08 nM, about 0.09 nM, about 0.1 nM, about 0.2 nM, about 0.3 nM, about 0.4 nM, about 0.5 nM, about 0.6 nM, about 0.7 nM, about 0.8 nM, about 0.9 nM, about 1 nM, about 2 nM, about 3 nM, about 4 nM, about 5 nM, about 6 nM, about 7 nM, about 8 nM, about 9 nM, about 10 nM, about 12nM, about 14 nM, about 16 nM, about 18 nM, about 20 nM, about 22 nM, about 24 nM, about 26 nM, about 28 nM or about 30 nM. The extracellular domain may comprise at least a portion of a single chain variable fragment (scFv). The extracellular domain may comprise avidin or a fragment thereof. The extracellular domain may not comprise avidin or fragment thereof. The antibody may comprise an anti-FITC antibody or fragment thereof. The anti-FITC antibody may be an anti-FITC scFv. The anti-FITC scFv may be selected from 4-4-20, 4D5Flu, 4M5.3 and FITC-E2. The anti-FITC scFv may be encoded by a sequence selected from SEQ ID NOs: 1-4.

[0114] The antibody to FITC or fragment thereof may have a binding affinity for FITC less than 0.1 pM. The antibody to FITC or fragment thereof may have a binding affinity for FITC between about 0.1 pM and about 1 pM. The antibody to FITC or fragment thereof may have a binding affinity for FITC between about 1 pM and about 10 pM. The antibody to FITC or fragment thereof may have a binding affinity for FITC of about 10 pM, about 20 pM, about 30 pM, about 40 pM, about 50 pM, about 60 pM, about 70 pM, about 80 pM, about 90 pM or about 100 pM. The antibody to FITC or fragment thereof may have a binding affinity for FITC of about 100 pM, about 200 pM, about 300 pM, about 400 pM, about 500 pM, about 600 pM, about 700 pM, about 800 pM, about 900 pM or about 1 nM. The antibody to FITC or fragment thereof may have a binding affinity for FITC of about 1 nM, about 2 nM, about 3 nM, about 4 nM, about 5 nM, about 6 nM, about 7 nM, about 8 nM, about 9 nM or about 10 nM. The antibody to FITC or fragment thereof may have a binding affinity for FITC of about 10 nM, about 15 nM, about 20 nM, about 25 nM, about 30 nM, about 35 nM, about 40 nM, about 45 nM or about 50 nM. The antibody to FITC or fragment thereof may have a binding affinity for FITC greater than 50 nM. The antibody to FITC may comprise an anti-FITC scFv or fragment thereof. The anti-FITC scFv may be selected from a group comprising 4-4-20, 4D5Flu, 4M5.3 and FITC-E2. The binding affinity of 4-4-20 may be about 0.2 nM. The binding affinity of 4D5Flu may be about 20 nM. The

binding affinity of 4M5.3 may be about 0.3 pM. The binding affinity of FITC-E2 may be about 0.3 nM.

[0115] The transmembrane domain and/or the intracellular domain may comprise at least a portion of a cytoplasmic signaling domain. The intracellular domain may comprise at least a portion of a signaling molecule selected from the group comprising CD3ξ, CD28, and 4-1BB. The intracellular domain may comprise an Fc receptor or a portion thereof. The Fc receptor or portion thereof may be CD16 or a portion thereof. The signaling molecule may comprise CD3ξ. The signaling molecule may comprise CD28. The signaling molecule may comprise 4-1BB. The intracellular domain may comprise at least a portion of CD3ξ. The intracellular domain may comprise at least a portion of CD28, The intracellular domain may comprise at least a portion of 4-1BB, The intracellular domain may comprise at least a portion of OX-40, The intracellular domain may comprise at least a portion of CD30, The intracellular domain may comprise at least a portion of CD40, The intracellular domain may comprise at least a portion of CD2. The intracellular domain may comprise at least a portion of CD27. The intracellular domain may comprise at least a portion of PD-1. The intracellular domain may comprise at least a portion of ICOS. The intracellular domain may comprise at least a portion of lymphocyte function-associated antigen-1 (LFA-1). The intracellular domain may comprise at least a portion of CD7. The intracellular domain may comprise at least a portion of LIGHT. The intracellular domain may comprise at least a portion of NKG2C. The intracellular domain may comprise at least a portion of B7-H3. The intracellular domain may comprise at least a portion of a cytoplasmic signaling domain from one or more signaling molecules. The intracellular domain may comprise at least a portion of two or more cytoplasmic signaling domains. The two or more cytoplasmic signaling domains may be from two or more different signaling molecules. The intracellular domain may comprise at least a portion of three or more cytoplasmic signaling domains. The intracellular domain may comprise at least a portion of four or more cytoplasmic signaling domains. The intracellular domain may comprise at least a portion of a ligand that binds to one or more signaling molecules. The intracellular domain may comprise at least a portion of a ligand that binds to CD83.

[0116] The CAR may comprise a hinge domain ("hinge"). The hinge domain may be located in the extracellular domain of the CAR. The hinge domain may be located between the transmembrane domain and a region that interacts with a chimeric antigen receptor

switch. The hinge may comprise a portion of the extracellular domain. The hinge may comprise a portion of the transmembrane domain. The hinge may be flexible (e.g. the hinge may fluctuate between different geometries or shapes in absence and/or presence of switch). The hinge may be rigid (e.g. the hinge comprises a beta sheet, coiled coil structure, or otherwise rigid structure). The hinge may provide a length, orientation, geometry or flexibility to the CAR that is necessary for an optimal immunological synapse. The optimal immunological synapse may provide for an optimal distance and/or orientation between the CAR-EC and the target cell. The optimal immunological synapse may provide for optimal and/or maximal cytotoxicity against the target cell. The hinge may comprise about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 12, about 14, about 16, about 18, about 20, about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 90 or about 100 amino acids. The hinge may comprise a sequence selected from SEQ ID NOS: 34-37. The hinge may comprise a sequence that is at least about 50% homologous to a sequence selected from 34-37.

[0117] The CAR may be expressed at relatively low levels (~10,000 to ~100,000 copies per cell) on the CAR-EC. The CAR may be expressed at less than about 10,000 copies per cell. The CAR may be expressed at relatively high levels on the CAR-EC (more than ~500,000 copies per cell). The CAR may be expressed at moderate levels (~100,000 to ~500,000 copies per cell). The CAR may be expressed under the control of a promoter selected from EF1a, IL-2, CMV, and synthetic promoters designed to increase or decrease CAR expression. The promoter may be constitutive. The promoter may be inducible.

Chimeric Antigen Receptor Effector Cells (CAR-EC)

[0118] The methods, platforms and kits disclosed herein may comprise one or more chimeric antigen receptor effector cells (CAR-EC) or uses thereof. The chimeric antigen receptor effector cells disclosed herein express a chimeric antigen receptor. The chimeric antigen receptor (CAR) may be any CAR disclosed herein. Wherein the methods, platforms or kits comprise two or more effector cells, the two or more effector cells may be of the same cell type. The two or more effector cells may be of a different cell type. The two or more effector cells may be of the same cell lineage. The two or more effector cells may be of different cell lineages. The two or more effector cells may comprise two or more identical

CARs. The two or more effector cells may comprise two or more different CARs. The two or more effector cells may comprise two or more similar CARs.

[0119] The effector cell may be a T cell. The effector cell may be a cell of a T cell lineage. The effector cell may be a mature T cell. The effector cell may be a precursor T cell. The effector cell may be a cytotoxic T cell. The effector cell may be a naive T cell. The effector cell may be a memory stem cell T cell (T_{MSC}). The effector cell may be a central memory T cell (T_{CM}). The effector cell may be an effector T cell (TE). The effector cell may be a CD4+ T cell. The T cell may be a CD8+ T cell. The effector cell may be a CD4+ and CD8+ cell. The effector cell may be an alpha-beta T cell. The effector cell may be a gamma-delta T cell. The effector cell may be a natural killer T cell. The effector cell may be a helper T cell.

[0120] While preferred embodiments of the present disclosure describe methods, kits and platforms comprising T cells, one skilled in the art may also understand that other cell types may be used in place of a T cell. The effector cell may be an effector cell that has an effect on a target or target cell when brought into proximity of the target or target cell. The effector cell may be a cell that has a cytotoxic effect on a target or target cell when brought into proximity of the target or target cell. The effector cell may be an immune cell. The effector cell may be selected from a B cell, a monocyte, a thrombocyte, a leukocyte, a neutrophil, an eosinophil, a basophil, or a lymphocyte. The effector cell may be a lymphocyte. The effector cell may be a macrophage. The effector cell may be a phagocytic cell. The effector cell may be an effector B cell. The effector cell may be a natural killer cell. The effector cell may isolated or derived from a subject suffering from a disease or condition. The effector cell may be a cell derived from a subject to be treated with a CAR-EC switch or CAR-EC platform disclosed herein.

[0121] The T cell may express a chimeric antigen receptor encoded by one or more polypeptide based on or derived from SEQ ID NOS: 7-8. The polypeptide may be at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% identical to one or more polypeptide based on or derived from SEQ ID NOS: 7-8. The polypeptide may be at least about 70% identical to one or more polypeptide based on or derived from SEQ ID NOS: 7-8.

CAR-EC platform

[0122] Disclosed herein are chimeric antigen receptor effector cell (CAR-EC) platforms comprising a an effector cell, wherein the effector cell comprises a polynucleotide encoding a chimeric antigen receptor (CAR); and a chimeric antigen receptor effector cell (CAR-EC)

switch, wherein the CAR-EC switch comprises a CAR binding peptidic antigen and a TID, wherein the TID comprises an anti-Her2 antibody fragment Fab (clone 4D5), or a fragment thereof, which Fab comprises an unnatural amino acid located at each of Glycine 212 of a light chain of the anti-Her2 antibody Fab (LG212X; SEQ ID NO: 2) and Lysine 221 of a heavy chain of the anti-Her2 antibody Fab (HK221X; SEQ ID NO: 1), and wherein each of LG212X and HK221X is attached or linked to a separate CAR interacting domain; and wherein the CAR-EC switch binds a cell surface molecule on a target cell. The CAR-EC switch may be selected from any CAR-EC switches disclosed herein.

[0123] The CAR-EC platforms may comprise two or more CAR-EC switches. The CAR-EC platforms may comprise 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more CAR-EC switches. The CAR-EC platforms may comprise may comprise more than 20, more than 25, more than 30, more than 35, more than 40, more than 45 or more than 50 CAR-EC switches. The two or more switches may be selected from one or more CAR-EC switches disclosed herein or a combination thereof.

[0124] The CAR-EC platforms disclosed herein may further comprise a first CAR-EC switch and a second CAR-EC switch, wherein the first CAR-EC switch comprises a first CAR-ID and a first TID wherein the TID comprises an anti-Her2 antibody fragment Fab (clone 4D5), or a fragment thereof, which Fab comprises an unnatural amino acid located at each of Glycine 212 of a light chain of the anti-Her2 antibody Fab (LG212X; SEQ ID NO: 2) and Lysine 221 of a heavy chain of the anti-Her2 antibody Fab (HK221X; SEQ ID NO: 1), and wherein each of LG212X and HK221X is attached or linked to a separate CAR interacting domain;and the second CAR-EC switch comprises a second CAR-ID and a second targeting polypeptide. The first CAR-ID and the second CAR-ID may be the same. The first CAR-ID and the second CAR-ID may be different. The first CAR-ID and the second CAR-ID may be about 99%, about 98%, about 97%, about 96%, about 95%, about 92%, about 90%, about 85%, about 80%, about 75%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, about 15%, about 10%, about 5% or about 2% homologous. The first targeting polypeptide and the second targeting polypeptide may be the same. The first targeting polypeptide and the second targeting polypeptide may be different. The first targeting polypeptide and the second targeting polypeptide may be about 99%, about 98%, about 97%, about 96%, about 95%, about 92%, about 90%, about 85%, about 80%, about 75%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, about 35%,

about 30%, about 25%, about 20%, about 15%, about 10%, about 5% or about 2% homologous.

Kits, Vectors and Polynucleotides

[0125] Disclosed herein are kits comprising one or more CAR-EC switches disclosed herein. The kit may further comprise two or more CAR-EC switches. The kit may comprise three CAR-EC switches. The kit may comprise about 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 24, 30, 35, 48, 50, 55, 60, 65, 70, 75, 80, 85, 90, 96, 100, 120, 150, 200, 300, 384, 400, 500, 600, 700, 800, 900 or 1000 CAR-EC switches. The kit may be employed for biological research. The kit may be used for diagnosing a disease or a condition. The kit may be used for treating a disease or condition. The CAR-EC switches of the kit may be used with CAR-EC cells disclosed herein or existing CAR T-cells clinically used or tested. The kit may further comprise one or more effector cells. The kit may further comprise one or more CAR-EC cells. The CAR-EC cell may be a T cell. The T cell may express one or more CARs. The kit may further comprise a polynucleotide encoding one or more CARs. The kit may further comprise a vector comprising a polynucleotide encoding one or more CARs. The CAR may be selected from any of the CARs disclosed herein. The kit may comprise one or more polynucleotide encoding a CAR-EC switch disclosed herein or a portion thereof (e.g. antibody, antibody fragment, peptide).

[0126] The present disclosure also includes vectors and polynucleotides encoding the target interacting domain of the CAR-EC switch. The polynucleotides may be DNA. The polynucleotides may be RNA. Unless otherwise specified, the terms “polynucleotide” and “vector,” as used herein, are used interchangeably. The targeting interacting domain may be an antibody or antibody fragment. The vector may comprise a sequence encoding a heavy chain of the antibody or antibody fragment. The vector may comprise a sequence encoding a light chain of the antibody or antibody fragment. The vector may comprise the sequence encoding the light chain of the antibody or antibody fragment and the sequence encoding the heavy chain of the antibody or antibody fragment. The light chain and the heavy chain may be expressed from the same vector. The light chain and the heavy chain may be expressed from two separate vectors.

[0127] The present disclosure also includes vectors and polynucleotides encoding chimeric antigen receptors, wherein the chimeric antigen receptors comprise an extracellular domain that binds to a peptide of a chimeric antigen receptor effector cell switch. The extracellular domain may comprise an antibody or antibody fragment. The antibody or

antibody fragment may bind a CAR-ID of a chimeric antigen receptor effector cell switch. The CAR-ID may be a small molecule. The CAR-ID may be a hapten. The CAR-ID may be FITC or a derivative thereof.

[0128] Vectors comprising sequences encoding chimeric antigen receptors and/or chimeric antigen receptor effector cell switches and portions thereof, disclosed herein, may be selected from any commercially available expression vector. The expression vector may be a prokaryotic expression vector. The expression vector may be a eukaryotic expression vector. The expression vector may be a mammalian expression vector. The expression vector may be a viral expression vector. The expression vector may have a constitutive promoter for constitutive expression of the CAR and/or CAR-EC switch encoding sequences. The expression vector may have an inducible promoter for conditional expression of the CAR and/or CAR-EC switch encoding sequences.

Therapeutic Use

[0129] Disclosed herein are methods, platforms and kits for treating a disease or condition in a subject in need thereof, the method comprising administering a chimeric antigen receptor effector cell (CAR-EC) switch to the subject, wherein the CAR-EC switch comprises: a CAR-interacting domain; and a TID, wherein the TID comprises an anti-Her2 antibody fragment Fab (clone 4D5), or a fragment thereof, which Fab comprises an unnatural amino acid located at each of Glycine 212 of a light chain of the anti-Her2 antibody Fab (LG212X; SEQ ID NO: 2) and Lysine 221 of a heavy chain of the anti-Her2 antibody Fab (HK221X; SEQ ID NO: 1), and wherein each of LG212X and HK221X is attached or linked to a separate CAR interacting domain. Disclosed herein are methods of treating a disease or condition in a subject in need thereof, the method comprising administering any one of the CAR-EC switches disclosed herein.

