Title: ENGINEERED CELL SURFACE PROTEINS AND USES THEREOF

Abstract: Disclosed herein are engineered cell surface proteins and effector cells expressing said engineered cell surface proteins. Further disclosed are methods of using the effector cells for the treatment of disease or condition in a subject in need thereof.
ENGINEERED CELL SURFACE PROTEINS AND USES THEREOF

BACKGROUND OF THE INVENTION

[001] Immunotherapies, once considered "magic bullets" by Nobel laureate Paul Ehrlich, are rapidly becoming attractive alternatives to chemotherapies. Specifically, immunotherapies that use genetically modified T cells to "retrain" the immune system to recognize and eliminate malignant tumor cells are producing exciting results in early stage clinical trials. Such gene therapy circumvents many mechanisms of chemotherapy resistance and is active against relapsed/refractory disease, offering a realistic hope for a curative therapy. However, gene therapy techniques have encountered significant risks in the clinic including chronic immune dysregulation and even death. In the search for improved immunotherapies we have established a method of selectively activating and deactivating genetically modified T cells, which is both safer and more versatile than effector therapies currently being tested in the clinic.

[002] Adoptive transfer of genetically engineered chimeric antigen receptor T cells (CAR-Ts) equips the immune system with the ability to recognize and eliminate tumor cells. Current CAR constructs used in clinical trials express a multi-domain ("chimeric") receptor that consists of an extracellular single chain variable fragment (scFv; defines antigen specificity), a transmembrane domain from CD8, and intracellular domains from CD28 or CD137 (also known as 4IBB; provides costimulatory signals) and CD3 (initiates signal transduction). This therapy has achieved sustained remissions in clinical trials for chronic lymphocytic leukemia (CLL) and acute lymphoblastic leukemia (ALL) patients, and is rapidly emerging as a powerful alternative to chemotherapy. Despite these successes, this therapy suffers from serious safety concerns due to persistent activity of the CAR-Ts leading to toxic lymphopenia, and chronic hypogammaglobulinemia for hematological targets, and fatal off-target cytolysis for solid tumor targets.

SUMMARY OF THE INVENTION

[003] In one aspect of the disclosure, provided are engineered cell surface proteins comprising an extracellular domain comprising a sequence of an extracellular portion of a cell surface protein; a transmembrane domain; and an intracellular domain comprising a sequence of an intracellular signaling protein; wherein the engineered cell surface protein is engineered by combining sequences from at least two different protein sources. In some embodiments, a first protein source is the cell surface protein and a second protein source is the intracellular signaling protein. In some embodiments, the extracellular domain does not comprise an antibody or fragment thereof. In some embodiments, the intracellular signaling protein is a costimulatory domain. In some embodiments,
the costimulatory domain comprises a 4IBB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a portion or combination thereof. In some embodiments, the costimulatory domain comprises a sequence of any of SEQ ID NOs: 6-15. In some embodiments, the intracellular domain further comprises a T cell receptor zeta chain. In some embodiments, the intracellular signaling protein is a T cell receptor zeta chain. In some embodiments, the T cell receptor zeta chain does not comprise an ITAMI domain, ITAM2 domain, ITAM3 domain, or a portion or combination thereof. In some embodiments, the cell surface protein is a protein expressed on the surface of a T cell. In some embodiments, the cell surface protein is CD3 epsilon. In some embodiments, the extracellular domain comprises a sequence from any of SEQ ID NOs: 1-3. In some embodiments, the transmembrane domain comprises a sequence from a CD8 transmembrane domain, a CD28 transmembrane domain, an FcR transmembrane domain, a CD3 epsilon transmembrane domain, or a combination thereof. In some embodiments, provided herein is a nucleic acid encoding for an engineered cell surface protein as described herein. In some embodiments, provided herein is an effector cell expressing an engineered cell surface protein as described herein. In some embodiments, the effector cell is a tumor-infiltrating lymphocyte. In some embodiments, provided herein are kits comprising an effector cell as described herein and an antibody comprising an antigen binding domain that binds to the extracellular domain of the engineered cell surface protein. In some embodiments, the antibody is a bispecific antibody that further comprises an antigen binding domain that binds to an antigen on a target cell.

[004] In one aspect of the disclosure, provided are engineered cell surface proteins comprising an extracellular domain comprising a CD3 epitope; a transmembrane domain; and an intracellular domain comprising a sequence of an intracellular signaling protein; wherein the engineered cell surface protein is engineered by combining the CD3 epitope with a protein other than CD3. In some embodiments, the protein other than CD3 is the intracellular signaling protein, the transmembrane domain, or both the intracellular signaling protein and the transmembrane domain. In some embodiments, the extracellular domain does not comprise an antibody or fragment thereof. In some embodiments, the CD3 epitope is from a CD3 epsilon domain. In some embodiments, the CD3 epitope comprises a sequence from the amino terminal portion of the CD3 epsilon domain. In some embodiments, the amino terminal portion of the CD3 epsilon domain comprises the first 30 amino acids of the CD3 epsilon domain. In some embodiments, the CD3 epitope comprises a sequence from any of SEQ ID NOs: 1-3. In some embodiments, the intracellular signaling protein is a costimulatory domain. In some embodiments, the costimulatory domain comprises a 4IBB signaling
domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a portion or combination thereof. In some embodiments, the costimulatory domain comprises a sequence from any of SEQ ID NOs: 6-15. In some embodiments, the intracellular domain further comprises a T cell receptor zeta chain. In some embodiments, the intracellular signaling protein is a T cell receptor zeta chain. In some embodiments, the intracellular signaling protein is a T cell receptor zeta chain. In some embodiments, the T cell receptor zeta chain does not comprise an ITAMI domain, ITAM2 domain, ITAM3 domain, or a portion or combination thereof. In some embodiments, the transmembrane domain comprises a sequence derived from a CD8 transmembrane domain, a CD28 transmembrane domain, an FcR transmembrane domain, a CD3 epsilon transmembrane domain, or a combination thereof. In some embodiments, provided herein is a nucleic acid encoding for an engineered cell surface protein as described herein. In some embodiments, provided herein is an effector cell expressing an engineered cell surface protein as described herein. In some embodiments, the effector cell is a tumor-infiltrating lymphocyte. In some embodiments, provided herein are kits comprising an effector cell as described herein and an antibody comprising an antigen binding domain that binds to the CD3 epitope of the engineered cell surface protein. In some embodiments, the antibody is a bispecific antibody that further comprises an antigen binding domain that binds to an antigen on a target cell.

[005] In one aspect of the disclosure, provided are engineered cell surface proteins comprising an extracellular domain comprising a sequence of an extracellular portion of a cell surface protein; a transmembrane domain; and an intracellular domain comprising a sequence of a costimulatory domain; wherein the engineered cell surface protein is engineered by combining sequences from at least two different protein sources. In some embodiments, a first protein source is the cell surface protein and a second protein source is a protein comprising the costimulatory domain. In some embodiments, the extracellular domain does not comprise an antibody or fragment thereof. In some embodiments, the costimulatory domain comprises a 4IBB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a portion or combination thereof. In some embodiments, the costimulatory domain comprises a sequence from any of SEQ ID NOs: 6-15. In some embodiments, the cell surface protein is a protein expressed on the surface of a T cell. In some embodiments, the cell surface protein is CD3 epsilon. In some embodiments, the extracellular domain comprises a sequence from any of SEQ ID NOs: 1-3. In some embodiments, the intracellular domain further comprises a T cell receptor zeta
chain. In some embodiments, the T cell receptor zeta chain does not comprise an ITAMI domain, ITAM2 domain, ITAM3 domain, or a portion or combination thereof. In some embodiments, the transmembrane domain comprises a sequence derived from a CD8 transmembrane domain, a CD28 transmembrane domain, an FcR transmembrane domain, a CD3 epsilon transmembrane domain, or a combination thereof. In some embodiments, provided herein is a nucleic acid encoding for an engineered cell