[0130] The methods may comprise administering a CAR-EC cell and one or more CAR-EC switches (e.g., any one of the CAR-EC switches disclosed herein). The methods may comprise administering about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 24, 30, 35, 48, 50, 55, 60, 65, 70, 75, 80, 85, 90, 96, 100, 120, 150, 200, 300, 384, 400, 500, 600, 700, 800, 900, 1000 or more CAR-EC switches (e.g., any one of the CAR-EC switches disclosed herein). The methods may comprise administering two or more CAR-EC switches. The two or more CAR-EC switches may comprise the same CAR-binding peptidic antigen. The two more CAR-EC switches may comprise the same cell targeting polypeptide. The two or more CAR-EC

switches may comprise one or more different CAR-binding peptidic antigens. The two more CAR-EC switches may comprise one or more different cell targeting polypeptides. The methods may comprise a plurality of CAR-EC cells and one or more CAR-EC switches. Administering the CAR-EC cell may comprise intravenous CAR-EC delivery. Administering the CAR-EC cell may comprise intraperitoneal CAR-EC delivery. Administering the CAR-EC cell may comprise intravenous CAR-EC delivery and intraperitoneal CAR-EC delivery. Administering the CAR-EC cell may occur once. Administering the CAR-EC cell may occur more than once (e.g. repeat injection). The CAR-ECs may be sorted to enrich a memory population of CAR-ECs before administering the CAR-ECs. The CAR-ECs may be subjected to iterative stimulation to enrich the memory population, as opposed to recursive stimulation which promotes exhaustion, provide for a long-lived, persistent phenotype. This rationale is based on natural acute infections with enrich long-lived memory cells through a 1-2 week long contraction phase that occurs after the challenge has been cleared. Similarly, the sCAR-T cell system in which adoptively transferred cells are rested following stimulation may more closely recapitulate a physiological duration of T cell activation.

[0131] The methods may comprise administering one or more chimeric antigen receptor effector cells to a subject in need thereof and then administering one or CAR-EC switches to a subject in need thereof. The amount or dose of CAR-EC switch may affect the magnitude of the chimeric antigen receptor effector cells toward the target cells, therefore the amount or dose of the CAR-EC switch may be titrated for a desired effect. For example, tumors may be targeted by titration of CAR-EC switch to achieve suitable therapeutic index. The response may be titrated "on" to avoid CRS (cytokine release syndrome) and TLS (tumor lysis syndrome) events, providing for personalized therapy. Furthermore, administration of a switch can be terminated in case of an adverse event, control of CAR-EC cell activity, titration of off-target reactivity, abrogation of tumor lysis syndrome (TLS), or attenuation of cytokine release syndrome (CRS). The amount or dose may start at one level for a specified time period and then the amount or dose may be increased or decreased to a second level for a second specified time period. For example, the initial amount or dose of the CAR-EC switch may be the lowest dose necessary to eliminate the tumor. The amount or dose of the CAR-EC switch may then be increased to a larger dose in order to eliminate any remaining tumor cells. The methods may comprise terminating the administration of the CAR-EC switch once the tumor cells are eliminated. The methods may comprise re-administering the CAR-EC switch if the tumor cells re-occur in the patient or if the patient relapses.

[0132] The methods may comprise administering one or more chimeric antigen receptor effector cells. The methods may comprise administering one or more T cells. The one or more effector cells may be selected from T cell is selected from a naive T cell, a memory stem cell T cell, a central memory T cell, an effector memory T cell, a helper T cell, a CD4+ T cell, a CD8+ T cell, a CD8/CD4+ T cell, an αβ T cell, a γδ T cell, a cytotoxic T cell, a natural killer T cell, a natural killer cell, a macrophage.

[0133] The CAR-EC switch may have a therapeutic effect that is at least partially dependent on bringing an effector cell in proximity of a target cell. The therapeutic effect on the intended indication of the CAR-EC switch may be at least partially due to the CAR-EC switch recruiting an effector cell to the target cell. The therapeutic effect on the intended indication of the CAR-EC switch may be predominantly due to the CAR-EC switch recruiting an effector cell to the target cell. The therapeutic effect of the CAR-EC switch may be at least partially dependent on stimulating an immune response in the CAR-EC cell.

[0134] Administering the CAR-EC switch may not have any therapeutic effect without further administering an effector cell. The CAR-EC switch may not have a significant, desirable and/or intended therapeutic effect without further administering an effector cell. The CAR-EC switch may not have any therapeutic effect towards an intended indication of the CAR-EC platform without further administering an effector cell. A portion or component of the CAR-EC switch (e.g. CAR-ID or TID) may not have a therapeutic effect towards the intended indication of the CAR-EC switch without being conjugated to a second portion or component of the CAR-EC switch (e.g. CAR-ID or TID). The dose of a portion or component of the CAR-EC switch (e.g. CAR-ID or TID) when administered as part of the CAR-EC platform to provide a therapeutic effect may not have a therapeutic effect when the portion or component of the CAR-EC switch is administered alone at that dose. The portion or component of the CAR-EC switch may not be intended to have any therapeutic effect besides recruiting the T cell to the target cell. Administering the portion or component of the CAR-EC switch alone may have a therapeutic effect on the target cell, wherein the therapeutic effect is negligible relative to the therapeutic effect of administering the CAR-EC switch and the CAR-EC cell. Administering the portion or component of the CAR-EC switch may have a therapeutic effect on the target cell, wherein the therapeutic effect is less than the therapeutic effect of administering the CAR-EC switch and the CAR-EC cell.

[0135] Disclosed herein are uses of CAR-EC switches disclosed herein to treat a disease or condition in a subject in need thereof. Further disclosed herein are uses of CAR-EC switches disclosed herein in the manufacture of a medicament for the treatment of a disease.

[0136] The disease or condition may be a cell proliferative disorder. The cell proliferative disorder may be selected from a solid tumor, a lymphoma, a leukemia, and a liposarcoma. The cell proliferative disorder may be acute, chronic, recurrent, refractory, accelerated, in remission, stage I, stage II, stage III, stage IV, juvenile or adult. The cell proliferative disorder may be selected from myelogenous leukemia, lymphoblastic leukemia, myeloid leukemia, an acute myeloid leukemia, myelomonocytic leukemia, neutrophilic leukemia, myelodysplastic syndrome, B-cell lymphoma, burkitt lymphoma, large cell lymphoma, mixed cell lymphoma, follicular lymphoma, mantle cell lymphoma, hodgkin lymphoma, recurrent small lymphocytic lymphoma, hairy cell leukemia, multiple myeloma, basophilic leukemia, eosinophilic leukemia, megakaryoblastic leukemia, monoblastic leukemia, monocytic leukemia, erythroleukemia, erythroid leukemia and hepatocellular carcinoma. The cell proliferative disorder may comprise a hematological malignancy. The hematological malignancy may comprise a B cell malignancy. The cell proliferative disorder may comprise a chronic lymphocytic leukemia. The cell proliferative disorder may comprise an acute lymphoblastic leukemia.

[0137] The disease or condition may be a cancer, a pathogenic infection, autoimmune disease, inflammatory disease, or genetic disorder.

[0138] In some instances, the one or more diseases comprises a cancer. The cancer may comprise a recurrent and/or refractory cancer. Examples of cancers include, but are not limited to, sarcomas, carcinomas, lymphomas or leukemias.

[0139] The cancer may comprise a neuroendocrine cancer. The cancer may comprise a pancreatic cancer. The cancer may comprise an exocrine pancreatic cancer. The cancer may comprise a thyroid cancer. The thyroid cancer may comprise a medullary thyroid cancer. The cancer may comprise a prostate cancer.

[0140] The cancer may comprise an epithelial cancer. The cancer may comprise a breast cancer. The cancer may comprise an endometrial cancer. The cancer may comprise an ovarian cancer. The ovarian cancer may comprise a stromal ovarian cancer. The cancer may comprise a cervical cancer. The cancer may comprise AML, ALL, CLL, multiple myeloma, neuroblastoma, pancreatic cancer, or colon cancer

[0141] The cancer may comprise a skin cancer. The skin cancer may comprise a neo-angiogenic skin cancer. The skin cancer may comprise a melanoma.

[0142] The cancer may comprise a kidney cancer.

[0143] The cancer may comprise a lung cancer. The lung cancer may comprise a small cell lung cancer. The lung cancer may comprise a non-small cell lung cancer.

[0144] The cancer may comprise a colorectal cancer. The cancer may comprise a gastric cancer. The cancer may comprise a colon cancer.

[0145] The cancer may comprise a brain cancer. The brain cancer may comprise a brain tumor. The cancer may comprise a glioblastoma. The cancer may comprise an astrocytoma.

[0146] The cancer may comprise a blood cancer. The blood cancer may comprise a leukemia. The leukemia may comprise a myeloid leukemia. The cancer may comprise a lymphoma. The lymphoma may comprise a non-Hodgkin's lymphoma.

[0147] The cancer may comprise a sarcoma. The sarcoma may comprise an Ewing's sarcoma.

[0148] Sarcomas are cancers of the bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue. Sarcomas include, but are not limited to, bone cancer, fibrosarcoma, chondrosarcoma, Ewing's sarcoma, malignant hemangioendothelioma, malignant schwannoma, bilateral vestibular schwannoma, osteosarcoma, soft tissue sarcomas (e.g. alveolar soft part sarcoma, angiosarcoma, cystosarcoma phylloides, dermatofibrosarcoma, desmoid tumor, epithelioid sarcoma, extraskeletal osteosarcoma, fibrosarcoma, hemangiopericytoma, hemangiosarcoma, Kaposi's sarcoma, leiomyosarcoma, liposarcoma, lymphangiosarcoma, lymphosarcoma, malignant fibrous histiocytoma, neurofibrosarcoma, rhabdomyosarcoma, and synovial sarcoma).

[0149] Carcinomas are cancers that begin in the epithelial cells, which are cells that cover the surface of the body, produce hormones, and make up glands. By way of non-limiting example, carcinomas include breast cancer, pancreatic cancer, lung cancer, colon cancer, colorectal cancer, rectal cancer, kidney cancer, bladder cancer, stomach cancer, prostate cancer, liver cancer, ovarian cancer, brain cancer, vaginal cancer, vulvar cancer, uterine cancer, oral cancer, penile cancer, testicular cancer, esophageal cancer, skin cancer, cancer of the fallopian tubes, head and neck cancer, gastrointestinal stromal cancer, adenocarcinoma, cutaneous or intraocular melanoma, cancer of the anal region, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, cancer of the urethra, cancer of the renal pelvis, cancer of the

ureter, cancer of the endometrium, cancer of the cervix, cancer of the pituitary gland, neoplasms of the central nervous system (CNS), primary CNS lymphoma, brain stem glioma, and spinal axis tumors. In some instances, the cancer is a skin cancer, such as a basal cell carcinoma, squamous, melanoma, nonmelanoma, or actinic (solar) keratosis.

[0150] In some instances, the cancer is a lung cancer. Lung cancer may start in the airways that branch off the trachea to supply the lungs (bronchi) or the small air sacs of the lung (the alveoli). Lung cancers include non-small cell lung carcinoma (NSCLC), small cell lung carcinoma, and mesotheliomia. Examples of NSCLC include squamous cell carcinoma, adenocarcinoma, and large cell carcinoma. The mesothelioma may be a cancerous tumor of the lining of the lung and chest cavity (pleura) or lining of the abdomen (peritoneum). The mesothelioma may be due to asbestos exposure. The cancer may be a brain cancer, such as a glioblastoma.

[0151] Alternatively, the cancer may be a central nervous system (CNS) tumor. CNS tumors may be classified as gliomas or nongliomas. The glioma may be malignant glioma, high grade glioma, diffuse intrinsic pontine glioma. Examples of gliomas include astrocytomas, oligodendrogliomas (or mixtures of oligodendroglioma and astocytoma elements), and ependymomas. Astrocytomas include, but are not limited to, low-grade astrocytomas, anaplastic astrocytomas, glioblastoma multiforme, pilocytic astrocytoma, pleomorphic xanthoastrocytoma, and subependymal giant cell astrocytoma. Oligodendrogliomas include low-grade oligodendrogliomas (or oligoastrocytomas) and anaplastic oligodendriogliomas. Nongliomas include meningiomas, pituitary adenomas, primary CNS lymphomas, and medulloblastomas. In some instances, the cancer is a meningioma.

[0152] The leukemia may be an acute lymphocytic leukemia, acute myelocytic leukemia, chronic lymphocytic leukemia, or chronic myelocytic leukemia. Additional types of leukemias include hairy cell leukemia, chronic myelomonocytic leukemia, and juvenile myelomonocytic leukemia.

[0153] Lymphomas are cancers of the lymphocytes and may develop from either B or T lymphocytes. The two major types of lymphoma are Hodgkin's lymphoma, previously known as Hodgkin's disease, and non-Hodgkin's lymphoma. Hodgkin's lymphoma is marked by the presence of the Reed-Sternberg cell. Non-Hodgkin's lymphomas are all lymphomas which are not Hodgkin's lymphoma. Non-Hodgkin lymphomas may be indolent lymphomas and aggressive lymphomas. Non-Hodgkin's lymphomas include, but are not limited to,

diffuse large B cell lymphoma, follicular lymphoma, mucosa-associated lymphatic tissue lymphoma (MALT), small cell lymphocytic lymphoma, mantle cell lymphoma, Burkitt's lymphoma, mediastinal large B cell lymphoma, Waldenström macroglobulinemia, nodal marginal zone B cell lymphoma (NMZL), splenic marginal zone lymphoma (SMZL), extranodal marginal zone B cell lymphoma, intravascular large B cell lymphoma, primary effusion lymphoma, and lymphomatoid granulomatosis.

[0154] The cancer may comprise a solid tumor. The cancer may comprise a sarcoma. The cancer may be selected from a group consisting of a bladder cancer, a breast cancer, a colon cancer, a rectal cancer, an endometrial cancer, a kidney cancer, a lung cancer, melanoma, a myeloma, a thyroid cancer, a pancreatic cancer, a glioma, a malignant glioma of the brain, a glioblastoma, an ovarian cancer, and a prostate cancer. The cancer may have non-uniform antigen expression. The cancer may have modulated antigen expression. The antigen may be a surface antigen. The cancer may not comprise a myeloma. The cancer may not comprise a melanoma. The cancer may not comprise a colon cancer. The cancer may be acute lymphoblastic leukemia (ALL). The cancer may be relapsed ALL. The cancer may be refractory ALL. The cancer may be relapsed, refractory ALL. The cancer may be chronic lymphocytic leukemia (CLL). The cancer may be relapsed CLL. The cancer may be refractory CLL. The cancer may be relapsed, refractory CLL.

[0155] The cancer may comprise a breast cancer. The breast cancer may be triple positive breast cancer (estrogen receptor, progesterone receptor and Her2 positive). The breast cancer may be triple negative breast cancer (estrogen receptor, progesterone receptor and Her2 negative). The breast cancer may be estrogen receptor positive. The breast cancer may be estrogen receptor negative. The breast cancer may be progesterone receptor positive. The breast cancer may be progesterone receptor negative. The breast cancer may comprise a Her2 negative breast cancer. The breast cancer may comprise a low-expressing Her2 breast cancer. The breast cancer may comprise a Her2 positive breast cancer. Cell lines expressing Her2 have been well-characterized for antigen density, reflecting clinical immunohistochemistry characterization which classifies malignancies as 0 (<20,000 Her2 antigens per cell), 1+ (100,000 Her2 antigens per cell), 2+ (500,000 Her2 antigens per cell), and 3+ (>2,000,000 Her2 antigens per cell). The present invention provides for methods of treating breast cancers of these classifications. The breast cancer may comprise a breast cancer classified as Her2 0. The breast cancer may comprise a breast cancer classified as

Her2 1+. The breast cancer may comprise a breast cancer classified as Her2 2+. The breast cancer may comprise a breast cancer classified as a Her2 3+.

[0156] The disease or condition may be a pathogenic infection. Pathogenic infections may be caused by one or more pathogens. In some instances, the pathogen is a bacterium, fungi, virus, or protozoan.