[006] In one aspect of the disclosure, provided are engineered cell surface proteins comprising an extracellular domain comprising a CD3 epitope; a transmembrane domain; and an intracellular domain comprising a sequence of a costimulatory domain; wherein the engineered cell surface protein is engineered by combining the CD3 epitope with a protein other than CD3. In some embodiments, the protein other than CD3 comprises the costimulatory domain. In some embodiments, the extracellular domain does not comprise an antibody or fragment thereof. In some embodiments, the CD3 epitope is from a CD3 epsilon domain. In some embodiments, the CD3 epitope comprises a sequence from the amino terminal portion of the CD3 epsilon domain. In some embodiments, the amino terminal portion of the CD3 epsilon domain comprises the first 30 amino acids of the CD3 epsilon domain. In some embodiments, the CD3 epitope comprises a sequence from any of SEQ ID NOs: 1-3. In some embodiments, the costimulatory domain comprises a 4IBB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a portion or combination thereof. In some embodiments, the costimulatory domain comprises a sequence from any of SEQ ID NOs: 6-15. In some embodiments, the intracellular domain further comprises a T cell receptor zeta chain. In some embodiments, the T cell receptor zeta chain does not comprise an ITAMI domain, ITAM2 domain, ITAM3 domain, or a portion or combination thereof. In some embodiments, the transmembrane domain comprises a sequence derived from a CD8 transmembrane domain, a CD28 transmembrane domain, an FcR transmembrane domain, a CD3 epsilon transmembrane domain, or a combination thereof. In some embodiments, provided herein is a nucleic acid encoding for an engineered cell
surface protein as described herein. In some embodiments, provided herein is an effector cell expressing an engineered cell surface protein as described herein. In some embodiments, the effector cell is a tumor-infiltrating lymphocyte. In some embodiments, provided herein are kits comprising an effector cell as described herein and an antibody comprising an antigen binding domain that binds to the CD3 epitope of the engineered cell surface protein. In some embodiments, the antibody is a bispecific antibody that further comprises an antigen binding domain that binds to an antigen on a target cell.

[007] In one aspect of the disclosure, provided are effector cells expressing an engineered cell surface protein comprising an extracellular domain comprising a sequence of an extracellular portion of the cell surface protein; a transmembrane domain; and an intracellular domain comprising a sequence of an intracellular signaling protein; wherein the engineered cell surface protein is engineered by combining sequences from at least two different protein sources. In some embodiments, a first protein source is the cell surface protein and a second protein source is the intracellular signaling protein. In some embodiments, the extracellular domain does not comprise an antibody or fragment thereof. In some embodiments, the intracellular signaling protein is a costimulatory domain. In some embodiments, the costimulatory domain comprises a 4IBB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a portion or combination thereof. In some embodiments, the intracellular domain further comprises a T cell receptor zeta chain. In some embodiments, the intracellular signaling protein is a T cell receptor zeta chain. In some embodiments, the T cell receptor zeta chain does not comprise an ITAMI domain, ITAM2 domain, ITAM3 domain, or a portion or combination thereof. In some embodiments, the cell surface protein is a protein expressed on the surface of a T cell. In some embodiments, the cell surface protein is CD3 epsilon. In some embodiments, the effector cell is a tumor-infiltrating lymphocyte. In some embodiments, provided herein are kits comprising an effector cell as described herein and an antibody comprising an antigen binding domain that binds to the extracellular domain of the engineered cell surface protein. In some embodiments, the antibody is a bispecific antibody that further comprises an antigen binding domain that binds to an antigen on a target cell.

[008] In one aspect of the disclosure, provided are effector cells comprising an engineered component of a TCR complex comprising a modification that improves an immune response. In some embodiments, the component of the TCR complex comprises an extracellular domain of CD3. In some embodiments, the modification comprises an addition of a costimulatory domain to the
component of the TCR complex. In some embodiments, the costimulatory domain comprises a sequence derived from a 41BB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a combination thereof. In some embodiments, the modification does not comprise an addition of an antibody or antibody fragment to the component of the TCR complex. In some embodiments, the modification does not comprise an addition of a CD3 zeta chain to the component of the TCR complex. In some embodiments, the effector cell is a tumor-infiltrating lymphocyte.

[009] In one aspect of the disclosure, provided are kits comprising: (a) an effector cell expressing an engineered cell surface protein comprising an extracellular domain comprising a sequence of an extracellular portion of a cell surface protein, a transmembrane domain, and an intracellular domain comprising a sequence of an intracellular signaling protein; wherein the engineered cell surface protein is engineered by combining sequences from at least two different protein sources; and (b) a bispecific antibody comprising an antigen binding domain that binds to the extracellular domain of the engineered cell surface protein. In some embodiments, a first protein source is the cell surface protein and a second protein source is the intracellular signaling protein. In some embodiments, the extracellular domain does not comprise an antibody or fragment thereof. In some embodiments, the intracellular signaling protein is a costimulatory domain. In some embodiments, the costimulatory domain comprises a 41BB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a portion or combination thereof. In some embodiments, the intracellular domain further comprises a T cell receptor zeta chain. In some embodiments, the intracellular signaling protein is a T cell receptor zeta chain. In some embodiments, the T cell receptor zeta chain does not comprise an ITAMI domain, ITAM2 domain, ITAM3 domain, or a portion or combination thereof. In some embodiments, the cell surface protein is CD3 epsilon. In some embodiments, the effector cell is a T cell. In some embodiments, the effector cell is a tumor-infiltrating lymphocyte. In some embodiments, the bispecific antibody comprises an antigen binding domain that binds to an antigen on a target cell. In some embodiments, the target cell is a tumor cell.

[010] In one aspect of the disclosure, provided are methods for treating a condition comprising administering to a subject in need thereof: (a) an effector cell that expresses an engineered cell surface protein, the engineered cell surface protein comprising: an extracellular domain comprising a sequence of an extracellular portion of a cell surface protein, a transmembrane domain, and an
intracellular domain comprising a sequence of an intracellular signaling protein; wherein the engineered cell surface protein is engineered by combining sequences from at least two different protein sources; and (b) a bispecific antibody comprising: a first antigen binding region that binds to the extracellular domain of the engineered cell surface protein, and a second antigen binding region that binds to an antigen on a target cell. In some embodiments, a first protein source is the cell surface protein and a second protein source is the intracellular signaling protein. In some embodiments, the extracellular domain does not comprise an antibody or fragment thereof. In some embodiments, the intracellular signaling protein is a costimulatory domain. In some embodiments, the costimulatory domain comprises a 41BB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a portion or combination thereof. In some embodiments, the intracellular domain further comprises a T cell receptor zeta chain. In some embodiments, the intracellular signaling protein is a T cell receptor zeta chain. In some embodiments, the cell surface protein is CD3 epsilon. In some embodiments, the effector cell is selected from a T cell, an effector B cell, a natural killer cell, a macrophage and a progenitor thereof. In some embodiments, the effector cell is a T cell 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. In some embodiments, the effector cell is a tumor-infiltrating lymphocyte. In some embodiments, the target cell is a tumor cell. In some embodiments, the condition is a cancer. In some embodiments, the effector cell and bispecific antibody are administered simultaneously. In some embodiments, the effector cell and bispecific antibody are administered sequentially.