[0157] Exemplary pathogens include but are not limited to: Bordetella, Borrelia, Brucella, Campylobacter, Chlamydia, Chlamydophila, Clostridium, Corynebacterium, Enterococcus, Escherichia, Francisella, Haemophilus, Helicobacter, Legionella, Leptospira, Listeria, Mycobacterium, Mycoplasma, Neisseria, Pseudomonas, Rickettsia, Salmonella, Shigella, Staphylococcus, Streptococcus, Treponema, Vibrio, or Yersinia. In some cases, the disease or condition caused by the pathogen is tuberculosis and the heterogeneous sample comprises foreign molecules derived from the bacterium Mycobacterium tuberculosis and molecules derived from the subject. In some instances, the disease or condition is caused by a bacterium is tuberculosis, pneumonia, which may be caused by bacteria such as Streptococcus and Pseudomonas, a foodborne illness, which may be caused by bacteria such as Shigella, Campylobacter and Salmonella, and an infection such as tetanus, typhoid fever, diphtheria, syphilis and leprosy. The disease or condition may be bacterial vaginosis, a disease of the vagina caused by an imbalance of naturally occurring bacterial flora. Alternatively, the disease or condition is a bacterial meningitis, a bacterial inflammation of the meninges (e.g., the protective membranes covering the brain and spinal cord). Other diseases or conditions caused by bacteria include, but are not limited to, bacterial pneumonia, a urinary tract infection, bacterial gastroenteritis, and bacterial skin infection. Examples of bacterial skin infections include, but are not limited to, impetigo which may be caused by Staphylococcus aureus or Streptococcus pyogenes; erysipelas which may be caused by a streptococcus bacterial infection of the deep epidermis with lymphatic spread; and cellulitis which may be caused by normal skin flora or by exogenous bacteria.

[0158] The pathogen may be a fungus, such as, *Candida, Aspergillus, Cryptococcus, Histoplasma, Pneumocystis,* and *Stachybotrys.* Examples of diseases or conditions caused by a fungus include, but are not limited to, jock itch, yeast infection, ringworm, and athlete's foot.

[0159] The pathogen may be a virus. Examples of viruses include, but are not limited to, adenovirus, coxsackievirus, Epstein-Barr virus, Hepatitis virus (e.g., Hepatitis A, B, and C), herpes simplex virus (type 1 and 2), cytomegalovirus, herpes virus, HIV, influenza virus,

measles virus, mumps virus, papillomavirus, parainfluenza virus, poliovirus, respiratory syncytial virus, rubella virus, and varicella-zoster virus. Examples of diseases or conditions caused by viruses include, but are not limited to, cold, flu, hepatitis, AIDS, chicken pox, rubella, mumps, measles, warts, and poliomyelitis.

[0160] The pathogen may be a protozoan, such as Acanthamoeba (e.g., A. astronyxis, A. castellanii, A. culbertsoni, A. hatchetti, A. polyphaga, A. rhysodes, A. healyi, A. divionensis), Brachiola (e.g., B connori, B. vesicularum), Cryptosporidium (e.g., C. parvum), Cyclospora (e.g., C. cayetanensis), Encephalitozoon (e.g., E. cuniculi, E. hellem, E. intestinalis), Entamoeba (e.g., E. histolytica), Enterocytozoon (e.g., E. bieneusi), Giardia (e.g., G. lamblia), Isospora (e.g., I. belli), Microsporidium (e.g., M. africanum, M. ceylonensis), Naegleria (e.g., N. fowleri), Nosema (e.g., N. algerae, N. ocularum), Pleistophora, Trachipleistophora (e.g., T. anthropophthera, T. hominis), and Vittaforma (e.g., V. corneae).

[0161] The disease or condition may be an autoimmune disease or autoimmune related disease. An autoimmune disorder may be a malfunction of the body's immune system that causes the body to attack its own tissues. Examples of autoimmune diseases and autoimmune related diseases include, but are not limited to, Addison's disease, alopecia areata, ankylosing spondylitis, antiphospholipid syndrome (APS), autoimmune aplastic anemia, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune myocarditis, Behcet's disease, celiac sprue, Crohn's disease, dermatomyositis, eosinophilic fasciitis, erythema nodosum, giant cell arteritis (temporal arteritis), Goodpasture's syndrome, Graves' disease, Hashimoto's disease, idiopathic thrombocytopenic purpura (ITP), IgA nephropathy, juvenile arthritis, diabetes, juvenile diabetes, Kawasaki syndrome, Lambert-Eaton syndrome, lupus (SLE), mixed connective tissue disease (MCTD), multiple sclerosis, myasthenia gravis, pemphigus, polyarteritis nodosa, type I, II, & III autoimmune polyglandular syndromes, polymyalgia rheumatica, polymyositis, psoriasis, psoriatic arthritis, Reiter's syndrome, relapsing polychondritis, rheumatoid arthritis, sarcoidosis, scleroderma, Sjogren's syndrome, sperm & testicular autoimmunity, stiff person syndrome, Takayasu's arteritis, temporal arteritis/giant cell arteritis, ulcerative colitis, uveitis, vasculitis, vitiligo, and Wegener's granulomatosis.

[0162] The disease or condition may be an inflammatory disease. Examples of inflammatory diseases include, but are not limited to, alveolitis, amyloidosis, angiitis, ankylosing spondylitis, avascular necrosis, Basedow's disease, Bell's palsy, bursitis, carpal tunnel syndrome, celiac disease, cholangitis, chondromalacia patella, chronic active hepatitis, chronic fatigue syndrome, Cogan's syndrome, congenital hip dysplasia, costochondritis,

Crohn's Disease, cystic fibrosis, De Quervain's tendinitis, diabetes associated arthritis, diffuse idiopathic skeletal hyperostosis, discoid lupus, Ehlers-Danlos syndrome, familial mediterranean fever, fascitis, fibrositis/fibromyalgia, frozen shoulder, ganglion cysts, giant cell arteritis, gout, Graves' Disease, HIV-associated rheumatic disease syndromes, hyperparathyroid associated arthritis, infectious arthritis, inflammatory bowel syndrome/ irritable bowel syndrome, juvenile rheumatoid arthritis, lyme disease, Marfan's Syndrome, Mikulicz's Disease, mixed connective tissue disease, multiple sclerosis, myofascial pain syndrome, osteoarthritis, osteomalacia, osteoporosis and corticosteroid-induced osteoporosis, Paget's Disease, palindromic rheumatism, Parkinson's Disease, Plummer's Disease, polymyalgia rheumatica, polymyositis, pseudogout, psoriatic arthritis, Raynaud's Phenomenon/Syndrome, Reiter's Syndrome, rheumatic fever, rheumatoid arthritis, sarcoidosis, sciatica (lumbar radiculopathy), scleroderma, scurvy, sickle cell arthritis, Sjogren's Syndrome, spinal stenosis, spondyloisthesis, Still's Disease, systemic lupus erythematosis, Takayasu's (Pulseless) Disease, Tendinitis, tennis elbow/golf elbow, thyroid associated arthritis, trigger finger, ulcerative colitis, Wegener's Granulomatosis, and Whipple's Disease.

[0163] Methods of treatment disclosed herein may comprise off-target activity as measured by cytokine levels. The method may reduce the off-target activity, as measured by cytokine levels, when compared to other CAR-EC therapies. The method may reduce the off-target activity as measured by interferon gamma levels. Other off-target activities that may be reduced include toxic lymphophenia, fatal cytolysis of solid tumor targets and chronic hypogammaglobulinemia for hematological targets.

[0164] The CAR-EC switch may be administered with one or more additional therapeutic agents. The one or more additional therapeutic agents may be selected from a group consisting of an immunotherapy, a chemotherapy and a steroid. The one or more additional therapeutic agents may be a chemotherapy drug. The chemotherapy drug may be an alkylating agent, an antimetabolite, an anthracycline, a topoisomerase inhibitor, a mitotic inhibitor, a corticosteroid or a differentiating agent. The chemotherapy drug may be selected from actinomycin-D, bleomycin, altretamine, bortezomib, busulfan, carboplatin, capecitabine, carmustine, chlorambucil, cisplatin, cladribine, clofarabine, cyclophosphamide, cytarabine, dacarbazine, daunorubicin, docetaxel, doxorubicin, epirubicin, etoposide, estramustine, floxuridine, fludarabine, fluorouracil, gemcitbine (Gemzar), hydroxyurea, idarubicin, ifosfamide, irinotecan (Camptosar), ixabepilone, L-asparaginase, lomustine,

mechlorethamine, melphalan, 6-mercaptopurine, methotrexate, mitomycin-C, paclitaxel (Taxol), pemetrexed, pentostatin, streptozocin, temozolomide, teniposide, thioguanine, thiotepa, topotecan (Hycamtin), vincristine, vinblastine, vinorelbine, retinoids, tretinoin (ATRA or Atralin®), bexarotene (Targretin®) and arsenic trioxide (Arsenox®). The chemotherapy may be administered as a pill to swallow, as an injection into the muscle or fat tissue, intravenously, topically or directly into a body cavity.

[0165] The one or more additional therapeutic agents may comprise an angiogenesis inhibitor. The angiogenesis inhibitor may be selected from bevacizumab, itraconazole, carboxyamidotriazole, TNP-470, CM101, IFN alpha, IL-12, platelet factor 4, suramin, SU5416, thrombospondin, a VEGFR antagonist, an angiostatic steroid with heparin, CAR-ECilage-derived angiogenesis inhibitory factor, matrix metalloprotease inhibitors, angiostatin, endostatin, sorafenib, sunitinib, pazopanib, everolimus, 2-methoxyestradiol, tecogalan, tetrathiomolybdate, thalidomide, prolactin, $\alpha v\beta_3$ inhibitor, linomide, tasquinimod, soluble VEGFR-1, soluble NRP-1, angiopoietin 2, vasostatin, calreticulin, TIMP, CDAI, Meth-1, Meth-2, interferon-alpha, interferon-beta, interferon-gamma, CXCL10, IL-4, IL-12, IL-18, prothrombin, antithrombin III fragment, prolactin, VEGI, SPARC, osteopontin, maspin, canstatin, proliferin-related protein and restin.

[0166] The one or more additional therapeutic agents may comprise a hormone therapy. The hormone therapy may be selected from an anti-estrogen (e.g. fulvestrant (Faslodex®), tamoxifen, toremifene (Fareston®)); an aromatase inhibitor (e.g. anastrozole (Arimidex®), exemestane (Aromasin®), letrozole (Femara®)); a progestin (e.g. megestrol acetate (Megace®)); an estrogen; an anti-androgen (e.g. bicalutamide (Casodex®), flutamide (Eulexin®), nilutamide (Nilandron®)); a gonadotropin-releasing hormone (GnRH) or luteinizing hormone-releasing hormone (LHRH) agonist or analog (e.g. leuprolide (Lupron®), goserelin (Zoladex®)).

[0167] The one or more additional therapeutic agents may comprise a steroid. The steroid may be a corticosteroid. The steroid may be cortisol or a derivative thereof. The steroid may be selected from prednisone, methylprednisolone (Solumedrol®) or dexamethasone.

[0168] The CAR-EC switch may be administered with one or more additional therapies. The one or more additional therapies may comprise laser therapy. The one or more additional therapies may comprise radiation therapy. The one or more additional therapies may comprise surgery.

[0169] Disclosed herein are platforms, kits and methods for treating a disease or condition in a subject. The subject may be a healthy subject. The subject may be suffering from a disease or condition. The subject may be suffering from more than one disease or condition. The subject may be suffering from chronic lymphocytic leukemia. The subject may be suffering from acute lymphoblastic leukemia. The subject may be an animal. The subject may be a mammal. The mammal may be a human, a chimpanzee, a gorilla, a monkey, a bovine, a horse, a donkey, a mule, a dog, a cat, a pig, a rabbit, a goat, a sheep, a rat, a hamster, a guinea pig or a mouse. The subject may be a bird or a chicken. The subject may be a human. The subject may be a child. The child may be suffering from acute lymphoblastic leukemia. The subject may be less than 6 months old. The subject may be about 1 year old, about 2 years old, about 3 years old, about 4 years old, about 5 years old, about 6 years old, about 7 years old, about 8 years old, about 9 years old, about 10 years old, about 11 years old, about 12 years old, about 13 years old, about 14 years old, about 15 years old, about 18 years old, about 20 years old, about 25 years old, about 30 years old, about 35 years old, about 40 years old, about 45 years old, about 50 years old, about 55 years old, about 60 years old, about 65 years old, about 70 years old, about 75 years old, about 80 years old, about 85 years old, about 90 years old, about 95 years old, about 100 years old or about 105 years old.

. Pharmaceutical Compositions

[0170] Disclosed herein is a pharmaceutical composition comprising one or more of the CAR-EC switches disclosed herein. The compositions may further comprise one or more pharmaceutically acceptable salts, excipients or vehicles. Pharmaceutically acceptable salts, excipients, or vehicles for use in the present pharmaceutical compositions include carriers, excipients, diluents, antioxidants, preservatives, coloring, flavoring and diluting agents, emulsifying agents, suspending agents, solvents, fillers, bulking agents, buffers, delivery vehicles, tonicity agents, cosolvents, wetting agents, complexing agents, buffering agents, antimicrobials, and surfactants.

[0171] Neutral buffered saline or saline mixed with serum albumin are exemplary appropriate carriers. The pharmaceutical compositions may include antioxidants such as ascorbic acid; low molecular weight polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and

other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween, pluronics, or polyethylene glycol (PEG). Also by way of example, suitable tonicity enhancing agents include alkali metal halides (preferably sodium or potassium chloride), mannitol, sorbitol, and the like. Suitable preservatives include benzalkonium chloride, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid and the like. Hydrogen peroxide also may be used as preservative. Suitable cosolvents include glycerin, propylene glycol, and PEG. Suitable complexing agents include caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxy-propyl-beta-cyclodextrin. Suitable surfactants or wetting agents include sorbitan esters, polysorbates such as polysorbate 80, tromethamine, lecithin, cholesterol, tyloxapal, and the like. The buffers may be conventional buffers such as acetate, borate, citrate, phosphate, bicarbonate, or Tris-HCl. Acetate buffer may be about pH 4-5.5, and Tris buffer may be about pH 7-8.5. Additional pharmaceutical agents are set forth in Remington's Pharmaceutical Sciences, 18th Edition, A. R. Gennaro, ed., Mack Publishing Company, 1990.

[0172] The composition may be in liquid form or in a lyophilized or freeze-dried form and may include one or more lyoprotectants, excipients, surfactants, high molecular weight structural additives and/or bulking agents (see, for example, U.S. Patent Nos. 6,685,940, 6,566,329, and 6,372,716). In one embodiment, a lyoprotectant is included, which is a non-reducing sugar such as sucrose, lactose or trehalose. The amount of lyoprotectant generally included is such that, upon reconstitution, the resulting formulation will be isotonic, although hypertonic or slightly hypotonic formulations also may be suitable. In addition, the amount of lyoprotectant should be sufficient to prevent an unacceptable amount of degradation and/or aggregation of the protein upon lyophilization. Exemplary lyoprotectant concentrations for sugars (*e.g.,* sucrose, lactose, trehalose) in the pre-lyophilized formulation are from about 10 mM to about 400 mM. In another embodiment, a surfactant is included, such as for example, nonionic surfactants and ionic surfactants such as polysorbates (*e.g.,* polysorbate 20, polysorbate 80); poloxamers (*e.g.,* poloxamer 188); poly(ethylene glycol) phenyl ethers (*e.g.*, Triton); sodium dodecyl sulfate (SDS); sodium laurel sulfate; sodium octyl glycoside; lauryl-, myristyl-, linoleyl-, or stearyl-sulfobetaine; lauryl-, myristyl-, linoleyl-or stearyl-sarcosine; linoleyl, myristyl-, or cetyl-betaine; lauroamidopropyl-, cocamidopropyl-, linoleamidopropyl-, myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-betaine (*e.g.,* lauroamidopropyl); myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-

dimethylamine; sodium methyl cocoyl-, or disodium methyl ofeyl-taurate; and the MONAQUAT™. series (Mona Industries, Inc., Paterson, N.J.), polyethyl glycol, polypropyl glycol, and copolymers of ethylene and propylene glycol (*e.g.,* Pluronics, PF68 etc). Exemplary amounts of surfactant that may be present in the pre-lyophilized formulation are from about 0.001-0.5%. High molecular weight structural additives (*e.g.,* fillers, binders) may include for example, acacia, albumin, alginic acid, calcium phosphate (dibasic), cellulose, carboxymethylcellulose, carboxymethylcellulose sodium, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, microcrystalline cellulose, dextran, dextrin, dextrates, sucrose, tylose, pregelatinized starch, calcium sulfate, amylose, glycine, bentonite, maltose, sorbitol, ethylcellulose, disodium hydrogen phosphate, disodium phosphate, disodium pyrosulfite, polyvinyl alcohol, gelatin, glucose, guar gum, liquid glucose, compressible sugar, magnesium aluminum silicate, maltodextrin, polyethylene oxide, polymethacrylates, povidone, sodium alginate, tragacanth microcrystalline cellulose, starch, and zein. Exemplary concentrations of high molecular weight structural additives are from 0.1% to 10% by weight. In other embodiments, a bulking agent (*e.g.,* mannitol, glycine) may be included.