[011] In one aspect of the disclosure, provided are chimeric antigen receptors comprising a chimeric antigen receptor comprising an extracellular domain that interacts with an anti-CD3 antibody or fragment thereof; a transmembrane domain; and an intracellular domain, wherein at least a portion of the transmembrane domain or at least a portion of the intracellular domain is not based on or derived from a CD3 protein. The effector cell may be a T cell. The effector cell may comprise a CD3 extracellular domain or a portion thereof. The extracellular domain may comprise a CD3 epsilon domain or a portion thereof. The extracellular domain may be based on or derived from a human CD3. The extracellular domain may comprise a sequence selected from SEQ ID NOs: 1-3. The extracellular domain may comprise a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% homologous or identical to a sequence selected from SEQ ID NOs: 1-3. The
extracellular domain may comprise at least about 5 consecutive amino acids of SEQ ID NO. 1. The extracellular domain may comprise at least about 20, 25, 27, 30, 35 or 50 consecutive amino acids of SEQ ID NO. 1. The transmembrane domain may comprise a sequence from SEQ ID NO. 4. The transmembrane domain may comprise a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to SEQ ID NO. 4. The intracellular domain may comprise a sequence selected from any of SEQ ID NOs: 5-15. The intracellular domain may comprise a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence of SEQ ID NOs: 5-15. The intracellular domain may not comprise a CD3 zeta chain signaling domain selected from ITAM1, ITAM2 and ITAM. The chimeric antigen receptor may complex with a native or endogenous T cell receptor complex or portion thereof. The anti-CD3 antibody or fragment thereof may comprise a monoclonal anti-human CD3 epsilon antibody or fragment thereof. The transmembrane domain may comprise a domain selected from a CD8 transmembrane domain, a CD28 transmembrane domain, an FcR transmembrane domain, a CD3 epsilon transmembrane domain and portions thereof. The chimeric antigen receptor may comprise a spacer. The spacer may be located between the extracellular domain and a transmembrane domain. The intracellular domain may comprise one or more signaling domain selected from a 4IBB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain and portions thereof. The chimeric antigen receptor may comprise an intracellular domain selected from CD3 zeta or a portion thereof. The chimeric antigen receptor may comprise a human CD3 epsilon extracellular domain or portion thereof, a transmembrane domain or portion thereof, a CD3 zeta intracellular domain or portion thereof and one or more signaling domains or portions thereof selected from signaling domains of 41BB, CD28, ICOS, OX40, CD27, CD30, CD150, DAP-10 and NKG2D. The CD3 zeta intracellular domain maybe a carboxy-terminal domain. The chimeric antigen receptor may comprise a human CD3 epsilon extracellular domain or portion thereof, a transmembrane domain or portion thereof and one or more signaling domains or portions thereof selected from signaling domains of 41BB, CD28, ICOS, OX40, CD27, CD30, CD150, DAP-10 and NKG2D. The transmembrane domain may be selected from a CD8 transmembrane domain, a CD28 transmembrane domain, an FcR transmembrane domain, a CD3 epsilon transmembrane domain and portions thereof. The extracellular domain may comprise the amino terminal 27 amino acids of human CD3 epsilon. The spacer may be located between the extracellular domain and the transmembrane domain.
In one aspect of the disclosure, provided are chimeric antigen receptors comprising: an extracellular domain that interacts with an anti-CD3 antibody or fragment thereof; a transmembrane domain; and an intracellular domain, wherein at least a portion of the transmembrane domain or at least a portion of the intracellular domain is not based on or derived from a CD3 protein. The extracellular domain may comprise a CD3 extracellular domain or portion thereof. The extracellular domain may comprise a CD3 epsilon extracellular domain or portion thereof. The extracellular domain may comprise a sequence selected from any of SEQ ID NOs: 1-3. The extracellular domain may comprise a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% homologous or identical to a sequence selected from any of SEQ ID NOs: 1-3. The extracellular domain may comprise at least 5 consecutive amino acids of SEQ ID NO. 1. The extracellular domain may comprise at least about 20, 25, 27, 30, 40 or 50 consecutive amino acids of SEQ ID NO. 1. The extracellular domain may be based on or derived from a human CD3. The transmembrane domain may comprise a sequence from SEQ ID NO. 4. The transmembrane domain may comprise a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to SEQ ID NO. 4. The intracellular domain may comprise a sequence from any of SEQ ID NOs: 5-15. The intracellular domain may comprise a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to any of SEQ ID NOs: 5-15. The intracellular domain may not comprise a CD3 zeta chain signaling domain selected from ITAM1, ITAM2 and ITAM. The anti-CD3 antibody or fragment thereof may comprise a monoclonal anti-human CD3 epsilon antibody or fragment thereof. The transmembrane domain may comprise a domain selected from a CD8 transmembrane domain, a CD28 transmembrane domain, an FcR transmembrane domain, a CD3 epsilon transmembrane domain and portions thereof. The chimeric antigen receptor may comprise a spacer. The spacer may be located between the extracellular domain and a transmembrane domain. The intracellular domain may comprise one or more signaling domains selected from a 4IBB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain and portions thereof. The chimeric antigen receptor may comprise an intracellular domain selected from CD3 zeta or a portion thereof. The chimeric antigen receptor may comprise a human CD3 epsilon extracellular domain or portion thereof, a transmembrane domain or portion thereof, a CD3 zeta intracellular domain or portion thereof and one or more signaling domains or portions thereof selected from signaling domains of 41BB, CD28, ICOS, OX40, CD27, CD30, CD150, DAP-10 and NKG2D. The CD3 zeta intracellular domain may be a carboxy-terminal domain. The chimeric antigen receptor may comprise a human CD3 epsilon
extracellular domain or portion thereof, a transmembrane domain or portion thereof and one or more signaling domains or portions thereof selected from signaling domains of 4IBB, CD28, ICOS, OX40, CD27, CD30, CD150, DAP-10 and NKG2D. The transmembrane domain may be selected from a CD8 transmembrane domain, a CD28 transmembrane domain, an FcR transmembrane domain, a CD3 epsilon transmembrane domain and portions thereof. The extracellular domain may comprise the amino terminal 27 amino acids of human CD3 epsilon. The spacer may be located between the extracellular domain and the transmembrane domain.