[0173] Compositions may be suitable for parenteral administration. Exemplary compositions are suitable for injection or infusion into an animal by any route available to the skilled worker, such as intraarticular, subcutaneous, intravenous, intramuscular, intraperitoneal, intracerebral (intraparenchymal), intracerebroventricular, intramuscular, intraocular, intraarterial, or intralesional routes. A parenteral formulation typically will be a sterile, pyrogen-free, isotonic aqueous solution, optionally containing pharmaceutically acceptable preservatives.

[0174] Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringers' dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, anti-microbials, anti-oxidants, chelating agents, inert gases and the like. See generally, Remington's Pharmaceutical Science, 16th Ed., Mack Eds., 1980.

[0175] Pharmaceutical compositions described herein may be formulated for controlled or sustained delivery in a manner that provides local concentration of the product (*e.g.,* bolus, depot effect) and/or increased stability or half-life in a particular local environment. The compositions may comprise the formulation of CAR-EC switches, polypeptides, nucleic acids, or vectors disclosed herein with particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, *etc.*, as well as agents such as a biodegradable matrix, injectable microspheres, microcapsular particles, microcapsules, bioerodible particles beads, liposomes, and implantable delivery devices that provide for the controlled or sustained release of the active agent which then may be delivered as a depot injection. Techniques for formulating such sustained-or controlled-delivery means are known and a variety of polymers have been developed and used for the controlled release and delivery of drugs. Such polymers are typically biodegradable and biocompatible. Polymer hydrogels, including those formed by complexation of enantiomeric polymer or polypeptide segments, and hydrogels with temperature or pH sensitive properties, may be desirable for providing drug depot effect because of the mild and aqueous conditions involved in trapping bioactive protein agents (*e.g.,* antibodies comprising an ultralong CDR3). See, for example, the description of controlled release porous polymeric microparticles for the delivery of pharmaceutical compositions in WO 93/15722. Suitable materials for this purpose include polylactides (see, *e.g.,* U.S. Patent No. 3,773,919), polymers of poly-(a-hydroxycarboxylic acids), such as poly-D-(-)-3-hydroxybutyric acid (EP 133,988A), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al.*, Biopolymers, 22: 547-556 (1983)), poly(2-hydroxyethyl-methacrylate) (Langer *et al.*, J. Biomed. Mater. Res., 15: 167-277 (1981), and Langer, Chem. Tech., 12: 98-105 (1982)), ethylene vinyl acetate, or poly-D(-)-3-hydroxybutyric acid. Other biodegradable polymers include poly(lactones), poly(acetals), poly(orthoesters), and poly(orthocarbonates). Sustained-release compositions also may include liposomes, which may be prepared by any of several methods known in the art (see, *e.g.,* Eppstein *et al.*, Proc. Natl. Acad. Sci. USA, 82: 3688-92 (1985)). The carrier itself, or its degradation products, should be nontoxic in the target tissue and should not further aggravate the condition. This may be determined by routine screening in animal models of the target disorder or, if such models are unavailable, in normal animals. Microencapsulation of recombinant proteins for sustained release has been performed successfully with human growth hormone (rhGH), interferon-(rhIFN-), interleukin-2, and MN rgp120. Johnson *et al.*, Nat. Med., 2:795-799 (1996); Yasuda, Biomed. Ther., 27:1221-1223 (1993); Hora *et al.*, Bio/Technology. 8:755-

758 (1990); Cleland, "Design and Production of Single Immunization Vaccines Using Polylactide Polyglycolide Microsphere Systems," in Vaccine Design: The Subunit and Adjuvant Approach, Powell and Newman, eds, (Plenum Press: New York, 1995), pp. 439-462; WO 97/03692, WO 96/40072, WO 96/07399; and U.S. Patent No. 5,654,010. The sustained-release formulations of these proteins were developed using poly-lactic-coglycolic acid (PLGA) polymer due to its biocompatibility and wide range of biodegradable properties. The degradation products of PLGA, lactic and glycolic acids may be cleared quickly within the human body. Moreover, the degradability of this polymer may be depending on its molecular weight and composition. Lewis, "Controlled release of bioactive agents from lactide/glycolide polymer," in: M. Chasin and R. Langer (Eds.), Biodegradable Polymers as Drug Delivery Systems (Marcel Dekker: New York, 1990), pp. 1-41. Additional examples of sustained release compositions include, for example, EP 58,481A, U.S. Patent No. 3,887,699, EP 158,277A, Canadian Patent No. 1176565, U. Sidman *et al.*, Biopolymers 22, 547 [1983], R. Langer *et al.*, Chem. Tech. 12, 98 [1982], Sinha *et al.*, J. Control. Release 90, 261 [2003], Zhu *et al.*, Nat. Biotechnol. 18, 24 [2000], and Dai *et al.*, Colloids Surf B Biointerfaces 41, 117 [2005].

[0176] Bioadhesive polymers are also contemplated for use in or with compositions of the present disclosure. Bioadhesives are synthetic and naturally occurring materials able to adhere to biological substrates for extended time periods. For example, Carbopol and polycarbophil are both synthetic cross-linked derivatives of poly(acrylic acid). Bioadhesive delivery systems based on naturally occurring substances include for example hyaluronic acid, also known as hyaluronan. Hyaluronic acid is a naturally occurring mucopolysaccharide consisting of residues of D-glucuronic and N-acetyl-D-glucosamine. Hyaluronic acid is found in the extracellular tissue matrix of vertebrates, including in connective tissues, as well as in synovial fluid and in the vitreous and aqueous humor of the eye. Esterified derivatives of hyaluronic acid have been used to produce microspheres for use in delivery that are biocompatible and biodegradable (see, for example, Cortivo *et al.*, Biomaterials (1991) 12:727-730; EP 517,565; WO 96/29998; Illum *et al.*, J. Controlled Rel. (1994) 29:133-141).

[0177] Both biodegradable and non-biodegradable polymeric matrices may be used to deliver compositions of the present disclosure, and such polymeric matrices may comprise natural or synthetic polymers. Biodegradable matrices are preferred. The period of time over which release occurs is based on selection of the polymer. Typically, release over a period ranging from between a few hours and three to twelve months is most desirable. Exemplary

synthetic polymers which may be used to form the biodegradable delivery system include: polymers of lactic acid and glycolic acid, polyamides, polycarbonates, polyalkylenes, polyalkylene glycols, polyalkylene oxides, polyalkylene terepthalates, polyvinyl alcohols, polyvinyl ethers, polyvinyl esters, poly-vinyl halides, polyvinylpyrrolidone, polyglycolides, polysiloxanes, polyanhydrides, polyurethanes and co-polymers thereof, poly(butic acid), poly(valeric acid), alkyl cellulose, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, polymers of acrylic and methacrylic esters, methyl cellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, hydroxybutyl methyl cellulose, cellulose acetate, cellulose propionate, cellulose acetate butyrate, cellulose acetate phthalate, carboxylethyl cellulose, cellulose triacetate, cellulose sulphate sodium salt, poly(methyl methacrylate), poly(ethyl methacrylate), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate), polyethylene, polypropylene, poly(ethylene glycol), poly(ethylene oxide), poly(ethylene terephthalate), poly(vinyl alcohols), polyvinyl acetate, poly vinyl chloride, polystyrene and polyvinylpyrrolidone. Exemplary natural polymers include alginate and other polysaccharides including dextran and cellulose, collagen, chemical derivatives thereof (substitutions, additions of chemical groups, for example, alkyl, alkylene, hydroxylations, oxidations, and other modifications routinely made by those skilled in the art), albumin and other hydrophilic proteins, zein and other prolamines and hydrophobic proteins, copolymers and mixtures thereof. In general, these materials degrade either by enzymatic hydrolysis or exposure to water *in vivo*, by surface or bulk erosion. The polymer optionally is in the form of a hydrogel (see, for example, WO 04/009664, WO 05/087201, Sawhney, *et al.*, Macromolecules, 1993, 26, 581-587) that can absorb up to about 90% of its weight in water and further, optionally is cross-linked with multi-valent ions or other polymers.

[0178] Delivery systems also include non-polymer systems that are lipids including sterols such as cholesterol, cholesterol esters and fatty acids or neutral fats such as mono-di- and tri-glycerides; hydrogel release systems; silastic systems; peptide based systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and the like. Specific examples include, but are not limited to: (a) erosional systems in which the product is contained in a form within a matrix such as those described in U.S. Patent Nos. 4,452,775, 4,675,189 and 5,736,152 and (b) diffusional systems in which a

product permeates at a controlled rate from a polymer such as described in U.S. Patent Nos. 3,854,480, 5,133,974 and 5,407,686. Liposomes containing the product may be prepared by methods known methods, such as for example (DE 3,218,121; Epstein *et al.,* Proc. Natl. Acad. Sci. USA, 82: 3688-3692 (1985); Hwang *et al.,* Proc. Natl. Acad. Sci. USA, 77: 4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; JP 83-118008; U.S. Patent Nos. 4,485,045 and 4,544,545; and EP 102,324).

[0179] Alternatively or additionally, the compositions may be administered locally via implantation into the affected area of a membrane, sponge, or other appropriate material on to which a CAR-EC switch disclosed herein has been absorbed or encapsulated. Where an implantation device is used, the device may be implanted into any suitable tissue or organ, and delivery of a CAR-EC switch, nucleic acid, or vector disclosed herein may be directly through the device via bolus, or via continuous administration, or via catheter using continuous infusion.

[0180] A pharmaceutical composition comprising a CAR-EC switch disclosed herein may be formulated for inhalation, such as for example, as a dry powder. Inhalation solutions also may be formulated in a liquefied propellant for aerosol delivery. In yet another formulation, solutions may be nebulized. Additional pharmaceutical composition for pulmonary administration include, those described, for example, in WO 94/20069, which discloses pulmonary delivery of chemically modified proteins. For pulmonary delivery, the particle size should be suitable for delivery to the distal lung. For example, the particle size may be from 1 μm to 5 μm; however, larger particles may be used, for example, if each particle is fairly porous.

[0181] Certain formulations containing CAR-EC switches disclosed herein may be administered orally. Formulations administered in this fashion may be formulated with or without those carriers customarily used in the compounding of solid dosage forms such as tablets and capsules. For example, a capsule may be designed to release the active portion of the formulation at the point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional agents may be included to facilitate absorption of a selective binding agent. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders also may be employed.

[0182] Another preparation may involve an effective quantity of a CAR-EC switch disclosed herein in a mixture with non-toxic excipients which are suitable for the

manufacture of tablets. By dissolving the tablets in sterile water, or another appropriate vehicle, solutions may be prepared in unit dose form. Suitable excipients include, but are not limited to, inert diluents, such as calcium carbonate, sodium carbonate or bicarbonate, lactose, or calcium phosphate; or binding agents, such as starch, gelatin, or acacia; or lubricating agents such as magnesium stearate, stearic acid, or talc.

[0183] Suitable and/or preferred pharmaceutical formulations may be determined in view of the present disclosure and general knowledge of formulation technology, depending upon the intended route of administration, delivery format, and desired dosage. Regardless of the manner of administration, an effective dose may be calculated according to patient body weight, body surface area, or organ size. Further refinement of the calculations for determining the appropriate dosage for treatment involving each of the formulations described herein are routinely made in the art and is within the ambit of tasks routinely performed in the art. Appropriate dosages may be ascertained through use of appropriate dose-response data.

CAR-EC Switch Production Methods

Methods of Producing Switches and Switch Intermediates

[0184] Disclosed herein are methods of producing CAR-EC switches. The method comprises attaching a chimeric antigen receptor-interacting domain (CAR-ID) to a target interacting domain TID wherein the TID comprises an anti-Her2 antibody fragment Fab (clone 4D5), or a fragment thereof, which Fab comprises an unnatural amino acid located at each of Glycine 212 of a light chain of the anti-Her2 antibody Fab (LG212X; SEQ ID NO: 2) and Lysine 221 of a heavy chain of the anti-Her2 antibody Fab (HK221X; SEQ ID NO: 1), and wherein each of LG212X and HK221X is attached or linked to a separate CAR interacting domain. Alternatively, the method may comprise attaching a switch intermediate comprising a CAR-ID and a linker to a TID. The method may comprise attaching a switch intermediate comprising a TID and a linker to a CAR-ID. The method may comprise attaching a first switch intermediate comprising a CAR-ID and a first linker to a second switch comprising a TID and a second linker.. Attachment in a site-specific manner may comprise attaching the TID to a predetermined site on the CAR-ID. Attachment of the CAR-ID to the TID may occur in a site-independent manner. Attachment in a site-independent manner may comprise attaching the CAR-ID to a random site on the TID. Attachment in a site-independent manner may comprise attaching the TID to a random site on the CAR-ID. The method may further comprise attaching one or more additional CAR-IDs to the TID. The

method may further comprise attaching or more additional TIDs to the CAR-ID. The method may further comprise using one or more additional linkers to connect the TID to the CAR-ID. Attaching the CAR-ID to the TID may comprise conducting one or more chemical reactions.

[0185] The method of producing a switch may comprise linking a TID based on or derived from an antibody or antibody fragment to a CAR-ID or a switch intermediate comprising a CAR-ID to produce a CAR-EC switch comprising (a) the TID; (b) one or more linkers; and (c) the CAR-ID, The one or more linkers may link the TID to the CAR-ID. Linking the TID to the CAR-ID may occur in a site-specific manner. The CAR-ID may be attached to a predetermined site on the TID via the one or more linkers. The TID may be attached to a predetermined site on the CAR-ID via the one or more linkers.

[0186] Disclosed herein are methods of producing a switch of Formula I: X-L1-Y or Formula IA: Y-L1-X, wherein X is a chimeric antigen receptor-interacting domain (CAR-ID), Y is a target interacting domain (TID) and L1 is a linker. X may be a CAR binding small molecule and Y may be an antibody or antibody fragment. X may be a CAR binding small molecule that does not comprise a peptide and Y may be a peptide that does not comprise an antibody or antibody fragment. X may be a CAR binding small molecule that does not comprise a peptide and Y may be a targeting small molecule that does not comprise a peptide. The method may comprise conducting one or more reactions to attach the CAR-ID to a predetermined site in the TID. Conducting the one or more reactions to attach the CAR-ID to the TID may comprise mixing a plurality of CAR-IDs with a plurality of TIDs. The method may comprise attaching one end of the linker to the TID, followed by attachment of the other end of the linker to the CAR-ID. The method may comprise attaching one end of the linker to the CAR-ID, followed by attachment of the other end of the linker to the TID. Attachment of the linker to the TID may occur in a site-specific manner. The linker may be attached to a predetermined amino acid of the TID. The amino acid may be an unnatural amino acid. The linker may comprise a functional group that interacts with the amino acid. Attachment of the linker to the TID may occur in a site-independent manner. The linker may be randomly attached to the TID. The linker may comprise a functional group that reacts with a functional group in the TID. Attachment of the linker to the CAR-ID may occur in a site-specific manner. Attachment of the linker to the CAR-ID may occur in a site-independent manner. The linker may comprise a functional group that reacts with a function group in the CAR-ID. Conducting the one or more reactions to attach the CAR-ID to the TID may comprise conducting an oxime ligation.

[0187] Alternatively, or additionally, the method may comprise conducting a reaction to attach the linker or a precursor of the linker to the CAR-ID to produce a switch intermediate comprising the linker conjugated to the CAR-ID. The switch intermediate may have the Formula II: X-L1 or Formula IIA: L1-X, wherein X is the CAR-ID and L1 is the linker or precursor of the linker. The linker may be conjugated to the CAR-ID in a site-specific manner. The linker may be conjugated to the CAR-ID in a site-independent manner. Conducting the one or more reactions to attach the CAR-ID to the TID may comprise attaching the linker portion of the switch intermediate to the TID. Conducting the one or more reactions to attach the CAR-ID to the TID may comprise contacting a plurality of switch intermediates comprising the linker or linker precursor conjugated to the CAR-ID with a plurality of TIDs. Attachment of the linker portion of the switch intermediate to the TID may occur in a site-specific manner. The TID may comprise one or more unnatural amino acids. The linker portion of the switch may be attached to the TID via the one or more unnatural amino acids. Attachment of the linker portion of the switch intermediate may occur in a site-independent manner.

[0188] Alternatively, or additionally, the method may comprise conducting a reaction to attach the linker or a precursor of the linker to the TID to produce a switch intermediate comprising the linker or precursor of the linker conjugated to the TID. The switch intermediate may be of Formula III: Y-L1 or Formula IIIA: L1-Y, wherein Y is the TID and L1 is the linker or linker precursor. The linker may be conjugated to the TID in a site-specific manner. The linker may be conjugated to the TID in a site-independent manner. Conducting the one or more reactions to attach the CAR-ID to the TID may comprise attaching the linker portion of the switch intermediate to the CAR-ID. Conducting the one or more reactions to attach the CAR-ID to the TID may comprise contacting a plurality of switch intermediates comprising the linker or linker precursor conjugated to the TID with a plurality of CAR-IDs. Attachment of the linker portion of the switch intermediate to the CAR-ID may occur in a site-specific manner. Attachment of the linker portion of the switch intermediate may occur in a site-independent manner.

[0189] The method may comprise coupling one or more linkers to the TID to produce a switch intermediate of Formula III: Y-L1 or Formula IIIA: L1-Y, wherein Y is the TID and L1 is the linker; and conjugating the switch intermediate to the CAR-ID, thereby producing the CAR-EC switch. The switch intermediate may be conjugated to the CAR-ID in a site-specific manner. The switch intermediate may be conjugated to the CAR-ID in a site-

independent manner. The method may further comprise incorporating one or more unnatural amino acids into the CAR-ID and/ or TID. The switch intermediate may be conjugated to the CAR-ID in a site-specific manner through the use of the unnatural amino acid.

[0190] The method may comprise coupling one or more linkers to the CAR-ID to produce a switch intermediate of Formula II: X-L1 or Formula IIA: L1-X, wherein X is the CAR-ID and L1 is the linker; and conjugating the switch intermediate to the TID, thereby producing the CAR-EC switch. The switch intermediate may be conjugated to the TID in a site-specific manner. The switch intermediate may be conjugated to the TID in a site-independent manner. The method may further comprise incorporating one or more unnatural amino acids into the CAR-ID and/ or TID. The switch intermediate may be conjugated to the TID in a site-specific manner through the use of the unnatural amino acid.

[0191] Conjugating the switch intermediate of Formula II: X-L1 or Formula IIA: L1-X, wherein X is the CAR-ID and L1, to the TID may comprise forming an oxime. Conjugating the switch intermediate of Formula III: Y-L1 or Formula IIIA: L1-Y, wherein Y is the TID and L1, to the CAR-ID may comprise forming an oxime. Forming an oxime may comprise conducting one or more reactions under acidic conditions. Forming an oxime may comprise conducting one or more reactions under slightly acidic conditions. Forming an oxime may comprise conducting one or more reactions under slightly neutral conditions.

[0192] A method of producing a switch may comprise (a) producing a target interacting domain TID comprising an unnatural amino acid; (b) attaching a first linker to the TID to produce a first switch intermediate comprising the TID and the first linker; (c) attaching a second switch intermediate comprising a chimeric antigen receptor-interacting domain (CAR-ID) and a second linker to the first switch intermediate, thereby producing the switch. The unnatural amino acid may be *p*-acetylphenalanine (*p*AcF). The TID may comprise a polypeptide based on or derived from an antibody or antibody fragment. The antibody may be selected from the group consisting of an anti-CD19 antibody, an anti-CD22 antibody, an anti-CD20 antibody, an anti-EGFR antibody, an anti-EGFRvIII antibody, an anti-Her2 antibody, an anti-CS1 antibody, an anti-BCMA antibody, an anti-CEA antibody, an anti-CLL-1 antibody and an anti-CD33 antibody. The antibody may be an anti-CD19 antibody. The antibody may be an anti-EGFR antibody. The antibody may be an anti-CD20 antibody. The antibody may be an anti-HER2 antibody. The TID may comprise an antibody fragment. The antibody may comprise an amino acid sequence of any one of SEQ ID NOs: 10-31. The antibody may be encoded by a nucleotide sequence of any one of SEQ ID NOs: 5-9. The first

linker may be a bifunctional linker. The linker may be a heterobifunctional linker. The linker may comprise one or more polyethylene glycol (PEG) subunits. The first linker may comprise cyclooctyne. The first linker may be a PEG-cyclooctyne linker. The linker may comprise an azide. The first linker may comprise triazole. The triazole may be 1,2,3-triazole. The triazole may be 1,2,4-triazole. The first linker may comprise an azide-PEG-aminoxy linker. The first linker may be attached to a ketone of the unnatural amino acid. The first linker may be attached to the TID via oxime ligation. The CAR-ID may comprise a small molecule. The CAR-ID may comprise FITC. The second linker may be a bifunctional linker. The linker may be a heterobifunctional linker. The linker may comprise one or more polyethylene glycol (PEG) subunits. The second linker may comprise cyclooctyne. The second linker may be a PEG-cyclooctyne linker. The linker may comprise an azide. The second linker may comprise triazole. The triazole may be 1,2,3-triazole. The triazole may be 1,2,4-triazole. The second linker may be a PEG-cyclooctyne linker. The second switch intermediate may be attached to the first switch intermediate via a click chemistry reaction. The second switch intermediate may be attached to the first switch intermediate through a cycloaddition reaction. The cycloaddition reaction may be a [3+2] cycloaddition reaction.

[0193] Conjugating the linker to the CAR-ID to produce the switch may comprise forming one or more bonds between the linker and the CAR-ID. Conjugating the linker to the TID to produce the switch may comprise forming one or more bonds between the linker and the TID. The one or more bonds may comprise an ionic bond, a covalent bond, a non-covalent bond or a combination thereof. Additional methods of conjugating the linker the CAR-ID and the TID may be performed as described in Roberts et al., Advanced Drug Delivery Reviews 54:459-476 (2002), which is included by reference in its entirety.

[0194] The CAR-ID may comprise any of the CAR-IDs disclosed herein. For example, the CAR-ID may comprise a small molecule. The CAR-ID may comprise FITC. The CAR-ID may be selected from the group consisting of DOTA, dinitrophenol, quinone, biotin, aniline, atrazine, an aniline-derivative, o-aminobenzoic acid, p-aminobenzoic acid, m-aminobenzoic acid, hydralazine, halothane, digoxigenin, benzene arsonate, lactose, trinitrophenol, biotin and derivatives thereof. The TID may comprise any of the TIDs disclosed herein. For example, the TID may comprise a small molecule. The TID may comprise 2-[3-(1,3-dicarboxypropyl)ureido] pentanedioic acid or a derivative thereof. The TID may comprise folate. The TID may be based on or derived from an antibody or antibody fragment. The antibody or antibody fragment may comprise anti-CD19. The antibody or

antibody fragment may be selected from the group consisting of anti-CD20, anti-CD22, anti-CD33, anti-BMSA, anti-carcinoembryonic antigen (CEA), anti-C type lectin-like molecule 1 (CLL1), anti-CS1, anti-epidermal growth factor receptor (EGFR), and anti-human epidermal growth factor receptor 2 (Her2). The linker may comprise any of the linkers disclosed herein. For example, the linker may comprise an aminooxy group, azide group cyclooctyne group, or a combination thereof at one or more termini. The linker may be a bifunctional linker. The linker may be a heterobifunctional linker. The linker may comprise one or more PEG subunits.

[0195] Disclosed herein are methods of producing a switch of Formula IV: X- L1-L2-Y, wherein in X is a CAR-ID, L1 is a first linker, L2 is a second linker and Y is a TID. The method may comprise (a) coupling L1 to X to produce a first switch intermediate of Formula II: X- L1; (b) coupling L2 to Y to produce a second switch intermediate of Formula V: L2-Y; and (c) linking the first switch intermediate of Formula II to the second switch intermediate of Formula: V, thereby producing the switch of Formula IV.

[0196] Disclosed herein are methods of producing a switch of Formula IVA: Y-L2-L1-X, wherein Y is a TID, L1 is a first linker, L2 is a second linker and X is a CAR-ID. The method may comprise (a) coupling L1 to X to produce a first switch intermediate of Formula IIA: L1-X; (b) coupling L2 to Y to produce a second switch intermediate of Formula VA: Y-L2; and (c) linking the first intermediate of Formula IIA to the second intermediate of Formula VA, thereby producing the CAR-EC switch of Formula IVA.

[0197] The methods may further comprise incorporating one or more unnatural amino acids into X and/or Y. The L1 may be coupled to X in a site-specific manner. The L1 may be coupled to X in a site-specific manner through the one or more unnatural amino acids. L2 may be coupled to Y in a site-specific manner. The L2 may be coupled to Y in a site-specific manner through the one or more unnatural amino acids. The method may further comprise modifying a nucleic acid encoding X to produce one or more amber codons in X. The method may further comprise modifying a nucleic acid encoding Y to produce one or more amber codons in Y.

[0198] Conjugating the linker to the CAR-ID to produce the first switch intermediate may comprise forming one or more bonds between the linker and the CAR-ID. Conjugating the linker to the TID to produce the second switch intermediate may comprise forming one or more bonds between the linker and the TID. The one or more bonds may comprise an ionic bond, a covalent bond, a non-covalent bond or a combination thereof. Additional methods of

conjugating the linker the CAR-ID and the TID may be performed as described in Roberts et al., Advanced Drug Delivery Reviews 54:459-476 (2002), which is included by reference in its entirety.

[0199] Linking the first switch intermediate to the second switch intermediate may comprise a Huisgen-cycloaddition, a Diels-Halder reaction, a hetero Diels-Alder reaction or an enzyme-mediated reaction. Linking the first switch intermediate to the second switch intermediate may produce an oxime, a tetrazole, a Diels Alder adduct, a hetero Diels Alder adduct, an aromatic substitution reaction product, a nucleophilic substitution reaction product, an ester, an amide, a carbamate, an ether, a thioether, a Michael reaction product, cycloaddition product, a metathesis reaction product, a metal-mediated cross-coupling reaction product, a radical polymerization product, an oxidative coupling product, an acyl-transfer reaction product, or a photo click reaction product. Linking the first switch intermediate to the second switch intermediate may produce a disulfide bridge or a maleimide bridge.

[0200] L1 and/or L2 may comprise a linker selected from a bifunctional linker, a cleavable linker, a non-cleavable linker, an ethylene glycol linker, a bifunctional ethylene glycol linker, a flexible linker, or an inflexible linker. L1 and/or L2 may comprise a linker selected from the group comprising cyclooctyne, cyclopropene, aryl/alkyl azides, trans-cyclooctene, norborene, and tetrazines. A terminus of L1 and/or a terminus of L2 may comprise an alkoxy-amine. A terminus of L1 and/or a terminus of L2 may comprise an azide or cyclooctyne group. X may be coupled to L1 by a chemical group selected from a cyclooctyne, cyclopropene, aryl/alkyl azide, trans-cyclooctene, norborene, and tetrazine. Linking the first switch intermediate (X-L1 or L1-X) and second switch intermediate (Y-L2 or L2-Y) may comprise conducting one or more copper-free reactions. Linking the first switch intermediate (X-L1 or L1-X) and second switch intermediate (Y-L2 or L2-Y) may comprise conducting one or more copper-containing reactions. Linking the first switch intermediate (X-L1 or L1-X) and second switch intermediate (Y-L2 or L2-Y) may comprise one or more cycloadditions. Linking the first switch intermediate (X-L1 or L1-X) and second switch intermediate (Y-L2 or L2-Y) may comprise one or more Huisgen-cycloadditions. Linking the first switch intermediate (X-L1 or L1-X) and second switch intermediate (Y-L2 or L2-Y) may comprise one or more Diels Alder reactions. Linking the first switch intermediate (X-L1 or L1-X) and second switch intermediate (Y-L2 or L2-Y) may comprise one or more Hetero Diels Alder reaction.

[0201] The methods disclosed herein may comprise coupling one or more linkers to one or more TIDs, CAR-IDs or combinations thereof to produce one or more switch intermediates. The switch intermediate may comprise a TID attached to a linker (e.g., TID switch intermediate). The switch intermediate may comprise a CAR-ID attached to a linker (e.g., CAR-ID switch intermediates). The methods may comprise coupling a first linker to TID to produce a TID switch intermediate. The methods may comprise coupling a linker to a CAR-ID to produce a CAR-ID switch intermediate.

[0202] Coupling of the one or more linkers to the TID and the CAR-ID may occur simultaneously. Coupling of the one or more linkers to the TID and the CAR-ID may occur sequentially. Coupling of the one or more linkers to the TID and the CAR-ID may occur in a single reaction volume. Coupling of the one or more linkers to the TID and the CAR-ID may occur in two or more reaction volumes.

[0203] Coupling one or more linkers to the TID and/or the CAR-ID may comprise forming one or more oximes between the linker and the TID and/or the CAR-ID. Coupling one or more linkers to the TID and/or the CAR-ID may comprise forming one or more stable bonds between the linker and the TID and/or the CAR-ID. Coupling one or more linkers to the TID and/or the CAR-ID may comprise forming one or more covalent bonds between the linker and the TID and/or the CAR-ID. Coupling one or more linkers to the TID and/or the CAR-ID may comprise forming one or more non-covalent bonds between the linker and TID and/or the CAR-ID. Coupling one or more linkers to the TID and/or the CAR-ID may comprise forming one or more ionic bonds between the linker and the TID and/or the CAR-ID.

[0204] Coupling one or more linkers to the TID and/or the CAR-ID may comprise site specifically coupling one or more linkers to the TID and/or the CAR-ID. Site-specific coupling may comprise linking the one or more linkers to the unnatural amino acid of the TID and/or the CAR-ID. Linking the one or more linkers to the unnatural amino acid of the TID and/or the CAR-ID may comprise formation of an oxime. Linking the one or more linkers to the unnatural amino acid of the TID and/or the CAR-ID may comprise, by way of non-limiting example, reacting a hydroxylamine of the one or more linkers with an aldehyde or ketone of an amino acid. The amino acid may be an unnatural amino acid.

[0205] Conducting the one or more reactions to site-specifically link the CAR-ID to the TID, to site-specifically attach the linker or a precursor of the linker to the CAR-ID, to site-specifically attach the linker or a precursor of the linker to the TID, to site-specifically attach

the CAR-ID switch intermediate to the TID, to site-specifically attach the TID switch intermediate to the CAR-ID or to site-specifically attach the TID switch intermediate to the CAR-ID switch intermediate may comprise conducting one or more reactions selected from a copper-free reaction, a cycloadditions, a Huisgen-cycloaddition, a copper-free [3+2] Huisgen-cycloaddition, a copper-containing reaction, a Diels Alder reactions, a hetero Diels Alder reaction, metathesis reaction, a metal-mediated cross-coupling reaction, a radical polymerization, an oxidative coupling, an acyl-transfer reaction, a photo click reaction, an enzyme-mediated reaction, a transglutaminase-mediated reaction.

[0206] The switches disclosed herein may comprise a CAR-ID comprising FITC or a derivative thereof. The method of producing such switches may comprise coupling a linker or precursor thereof, a switch intermediate comprising a TID (e.g., TID switch intermediate), or a TID to the CAR-ID. Coupling the linker or precursor thereof, the TID switch intermediate to the CAR-ID may comprise conjugation of an isothiocyanate of FITC to the linker or precursor thereof, TID switch intermediate or TID. The TID may be based on or derived from a polypeptide. The polypeptide may be an antibody or antibody fragment. Coupling a TID to the CAR-ID may comprise conjugating the isothiocyanate of FITC to an amino acid of the TID. The amino acid may be a lysine. The method may comprise coupling or more CAR-IDs to the TID. The method may comprise conjugating FITC from two or more CAR-IDs to two or more amino acids of the TID. The two or more amino acids may be lysine.

[0207] Producing a switch disclosed herein may comprise ester coupling. Ester coupling may comprise forming an amide bond between the CAR-ID and the TID. Ester coupling may comprise forming an amide bond between a switch intermediate and the TID. The switch intermediate may comprise a CAR-ID attached to a linker. The amide bond may be formed between the linker of the switch intermediate and the TID. The linker may be a NHS-ester linker. The amide bond may be formed between the linker of the switch intermediate and an amino acid of the TID. The CAR-ID may comprise a small molecule. The small molecule may be FITC. The TID may be based on or derived from a polypeptide. The polypeptide may be an antibody or antibody fragment. The TID may comprise a small molecule.