[013] In one aspect of the disclosure, provided are methods of treating a condition comprising administering to a subject in need thereof chimeric antigen receptor effector cells comprising a chimeric antigen receptor, wherein the chimeric antigen receptor comprises an extracellular domain that interacts with an anti-CD3 antibody or fragment thereof; a transmembrane domain; and an intracellular domain, wherein at least a portion of the transmembrane domain or at least a portion of the intracellular domain is not based on or derived from a CD3 protein; and chimeric antigen receptor-effector cell switches comprising an anti-CD3 antibody and a targeting region. The condition may be an infection. The condition may be a cancer. The cancer may be selected from a relapsed cancer, a refractory cancer, a glioblastoma, an ovarian cancer, a prostate cancer, a hormone refractory prostate cancer and a breast cancer. The cancer may comprise a tumor selected from a liquid tumor or a solid tumor. The method may comprise administering a first chimeric antigen receptor-effector cell switch and a second chimeric antigen receptor-effector cell switch, wherein a first chimeric antigen receptor-effector cell switch targeting region binds a first cell surface marker on a first target and the second chimeric antigen receptor-effector cell switch targeting region binds a second cell surface marker on a second target. The first chimeric antigen receptor-effector cell switch and the second chimeric antigen receptor-effector cell switch may be administered simultaneously. The first chimeric antigen receptor-effector cell switch and the second chimeric antigen receptor-effector cell switch may be administered sequentially. The cancer may comprise a heterogeneous tumor. The chimeric antigen receptor effector cell may be selected from a T cell, an effector B cell, a natural killer cell, a macrophage and a progenitor thereof. The effector cell may be a T cell. The T cell may be 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. The extracellular domain may be selected from a CD3 extracellular domain, a homolog thereof and a portion thereof. The extracellular domain may be selected from a CD3 epsilon domain. The
intracellular domain may comprise one or more signaling domains selected from signaling domains of 41BB, CD28, ICOS, OX40, CD27, CD30, CD150, DAP-10 and NKG2D.

[014] In one aspect of the disclosure, provided are methods of producing chimeric antigen receptors comprising expressing a protein from a polynucleotide, wherein the protein comprises an extracellular domain that interacts with an anti-CD3 antibody or fragment thereof; a transmembrane domain; and an intracellular domain, wherein at least a portion of the transmembrane domain or at least a portion of the intracellular domain is not based on or derived from a CD3 protein. The extracellular domain may be selected from a CD3 extracellular domain, a homolog thereof and a portion thereof. The extracellular domain may be selected from a CD3 epsilon domain. The extracellular domain may comprise a sequence selected from SEQ ID NOs: 1-3. The extracellular domain may comprise a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% homologous or identical to a sequence selected from SEQ ID NOs: 1-3. The extracellular domain may comprise at least 5 consecutive amino acids of SEQ ID NO. 1. The extracellular domain may comprise at least about 20, 25, 27, 30, 35, 40 or 50 consecutive amino acids of SEQ ID NO. 1. The transmembrane domain may comprise a sequence selected from SEQ ID NO. 4. The transmembrane domain may comprise a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to SEQ ID NO. 4. The intracellular domain may comprise a sequence selected from any of SEQ ID NOs: 5-15. The intracellular domain may comprise a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to any of SEQ ID NOs: 5-15. The extracellular domain may be based on or derived from a human CD3. The chimeric antigen receptor may not comprise a CD3 zeta chain signaling domain selected from ITAM1, ITAM2 and ITAM. The intracellular domain may comprise one or more signaling domains selected from i signaling domains of 41BB, CD28, ICOS, OX40, CD27, CD30, CD150, DAP-10 and NKG2D.