[0208] The method of producing a switch disclosed herein may comprise: (a) obtaining a switch intermediate comprising (i) a chimeric antigen receptor-interacting domain (CAR-ID); and (ii) a linker; and (b) contacting the switch intermediate with a target interacting domain TID, thereby producing the switch. Contacting the switch intermediate with the TID may comprise performing an ester coupling reaction. The linker may comprise a NHS-ester linker.

The TID may comprise one or more amino acids. Performing the ester coupling reaction may comprise forming an amide bond between the NHS-ester linker of the switch intermediate and the one or more amino acids of the TID. The method may further comprise producing a plurality of switches. Two or more switches of the plurality of switches may comprise two or more switch intermediates attached to two or more different amino acids of the TID. For example, a first switch intermediate may be attached to a lysine residue of a first TID and a second switch intermediate may be attached to a glycine residue of a second TID. Two or more switches of the plurality of switches may comprise two or more switch intermediates attached to the same amino acid of the TID. For example, the two or more switch intermediates may be attached to a lysine residue of a first and second TID. Two or more switches of the plurality of switches may comprise two or more switch intermediates attached to the same amino acid located at two or more different positions in the TID. For example, a first switch intermediate may be attached to lysine 10 of a first TID and the second switch intermediate may be attached to lysine 45 of a second TID. Two or more switches of the plurality of switches may comprise two or more switch intermediates attached to the same amino acid located at the same position in the TID. For example, a first switch intermediate may be attached to lysine 10 of a first TID and the second switch intermediate may be attached to lysine 10 of a second TID.

[0209] Methods of producing a switch disclosed herein may comprise using one or more unnatural amino acids. The method may comprise incorporating one or more unnatural amino acids into the CAR-ID. The CAR-ID may be based on or derived from a polypeptide that can interact with a chimeric antigen receptor on an effector cell. The polypeptide may be a non-antibody based polypeptide. Generally, a non-antibody based polypeptide is a polypeptide that does not comprise an antibody or antibody fragment. The unnatural amino acid may be incorporated into the non-antibody based polypeptide. The unnatural amino acid may replace an amino acid of the non-antibody based polypeptide. Alternatively, or additionally, the method may comprise incorporating one or more unnatural amino acids into the TID. The TID may be based on or derived from a polypeptide. The polypeptide may be an antibody. The polypeptide may be a non-antibody based polypeptide. The unnatural amino acid may be incorporated into the polypeptide. The unnatural amino acid may replace an amino acid of the polypeptide.

[0210] The method of producing the switch may further comprise modifying one or more amino acid residues in polypeptide from which the CAR-ID is based or derived. The method

of producing the switch may comprise modifying one or more amino acid residues in polypeptide from which the TID is based or derived. Modifying the one or more amino acid residues may comprise mutating one or more nucleotides in the nucleotide sequence encoding the polypeptide. Mutating the one or more nucleotides in the nucleotide sequence encoding may comprise altering a codon encoding an amino acid to a nonsense codon.

[0211] Incorporating one or more unnatural amino acids into the polypeptide from which the CAR-ID is based or derived may comprise modifying one or more amino acid residues in the polypeptide to produce one or more amber codons in the antibody or antibody fragment. Incorporating one or more unnatural amino acids into the polypeptide from which the TID is based or derived may comprise modifying one or more amino acid residues in the polypeptide to produce one or more amber codons in the antibody or antibody fragment.

[0212] The one or more unnatural amino acids may be incorporated into the polypeptide in response to an amber codon. The one or more unnatural amino acids may be site-specifically incorporated into the polypeptide.

[0213] Incorporating one or more unnatural amino acids into the polypeptide from which the CAR-ID and the TID are based or derived may comprise use of one or more genetically encoded unnatural amino acids with orthogonal chemical reactivity relative to the canonical twenty amino acids to site-specifically modify the antibody, antibody fragment, or targeting peptide. Incorporating one or more unnatural amino acids may comprise the use of one or more tRNA synthetases. The tRNA synthetase may be an aminoacyl tRNA synthetase. The tRNA synthetase may be a mutant tRNA synthesis. Incorporating one or more unnatural amino acids may comprise a tRNA/tRNA synthetase pair. The tRNA/tRNA synthetase pair may comprise a tRNA/aminoacyl-tRNA synthetase pair. The tRNA/tRNA synthetase pair may comprise a tRNATyr/tyrosyl-tRNA synthetase pair. Incorporating the one or more unnatural amino acids may comprise use of an evolved tRNA/aminoacyl-tRNA synthetase pair to site-specifically incorporate one or more unnatural amino acids at defined sites in the polypeptide in response to one or more amber nonsense codon.

[0214] Additional methods for incorporating unnatural amino acids include, but are not limited to, methods disclosed in Chatterjee et al. (A Versatile Platform for Single- and Multiple-Unnatural Amino Acid Mutagenesis in Escherichia coli, *Biochemistry*, 2013), Kazane et al. (*J Am Chem Soc*, 135(1):340-6, 2013), Kim et al. (*J Am Chem Soc*, 134(24):9918-21, 2012), Johnson et al. (*Nat Chem Biol*, 7(11):779-86, 2011) and Hutchins et al. (*J Mol Biol*, 406(4):595-603, 2011).

[0215] A method of producing a switch for activating a chimeric antigen receptor-effector cell (CAR-EC) may comprise (a) obtaining a target interacting domain TID comprising an unnatural amino acid; and (b) attaching a chimeric antigen receptor-interacting domain (CAR-ID) to the TID, thereby producing the switch.

[0216] Attaching the CAR-ID to the TID may comprise one or cycloadditions. The one or more cycloadditions may comprise a Huisgen cycloaddition. The one or more cycloadditions may comprise a [3+2] cycloaddition. The one or more cycloadditions may comprise a [3+2] Huisgen cycloaddition. The one or more cycloadditions may comprise a copper-free cycloaddition. Attaching the CAR-ID to the TID may comprise a copper free reaction. Attaching the CAR-ID to the TID may comprise one or more copper-containing reactions. Attaching the CAR-ID to the TID may comprise one or more Diels Alder reactions. Attaching the CAR-ID to the TID may comprise one or more hetero Diels Alder reactions. Attaching the CAR-ID to the TID may comprise one or more ester couplings. Attaching the CAR-ID to the TID may comprise one or more isothiocyanate couplings. Attaching the CAR-ID to the TID may comprise attaching the CAR-ID to an amino acid of TID. The amino acid may be an unnatural amino acid. Attaching the CAR-ID to the TID may comprise one or more bioorthogonal reactions. The CAR-ID may be attached to the TID in a site-specific manner. The CAR-ID may be attached to a predetermined site in the TID. The CAR-ID may be attached to the TID in a site-independent manner.

[0217] The method may further comprise attaching a first linker to the TID to produce first switch intermediate. Attaching the first linker to the TID may comprise one or cycloadditions. Attaching the first linker to the TID may comprise a copper free reaction. Attaching the first linker to the TID may comprise one or more copper-containing reactions. Attaching the first linker to the TID may comprise one or more Diels Alder reactions. Attaching the first linker to the TID may comprise one or more hetero Diels Alder reactions. Attaching the first linker to the TID may comprise one or more ester couplings. Attaching the first linker to the TID may comprise oxime ligation. Attaching the first linker to the TID may comprise forming one or more oximes between the first linker and the TID. Attaching the first linker to the TID may comprise forming one or more stable bonds between the first linker and the TID. Attaching the first linker to the TID may comprise forming one or more covalent bonds between the first linker and the TID. Attaching the first linker to the TID may comprise forming one or more non-covalent bonds between the first linker and the TID. Attaching the first linker to the TID may comprise forming one or more ionic bonds between

the first linker and the TID. Attaching the first linker to the TID may comprise attaching the linker to an amino acid of TID. The amino acid may be an unnatural amino acid. Attaching the first linker to the TID may comprise one or more bioorthogonal reactions.

[0218] Attaching the CAR-ID to the TID may comprise attaching the first switch intermediate to the CAR-ID. Attaching the first switch intermediate to the CAR-ID may comprise one or cycloadditions. The one or more cycloadditions may comprise a Huisgen cycloaddition. The one or more cycloadditions may comprise a [3+2] cycloaddition. The one or more cycloadditions may comprise a [3+2] Huisgen cycloaddition. The one or more cycloadditions may comprise a copper-free cycloaddition. Attaching the first switch intermediate to the CAR-ID may comprise a copper free reaction. Attaching the first switch intermediate to the CAR-ID may comprise one or more copper-containing reactions. Attaching the first switch intermediate to the CAR-ID may comprise one or more Diels Alder reactions. Attaching the first switch intermediate to the CAR-ID may comprise one or more hetero Diels Alder reactions. Attaching the first switch intermediate to the CAR-ID may comprise one or more ester couplings. Attaching the first switch intermediate to the CAR-ID may comprise one or more isothiocyanate couplings.

[0219] The method may further comprise attaching a second linker to the CAR-ID to produce a second switch intermediate. Attaching the second linker to the CAR-ID may comprise one or cycloadditions. Attaching the second linker to the CAR-ID may comprise a copper free reaction. Attaching the second linker to the CAR-ID may comprise one or more copper-containing reactions. Attaching the second linker to the CAR-ID may comprise one or more Diels Alder reactions. Attaching the second linker to the CAR-ID may comprise one or more hetero Diels Alder reactions. Attaching the second linker to the CAR-ID may comprise one or more ester couplings. Attaching the second linker to the CAR-ID may comprise oxime ligation. Attaching the second linker to the CAR-ID may comprise forming one or more oximes between the second linker and the CAR-ID. Attaching the second linker to the CAR-ID may comprise forming one or more stable bonds between the second linker and the CAR-ID. Attaching the second linker to the CAR-ID may comprise forming one or more covalent bonds between the second linker and the CAR-ID. Attaching the second linker to the CAR-ID may comprise forming one or more non-covalent bonds between the second linker and the CAR-ID. Attaching the second linker to the CAR-ID may comprise forming one or more ionic bonds between the second linker and the CAR-ID.

[0220] Attaching the CAR-ID to the TID may comprise attaching the second switch intermediate to the TID. Attaching the second switch intermediate to the TID may comprise one or cycloadditions. The one or more cycloadditions may comprise a Huisgen cycloaddition. The one or more cycloadditions may comprise a [3+2] cycloaddition. The one or more cycloadditions may comprise a [3+2] Huisgen cycloaddition. The one or more cycloadditions may comprise a copper-free cycloaddition. Attaching the second switch intermediate to the TID may comprise a copper free reaction. Attaching the second switch intermediate to the TID may comprise one or more copper-containing reactions. Attaching the second switch intermediate to the TID may comprise one or more Diels Alder reactions. Attaching the second switch intermediate to the TID may comprise one or more hetero Diels Alder reactions. Attaching the second switch intermediate to the TID may comprise one or more ester couplings. Attaching the second switch intermediate to the TID may comprise one or more isothiocyanate couplings. Attaching the second switch intermediate to the TID may comprise attaching the linker to an amino acid of CAR-ID. The amino acid may be an unnatural amino acid. Attaching the second switch intermediate to the TID may comprise one or more bioorthogonal reactions.

[0221] Attaching the CAR-ID to the TID may comprise attaching the first switch intermediate to the second switch intermediate. Attaching the first switch intermediate to the second switch intermediate may comprise one or cycloadditions. The one or more cycloadditions may comprise a Huisgen cycloaddition. The one or more cycloadditions may comprise a [3+2] cycloaddition. The one or more cycloadditions may comprise a [3+2] Huisgen cycloaddition. The one or more cycloadditions may comprise a copper-free cycloaddition. Attaching the first switch intermediate to the second switch intermediate may comprise a copper free reaction. Attaching the first switch intermediate to the second switch intermediate may comprise one or more copper-containing reactions. Attaching the first switch intermediate to the second switch intermediate may comprise one or more Diels Alder reactions. Attaching the first switch intermediate to the second switch intermediate may comprise one or more hetero Diels Alder reactions. Attaching the first switch intermediate to the second switch intermediate may comprise one or more ester couplings. Attaching the first switch intermediate to the second switch intermediate may comprise one or more isothiocyanate couplings.

Purification of CAR-EC switches and portions thereof

[0222] Disclosed herein are methods of purifying CAR-EC switches disclosed herein, comprising separating the CAR-EC switches disclosed herein from components of a CAR-EC switch production system (e.g. cellular debris, free amino acids). Purifying the CAR-EC switch may comprise use of one or more concentrator columns, electrophoresis, filtration, centrifugation, chromatography or a combination thereof. Chromatography may comprise size-exclusion chromatography. Additional chromatography methods include, but are not limited to, hydrophobic interaction chromatography, ion exchange chromatography, affinity chromatography, metal binding, immunoaffinity chromatography, and high performance liquid chromatography or high pressure liquid chromatography. Electrophoresis may comprise denaturing electrophoresis or non-denaturing electrophoresis.

[0223] The CAR-EC switches may comprise one or more peptide tags. The methods of purifying CAR-EC switches may comprise binding one or more peptide tags of the CAR-EC switches to a capturing agent. The capturing agent may be selected from an antibody, a column, a bead and a combination thereof. The one or more tags may be cleaved by one or more proteases. Examples of tags include, but are not limited to, polyhistidine, FLAG® tag, HA, c-myc, V5, chitin binding protein (CBP), maltose binding protein (MBP), and glutathione-S-transferase (GST).

[0224] The methods may further comprise lyophilization or ultracentrifugation of the CAR-IDs, TIDs and/or the CAR-EC switches.

[0225] The purity of the CAR-IDs, TIDs and/or the CAR-EC switches may be equal to or greater than 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more. The purity of CAR-IDs, TIDs and/or the CAR-EC switches may be equal to or greater than 85%. The purity of the CAR-IDs, targeting polypeptides and/or the CAR-EC switches may be equal to or greater than 90%. The purity of the CAR-IDs, TIDs and/or the CAR-EC switches may be equal to or greater than 95%. The purity of the CAR-IDs, TIDs and/or the CAR-EC switches may be equal to or greater than 97%.

[0226] The methods of producing CAR-EC switches disclosed herein may comprise producing CAR-EC switches that are structurally homogeneous. The method of producing the CAR-EC switch from a polynucleotide may result in one or more CAR-EC switches that have the same or similar form, features, binding affinities (e.g. for the CAR or the target), geometry and/or size. The homogeneity of the CAR-EC switches may be equal to or greater than 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,

97%, 98%, 99% or more. The homogeneity of the CAR-EC switches may be equal to or greater than 85%. The homogeneity CAR-EC switches may be equal to or greater than 90%. The homogeneity of the CAR-EC switches may be equal to or greater than 95%. The homogeneity of the CAR-EC switches may be equal to or greater than 97%. The homogeneity may be a structural homogeneity. The homogeneity may be a structural homogeneity prior to administering the cell to a subject. The homogeneity may be a structural homogeneity prior to modifications to the CAR-EC switch by cellular activities (methylation, acetylation, glycosylation, etc.). These high percentages of homogeneity may provide a more predictable effect of the CAR-EC switch. These high percentages of homogeneity may provide for less off-target effects of the CAR-EC switch, when combined with a CAR-EC to treat a condition in a subject.

CAR-EC switch target interacting domains (antibodies) with FITC –Amino Acid Sequence		
Name	**Light chain**	**Heavy Chain**
anti-Her2 Fab (LG212HK221)	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRXEC (SEQ ID NO:2)	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPXSCDKTHT (SEQ ID NO:1)
anti-Her2 Fab wild type	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:3)	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO:4)

CAR Switches	
Name	**Sequence**
CAR-Her2 (CD8 hinge)	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSG VPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTGGGGSGGGGSGG GGSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTR YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS AAATTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLS LVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQG QNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO:5
CAR-Her2 (IgG4m hinge)	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSG VPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTGGGGSGGGGSGG GGSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTR YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS AAAESKYGPPCPPCPDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEED GCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQ ALPPR (SEQ ID NO:6)
CAR-FITC (CD8 hinge)	QVQLVESGGNLVQPGGSLRLSCAASGFTFGSFSMSWVRQAPGGGLEWVAGLSARS SLTHYADSVKGRFTISRDNAKNSVYLQMNSLRVEDTAVYYCARRSYDSSGYWGHFYSYMDVW GQGTLVTVSGGGGSGGGGSGGGGSSVLTQPSSVSAAPGQKVTISCSGSTSNIGNNYVSWYQ QHPGKAPKLMIYDVSKRPSGVPDRFSGSKSGNSASLDISGLQSEDEADYYCAAWDDSLSEFLF GTGTKLTVLGTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGT CGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADA PAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEA YSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO:7)
CAR-FITC (IgG4m hinge)	QVQLVESGGNLVQPGGSLRLSCAASGFTFGSFSMSWVRQAPGGGLEWVAGLSARS SLTHYADSVKGRFTISRDNAKNSVYLQMNSLRVEDTAVYYCARRSYDSSGYWGHFYSYMDVW GQGTLVTVSGGGGSGGGGSGGGGSSVLTQPSSVSAAPGQKVTISCSGSTSNIGNNYVSWYQ QHPGKAPKLMIYDVSKRPSGVPDRFSGSKSGNSASLDISGLQSEDEADYYCAAWDDSLSEFLF GTGTKLTVLGESKYGPPCPPCPDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPV QTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRG RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTY

CAR Switches	
Name	**Sequence**
	DALHMQALPPR (SEQ ID NO:8)

[0227] SEQ ID NO: 2 AND SEQ ID NO: 1 may be combined to make a switch, as disclosed herein, wherein the resulting polypeptide comprises CAR-ID attached or linked to the unnatural amino acids in said sequences.

EXAMPLES

[0228] The following illustrative examples are representative of embodiments of the software applications, systems, and methods described herein and are not meant to be limiting in any way.

Example 1: ***Design of FITC CAR switches Targeting Herceptin***

[0229] The standard of care for patients with $Her2^+$ cancer, trastuzumab, is not approved in patients with low levels of Her2 expression (Her2 1+), which occurs in ~35% of breast cancer patients and represents a major unmet medical need. This example demonstrates an sCAR-T cell-switch that potently lysed Her2 positive tumors, *in vitro,* and with efficacy that varied depending on the hinge of the CAR with which it was utilized. These data suggest that switch / sCAR combination may be optimized to achieve superior sCAR induced-cytotoxicity.

[0230] To develop switches that redirect the activity of sCAR-T cells to Her2-expressing cancer cells, anti-Her2 (4D5 Fab) switches were conjugated to FITC at defined sites in the variable or constant regions of antibody antigen binding fragment (Fab). These tag positions were chosen to provide switches that formed the ternary complex between the sCAR-T cell, switch, and the target cell, thus allowing formation of an immunological synapse.

[0231] For FITC-based switches, unnatural amino acid (UAA) methodology was deployed to site-specifically conjugate FITC to the 4D5 Fab. Briefly, a mutant 4D5 Fab with the TAG nonsense codon at select residues was co-expressed in *Escherichia coli* (*E.coli*) with an orthogonal *Methanococcus jannaschii*-derived tRNA/aminoacyl-tRNA synthetase

($tRNA_{CUA}$/pAzFRS) pair that selectively incorporates p-azidophenylalanine (pAzF) into proteins in response to the TAG codon. The pAzF residue was incorporated at both the light chain residue G212 (LG212X) and the heavy chain residue K221 (HK221X) simultaneously to create a bivalent switch (LG212X/HK221X) (**FIG. 3**). To chemically conjugate the Fab, a linker-modified FITC harboring a cyclooctyne group (BCN-PEG4-FITC) was attached via a "click" chemistry reaction (**FIG. 2**), resulting in FITC-conjugates that were 11.6 angstroms apart

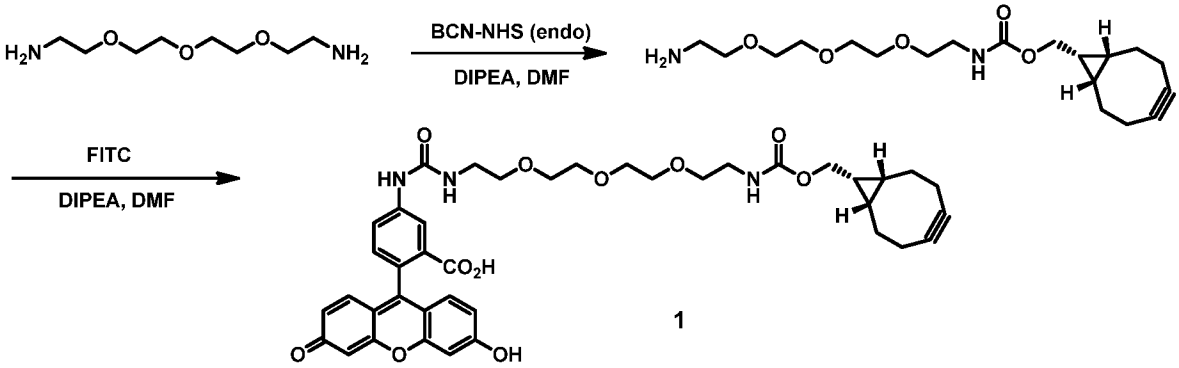
Example 2: *In vitro* analyses of LG212X/HK221X FITC switches

[0232] The LG212X/HK221X FITC switch generated in Example 1 was used to investigate functional differences in the sCD8 and IgG4m hinge designs for anti-FITC sCARs. *In vitro* cytotoxicity was measured against a multiple $Her2^+$ breast cancer cell lines. The LG212X/HK221X FITC switch afforded greater cytotoxic activity against MDA MB435 Her2 1+ cells with the anti-FITC sCAR-T construct harboring the IgG4m hinge compared with the CD8 hinge (FIG. 4A, IgG4m hinge, white bars; CD8 hinge, black bars). These results were confirmed by measuring the cytoxicity induced in MDA MB231 Her2 1+ cancer cells (**FIG. 4B**).Similarly, this FITC switch exhibited more robust sCAR-T cell activation and stimulated greater release of inflammatory cytokines with anti-FITC sCAR-T cell harboring IgG4m hinge (**FIG. 5**). These results demonstrate that LG212X/HK221X FITC switches activate anti-FITC sCAR-T cells and are capable of inducing cytotoxicity in Her2-expressing cancer cell lines. Further, these data indicate that certain switch/sCAR-T cell hinge design combinations are more effective at both activating sCAR-effector cells and inducing cytotoxicity in the target cells.

Example 3: ***Making and Using CAR-T cell switches***

Synthesis of FITC CAR Switches

[0233] Synthesis of BCN-PEG$_4$-FITC (**1**) is shown below:



[0234] A solution of BCN-NHS (endo) (130 mg, 0.446 mmol) in anhydrous DMF (3 mL) was added dropwise to a solution of 1,11-diamino-3,6,9-trioxaundecane (515 mg, 2.68 mmol) and N,N-diisopropylethylamine (0.39 mL, 2.23 mmol) in DMF (5 mL). After stirring for 15 min, the reaction mixture was concentrated in vacuo. The remaining residue was dissolved in dichloromethane (100 mL) and washed with 1N NaOH (10 mL × 2) and H_2O (10 mL) sequentially. The dichloromethane layer was dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo.

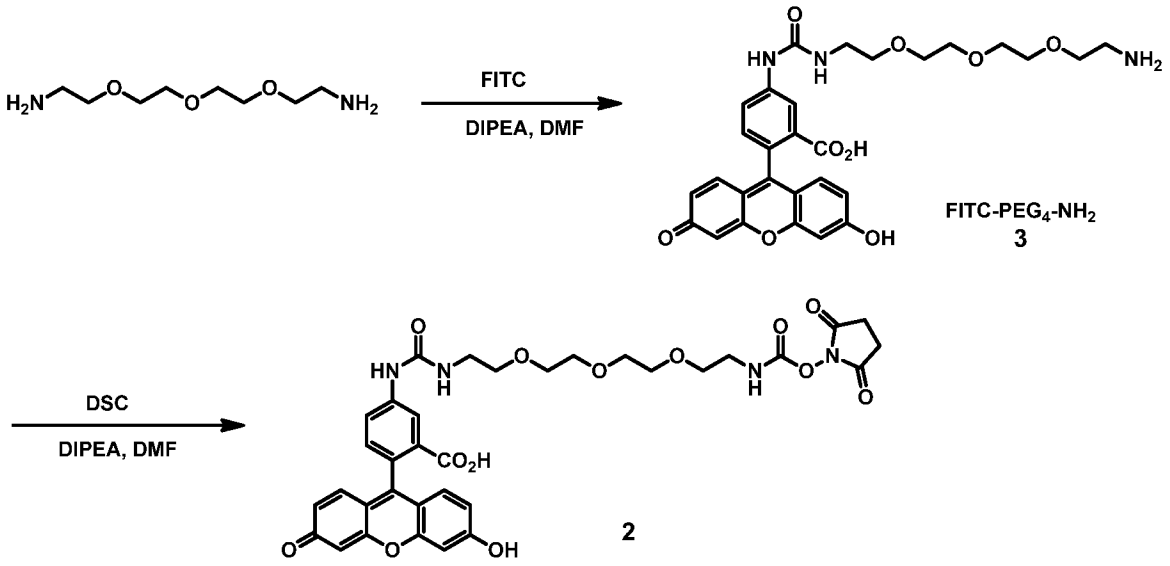
[0235] The crude residue was dissolved in DMF (5 mL), followed by the addition of N,N-diisopropylethylamine (0.23 mL, 1.32 mmol). A solution of FITC (171 mg, 0.40 mmol) in DMF (4 mL) was added dropwise to the mixture over 5 min. After 1 hour of stirring, the reaction mixture was concentrated in vacuo and purified by column chromatography to yield the desired product (215 mg, 0.284 mmol, 64% yield for 2 steps).

[0236] ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ (ppm) 10.07 (s, br, 2H), 9.99 (s, br, 1H), 8.24 (s, 1H), 8.05 (s, br, 1H), 7.70 (d, 1H, J = 8 Hz), 7.14 (d, 1H, J = 8 Hz), 7.02 (s, br, 1H), 6.64 – 6.63 (m, 2H), 6.58 – 6.51 (m ,4H), 4.29 – 4.25 (m, 1H), 3.98 (d, 2H, J = 8 Hz), 3.68 – 3.63 (m, 2H), 3.58 – 3.55 (m, 2H), 3.49 – 3.45 (m, 4H), 3.36 – 3.29 (m, 4H), 3.17 – 3.14 (m, 1H), 3.09 – 3.05 (m, 2H), 2.21 – 2.08 (m, 5H), 1.70 – 1.68 (m, 1H), 1.48 – 1.46 (m, 2H), 1.22 - 1.17 (m, 1H), 0.83 – 0.77 (m, 2H).

[0237] ^{13}C NMR (DMSO-d$_6$, 100 MHz) δ (ppm) 181.23, 169.33, 160.33, 157.26, 152.71, 129.85, 113.41, 110.56, 103.06, 99.81, 70.57, 70.37, 69.97, 67.53, 62.20, 41.27, 29.81, 29.42, 26.01, 21.01, 20.38, 18.49.

[0238] HRMS Calcd. for $C_{40}H_{44}N_3O_{11}S$ ($[M + H]^+$): 742.2970. Found: 742.2971.

[0239] Synthesis of FITC-PEG_4-NHS (**2**) is shown below:



[0240] A solution of FITC (400 mg, 0.925 mmol) in anhydrous DMF (20 mL) was added dropwise to a solution of 1,11-diamino-3,6,9-trioxaundecane (1.0 g, 5.20 mmol) and N,N-diisopropylethylamine (1.0 mL, 5.73 mmol) in DMF (15 mL). After stirring for 30 min, the reaction mixture was concentrated in vacuo, and purified by preparative HPLC to yield FITC-PEG_4-NH_2 as TFA salt (compound **3**, 574 mg, 0.826 mmol, 89%).

[0241] Compound **3** (TFA salt, 146 mg, 0.210 mmol) was dissolved in DMF (5 mL), followed by the addition of N,N-diisopropylethylamine (0.18 mL, 1.05 mmol). Next, N,N′-disuccinimidyl carbonate (81 mg, 0.315 mmol) was added to the reaction mixture. After stirring for 15 min, the reaction mixture was concentrated in vacuo, and purified by preparative HPLC to yield the desired product (**2**) (50.5 mg, 0.0699 mmol,33%).

[0242] ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 10.06 (s, br, 1H), 8.35 (s, br, 1H), 8.28 (s, 1H), 7.74 (d, 1H, J = 8 Hz), 7.18 (d, 1H, J = 8 Hz), 6.76 – 6.75 (m, 2H), 6.68 – 6.55 (m, 4H), 3.71 – 3.67 (m, 2H), 3.62 – 3.60 (m, 2H), 3.58 – 3.52 (m, 8H), 3.47 – 3.44 (m, 2H), 3.23 – 3.17 (m, 2H), 2.75 (s, 4H).

[0243] ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm) 182.32, 171.69, 169.33, 160.37, 152.71, 135.10, 129.86, 113.42, 110.55, 103.06, 70.60, 70.45, 70.40, 70.32, 70.20, 69.34, 41.84, 33.92, 26.09.

[0244] HRMS Calcd. for $C_{34}H_{35}N_4O_{13}S$ ($[M + H]^+$): 723.1967. Found: 723.1970.

Expression and generation of bifunctional FITC switch molecules

[0245] Anti-Her2 sequences were cloned into pBAD vectors and site-specific mutations to introduce TAG amber nonsense codon were generated using Quikchange Site-directed

Mutagenesis Kit (Stratagene). Antibodies were expressed in Escherichia coli (E.coli) with an orthogonal Methanococcus jannaschii tRNA/aminoacyl-tRNA synthetase specific for p-azido phenylalanine (pAzF) and purified. Purity and incorporation of UAA was confirmed by SDS-PAGE gel and mass spectrometry (QTOF).

[0246] Mutant antibodies containing pAzF ($\leq$ 1 mg/mL) were conjugated with 30-fold molar excess of BCN-PEG_4-FITC (**1**) in phosphate-buffered saline (PBS) pH 7.4 and incubated overnight at 37°C. The next day, completion of conjugation reaction was confirmed by QTOF, excess linkers were removed by size filtration (Amicon, 10K and 30K), and the size and purity of the final products were confirmed by SDS-PAGE gel.

Cell lines and culturing conditions

[0247] Breast cancer cell lines (MB MDA 231 and MB MDA 435) were purchased from ATCC and maintained in RPMI 1640 media supplemented with 10% heat-inactivated fetal bovine serum (FBS, Hyclone) and 1 mM sodium pyruvate (Life Technologies). Virus producing cell lines, HEK293T and Platinum E (Plat E, Cell Biolabs), were maintained in DMEM media with the following additions: 10% FBS, 2 mM Glutamax, MEM non-essential amino acids, and 1 mM sodium pyruvate. Transduced CAR-T cells were cultured in AIM V media (Life Technologies) with added 5% human AB serum (Valley Biomedical). All media contained 100 units/mL of penicillin and 100 μg/mL of streptomycin. Unless specified, all media and supplements were purchased from Life Technologies.

Virus production and generation of CAR-T cells

[0248] A gene cassette containing the human anti-FITC scFv, CD8α hinge and transmembrane region, and the cytoplasmic domains of 41BB and CD3ζ was synthesized by Genescript and cloned into the LV-vector. Lentivirus production and transduction of human T cells were performed. Briefly, HEK293FT cells were transfected with anti-FITC CAR plasmid and viral packaging vectors and 48 hours later, supernatants containing lentivirus were harvested or frozen at -80°C until ready for use. PBMCs were isolated from normal healthy blood using Ficoll-Paque density gradient approach (GE healthcare), and activated with CD3/CD28 activation beads (Life Technologies) at 3:1 bead to cell ratio for 24 hours. Activated T cells were mixed with supernatant containing lentivirus in the presence of protamine sulfate (1 ug/ml), centrifuged at 1000 x g for 90 min, and incubated overnight at 37°C. The next day, viral supernatant were replaced with fresh media containing recombinant human IL-2 (300 IU/mL; R&D systems). Transduced T cells were maintained at 0.125-2 x 10^6 cells/mL in media containing IL-2, which was replenished every 2-3 days.

[0249] Transduction efficiency was verified by flow cytometry using Alexa Fluor®647 conjugated anti-mouse or anti-human IgG F(ab)'2 antibodies (Jackson ImmunoResearch). Anti-FITC CAR expression was also confirmed using a FITC-labeled mouse IgG1 isotype control antibody (Biolegend). Non-transduced T cells labeled with F(ab)'2 antibodies served as background controls.