[015] In one aspect of the disclosure, provided are vectors encoding chimeric antigen receptors, wherein the chimeric antigen receptor comprises an extracellular domain that interacts with an anti-CD3 antibody or fragment thereof; a transmembrane domain; and an intracellular domain, wherein at least a portion of the transmembrane domain or at least a portion of the intracellular domain is not based on or derived from a CD3 protein.

[016] In one aspect of the disclosure, provided are chimeric antigen receptor effector cell platforms comprising: a chimeric antigen receptor effector cell comprising a chimeric antigen receptor, wherein the chimeric antigen receptor comprises an extracellular domain that interacts with an anti-CD3 antibody or fragment thereof; a transmembrane domain; and an intracellular domain, wherein at
least a portion of the transmembrane domain or at least a portion of the intracellular domain is not based on or derived from a CD3 protein; and a chimeric antigen receptor effector cell switch comprising an anti-CD3 antibody and a targeting region. The extracellular domain may be selected from a CD3 extracellular domain, a homolog thereof and a portion thereof. The extracellular domain may be selected from a CD3 epsilon domain. The extracellular domain may comprise a sequence selected from SEQ ID NOs: 1-3. The extracellular domain may comprise a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% homologous or identical to a sequence selected from SEQ ID NOs: 1-3. The extracellular domain may comprise at least 5 consecutive amino acids of SEQ ID NO. 1. The extracellular domain may comprise at least about 20, 25, 27, 30, 40 or 50 consecutive amino acids of SEQ ID NO. 1. The transmembrane domain may comprise a sequence selected from SEQ ID NO. 4. The transmembrane domain may comprise a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to SEQ ID NO. 4. The intracellular domain may comprise a sequence selected from any of SEQ ID NOs: 5-15. The intracellular domain may comprise a sequence that is at least about 50%>, 60%>, 70%>, 80%>, 90%>, 95%, 98%, or 99% identical to any of SEQ ID NOs: 5-15. The intracellular domain may not comprise a CD3 zeta chain signaling domain selected from ITAM1, ITAM2 and ITAM. The intracellular domain may comprise one or more signaling domains selected from signaling domains of 41BB, CD28, ICOS, OX40, CD27, CD30, CD150, DAP-10 and NKG2D. The effector cell may be a T cell.

**BRIEF DESCRIPTION OF THE DRAWINGS**

[017] Figure 1A shows exemplary chimeric antigen receptors with different combinations of signaling domains and an extracellular CD3 epsilon domain. Costimulatory domains 1 & 2 may be signaling domains of 41BB, CD28, ICOS, OX40, CD27, CD30, CD150, DAP-10, and NKG2D. Spacer and transmembrane (TM) may be derived from human CD8, human CD28, human FcRs, and/or human CD3 epsilon.

[018] Figure 1B shows exemplary chimeric antigen receptors comprising a CD3 epsilon extracellular domain, a CD3 epsilon transmembrane domain, and intracellular stimulatory domains. The intracellular domains shown comprise 41BB and CD3 zeta (CD3ε-41BBz), 41BB (CD3ε-41BB), CD3 ε CY (CD3e-c), 41BB and CD3 ε CY (CD3e-41BBε), CD28 and CD3 ε CY (CD3ε-CD28ε), and CD28, 41BB and CD3 ε CY (CD3e-CD28 41BBε). A GCN4 peptide (NYHLENEVARLKK) is located at the amino terminal of the receptors to distinguish the receptors from endogenous CD3 epsilon protein.
Figure 2 exemplifies activation of native T cells by anti-CD3/anti-tumor associated antigen bispecific antibodies.

Figure 3 exemplifies activation of a chimeric CD3 epsilon receptor (ε*)-engineered T cell comprising an additional CD3 zeta domain by an anti-CD3 bispecific antibody.