Colorimetric-based Cytotoxicity Assays

[0250] Co-cultures containing 1 x 10^4 target cells (murine CD19 overexpressing Myc5 cells, and 1 x 10^5 anti-FITC CAR-T cells with different concentrations of anti-CD19 (1D3) FITC switch were incubated at 37°C for 6 hours. Cytotoxicity was measured using the Cytotox-96 Nonradioactive cytotoxicity assay kit (Promega), which quantifies the amount of lactate dehydrogenase (LDH) released from lysed cells into the supernatant. The percent lytic activity was calculated with the following formula: (values used represent absorbance at 490nM) % Cytotoxicity = 100 x [((Target cells + Effector cells + Switch) - (Target cells + Effector cells only))/((Maximum target cell lysis) – (Target cells only))].

Cytokine Release Assays

[0251] Cytokines in cultured media from activation studies were quantified using BD CBA Human Th1/Th2 Kit II (BD Biosciences) according to manufacturer's protocol.

Statistical Analysis

[0252] All graphs and statistics were conducted using the Graphpad Prism 6.0 software.

[0253] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention.

CLAIMS

What is claimed is:

1. A chimeric antigen receptor switch comprising:

(a) a chimeric antigen receptor interacting domain (CAR-ID); and

(b) a target interacting domain (TID),

wherein the TID comprises an anti-Her2 antibody fragment Fab (clone 4D5), or a fragment thereof, which Fab comprises an unnatural amino acid located at each of Glycine 212 of a light chain of the anti-Her2 antibody Fab (LG212X; SEQ ID NO: 2) and Lysine 221 of a heavy chain of the anti-Her2 antibody Fab (HK221X; SEQ ID NO: 1), and wherein each of LG212X and HK221X is attached or linked to a separate CAR interacting domain.

2. The chimeric antigen receptor switch of claim 1, wherein the heavy chain has sequence that comprises or consists of SEQ ID NOS: 1.

3. The chimeric antigen receptor switch of claim 1, wherein the heavy chain has a sequence that is at least about 50% homologous to a sequence selected from SEQ ID NOS: 1.

4. The chimeric antigen receptor switch of claim 1, , wherein the light chain has sequence that comprises or consists of SEQ ID NOS: 2.

5. The chimeric antigen receptor switch of claim 1, wherein the light chain has a sequence that is at least about 50% homologous to a sequence selected from SEQ ID NOS: 2.

6. The chimeric antibody antigen receptor switch of any one of claims 1-5, wherein the chimeric antigen receptor interacting domain is a small molecule.

7. The chimeric antibody antigen receptor switch of any one of claims 1-5, wherein the chimeric antigen receptor interacting domain is a hapten.

8. The chimeric antibody antigen receptor switch of any one of claims 1-5, wherein the chimeric antigen receptor interacting domain is selected from FITC, biotin, and dinitrophenol.

9. The chimeric antibody antigen receptor switch of any one of claims 1-5, wherein the chimeric antigen receptor interacting domain is FITC.

10. The chimeric antigen receptor switch of claim 1, further comprising a linker, wherein the linker connects the chimeric antigen receptor interacting domain and the targeting antibody or antibody fragment.

11. A pharmaceutical composition comprising the chimeric antibody antigen receptor switch of any one of claims 1-10.

12. A method of treating a disease or condition in a subject in need thereof, comprising administering the chimeric antigen receptor switch of claims 1-10 and a chimeric antigen receptor effector cell, wherein the chimeric antigen receptor switch and/or chimeric antigen receptor effector cell is administered by a method selected from intraperitoneal injection and intravenous injection.

13. The method of claim 12, comprising administering the chimeric antigen receptor switch and/or the chimeric antigen receptor effector cell multiple times.

14. The method of claim 12 or 13, wherein the disease or condition is cancer

15. The method of claim 14, wherein the cancer is AML, ALL, CLL, multiple myeloma, breast cancer, Her2+ breast cancer, neuroblastoma, pancreatic cancer, endometrial cancer, ovarian cancer, or colon cancer.

16. The method of claim 14, wherein the cancer is selected from an epithelial cancer, a breast cancer, an endometrial cancer, an ovarian cancer, stromal ovarian cancer,and cervical cancer

17. The method of any one of claims 12-15, comprising administering a first chimeric antigen receptor switch comprising a first targeting antibody or antibody fragment and a second chimeric antigen receptor switch comprising a second targeting antibody or antibody fragment, wherein the first targeting antibody or antibody fragment binds a first antigen and the second targeting antibody or antibody fragment binds a second antigen, wherein the first antigen and the second antigen are different.

18. The method of claim 16, wherein the first and/or second antigen is Her2.

19. A method of treating cancer in a subject comprising administering a chimeric antigen receptor effector cell and the chimeric antigen receptor switch of any one of claims 1-10.

20. The method of claim 18, wherein the chimeric antigen receptor effector cell is administered to the subject prior to administration of the chimeric antigen receptor switch.

21. The method of any one of claims 18 to 19, wherein the chimeric antigen receptor switch is administered in an amount titrated in the subject to optimize the treatment of the cancer.

22. The method of claim 20, wherein the chimeric antigen receptor switch is administered at an initial dose, wherein the initial dose is the smallest dose necessary to treat the cancer.

23. The method of claim 20 or 21, wherein the chimeric antigen receptor switch is administered at a second dose after about 1, 2, 3, 4, 5, 6, 7, 8, or more weeks of treatment at the initial dose, wherein the second dose is larger than the initial dose.

24. The method of any one of claims 18-23, wherein administration of the chimeric antigen receptor switch is terminated after one or more of a) adverse effects or b) elimination of the cancer.

25. The method of claim 23 wherein adverse effects comprise one or more of tumor lysis syndrome or cytokine release syndrome.

26. The method of claim 23, wherein administration of the chimeric antigen receptor switch is continued after recurrence of the cancer.

FIG. 1

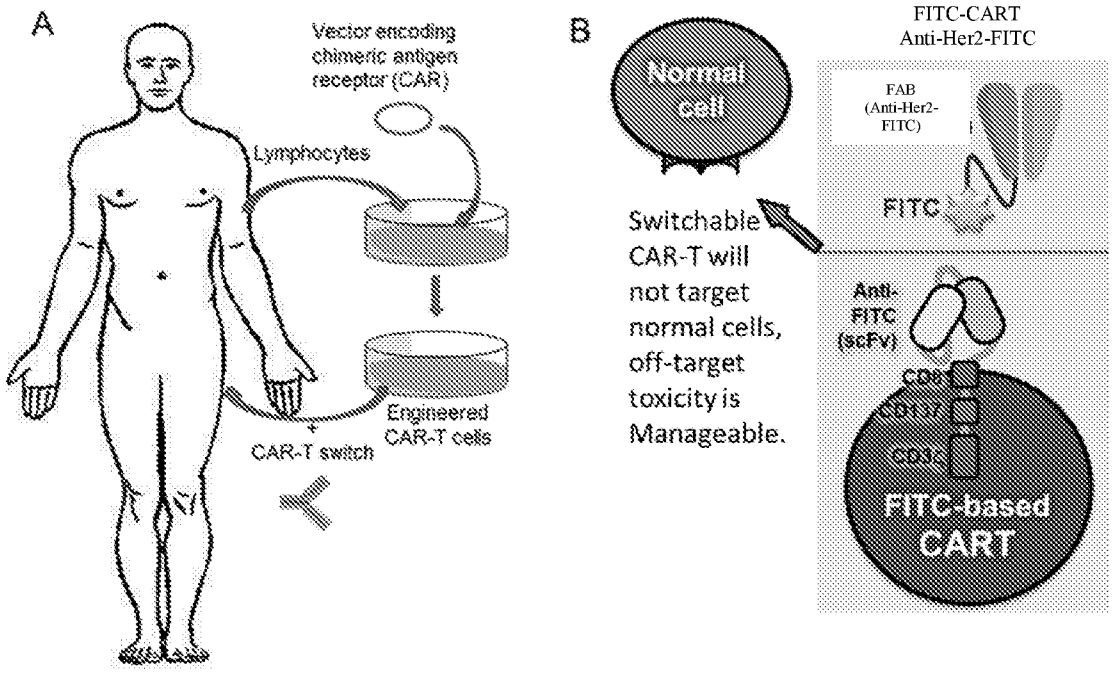

A
Vector encoding chimeric antigen receptor (CAR)
Lymphocytes
Engineered CAR-T cells
CAR-T switch
B
FITC-CART
Anti-Her2-FITC
Normal cell
Switchable CAR-T will not target normal cells, off-target toxicity is Manageable.
FAB (Anti-Her2-FITC)
FITC
Anti-FITC (scFv)
CD3ζ
FITC-based CART

FIG. 2

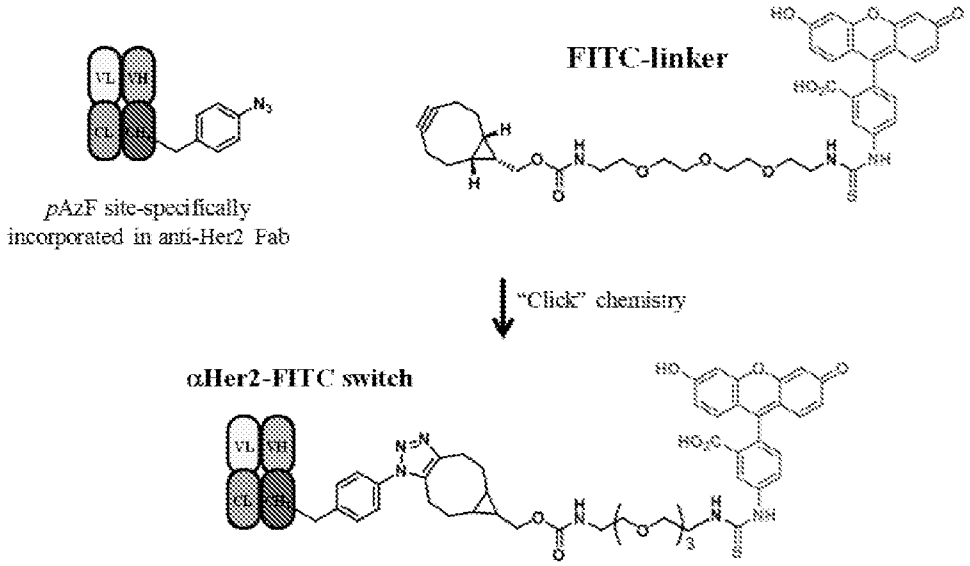

FITC-linker
pAzF site-specifically incorporated in anti-Her2 Fab
"Click" chemistry
αHer2-FITC switch

FIG. 3

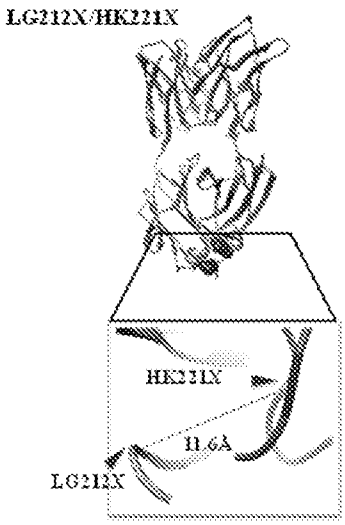

LG212X/HK221X
HK221X
H1.6A
LG212X

FIG. 4A

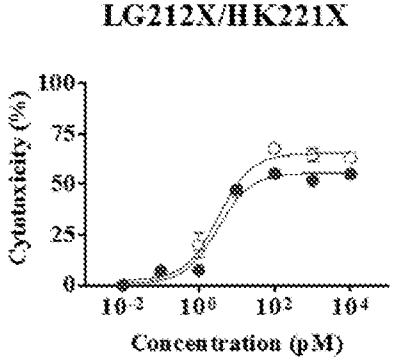
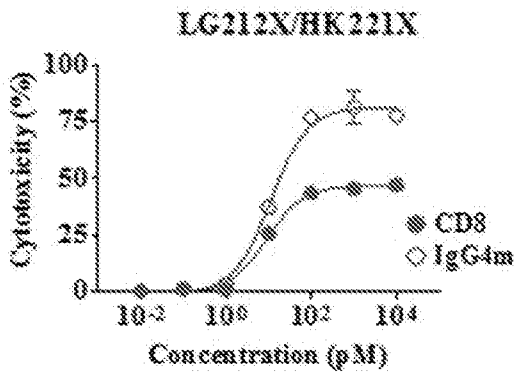
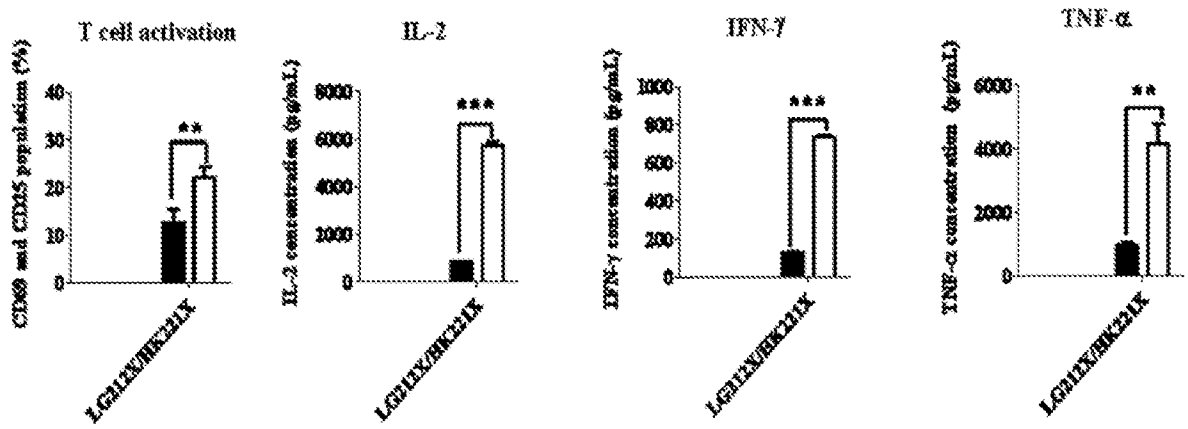


FIG. 4B



	Maximal killing (%)	EC_{50}(pM)
CD8	46.7±2.2	18.8±0.2
IgG4m	81.2±3.3	10.2±0.3

FIG. 5


T cell activation
CD69 and CD25 population (%)
40
30
20
10
0
**
LG01EX/HK21EX
IL-2
IL-2 concentration (pg/mL)
8000
6000
4000
2000
0
***
LG01EX/HK21EX
IFN-γ
IFN-γ concentration (pg/mL)
1000
800
600
400
200
0
***
LG01EX/HK21EX
TNF-α
TNF-α concentration (pg/mL)
6000
4000
2000
0
**
LG01EX/HK21EX

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/027993

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/395; A61K 47/48; C07C 275/16; C07C 275/24; C07D 249/04 (2016.01)
CPC - C07K 16/2803; C07K 16/2806; C07K 16/2851; C07K 16/32 (2016.05)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC - A61K 39/395; A61K 47/48; C07C 275/16; C07C 275/24; C07D 249/04
CPC - C07K 16/2803; C07K 16/2806; C07K 16/2851; C07K 16/32

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 424/133.1; 424/136.1; 530/387.3 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Patbase, Google Patents, PubMed, Google
Search terms used: G212X G212PAcF substitute p-acetylphenalanine her2 erbb2 Her/Neu2 antibody heavy chain light chain

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	AXUP et al. "Synthesis of site-specific antibody-drug conjugates using unnatural amino acids," Proceedings of the National Academy of Sciences, 02 October 2012 (02.10.2012), Vol. 109, No. 40, Pgs. 16101-16106. entire document	1-10
A	JACKSON et al. "In Vitro and In Vivo Evaluation of Cysteine and Site Specific Conjugated Herceptin Antibody-Drug Conjugates," PLoS One, 14 January 2014 (14.01.2014), Vol. 9, Iss. 1, Pgs. 1-14. entire document	1-10
A	US 2006/0018899 A1 (KAO et al) 26 January 2006 (26.01.2006) entire document	1-10
P, A	US 2015/0238631 A1 (THE CALIFORNIA INSTITUTE FOR BIOMEDICAL RESEARCH et al) 27 August 2015 (27.08.2015) entire document	1-10
A	WO 2014/153002 A1 (THE CALIFORNIA INSTITUTE FOR BIOMEDICAL RESEARCH et al) 25 September 2014 (25.09.2014) entire document	1-10

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
06 July 2016	29 AUG 2016
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/027993

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

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

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

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

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

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

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

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

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

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

3. Additional comments:

SEQ ID NOs: 1-8 were searched.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/027993

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

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

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

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

3. ☒ Claims Nos.: 11-26
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

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

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

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.