Figure 4 exemplifies activation of a chimeric CD3 epsilon receptor (ε*)-engineered T cell comprising two costimulatory domains (no additional CD3 zeta domain) by an anti-CD3 bispecific antibody.

Figure 5 exemplifies activation of a chimeric CD3 epsilon receptor (ε*)-engineered T cell through its endogenous T cell receptor that recognize a MHC-peptide complex presented on tumor cells.

Figure 6 shows binding of anti-PSMA (DUPA)/anti-GCN4 bispecific antibody to PSMA+ (C4-2) or PSMA- (DU145) cells as measured by flow cytometry.

Figure 7 is a flow cytometry histogram showing the expression of CD3e-41BB and CD3e-41BBz eCARs in T cells.

Figure 8A is a dose-response curve showing the percentage of PSMA+ cell death after treatment with T cells expressing CD3e-41BB or CD3e-41BBz and varying concentrations of bispecific anti-PSMA (DUPA)/anti-GCN4 antibody.

Figure 8B is a dose-response curve showing the percentage of PSMA- cell death after treatment with T cells expressing CD3e-41BB or CD3e-41BBz and varying concentrations of bispecific anti-PSMA (DUPA)/anti-GCN4 antibody.

Figure 9 provides flow cytometry plots showing the efficiency of transducing chimeric CD3 epsilon receptors into T cells.

Figure 10 is a dose-response curve showing the percentage of specific C4-2 cell death after treatment with T cells expressing CD3e-e, CD3e-41BBe, or CD3e-CD283 and varying concentrations of bispecific antibody specific for the T cells and the C4-2 cells.

Figure 11 shows the concentrations of cytokines released into cultured supernatants during a cytotoxic activity assay involving the incubation of C4-2 cells with CAR T cells and varying concentrations of bispecific antibody specific for the CAR T cells and the C4-2 cells.

Figure 12 shows the concentrations of cytokines induced in T cells as a function of bispecific antibody concentration.
Figure 13 is a graph of showing T cell proliferation in a population of CAR T cell positive cellular populations.

Figure 14 provides graphs showing the preferential expansion of CD8-positive cytotoxic T cells over CD4-positive T cells in CD3 e-41BBe and CD3e-CDSe CAR transduced T cells in comparison with control T cells (CD3e-e).

DETAILED DESCRIPTION OF THE INVENTION

Current CAR-T therapies suffer from cumbersome methods and unacceptable safety risks. These major limitations are often the result of insufficient methods to control CAR-T activity.

Disclosed herein, in various embodiments, are chimeric antigen receptors comprising an extracellular domain recognized by an antibody that targets a cell surface protein of a T cell, a transmembrane domain, and an intracellular domain. In some embodiments, the antibody that targets a cell surface protein of a T cell is a bispecific antibody that also targets an antigen of a tumor cell. In some cases, the antibody recognizes a cluster of differentiation protein 3 (CD3), or a fragment thereof, of the extracellular domain. In some embodiments, the intracellular domain comprises one or more costimulatory domains. A costimulatory domain includes, without limitation, 41BB, CD28, ICOS, OX40, CD27, CD30, CD150, DAP-10, NKG2D, or a combination or fragment thereof. In some embodiments, the intracellular domain is derived from a different protein than the extracellular domain. In some embodiments, the intracellular domain does not comprise a signaling domain of a CD3 zeta chain (e.g., ITAM1, ITAM2, and/or ITAM3). In some embodiments, the chimeric antigen receptors are expressed by effector cells. In additional aspects, provided herein are chimeric antigen receptors and switches, wherein a switch is an entity that binds to the chimeric antigen receptor. For example, the switch is a bispecific antibody. In some cases, the switch also binds to an antigen on a tumor cell.

Disclosed herein, in various embodiments, are chimeric antigen receptors comprising an extracellular domain comprising a cell surface protein of a T cell, or a portion thereof; a transmembrane domain; and an intracellular domain. In some cases, the cell surface protein comprises a sequence of an endogenous T cell surface protein. In some cases, the cell surface protein comprises a recombinant or engineered sequence that is not endogenous to a T cell surface protein. In some cases, the cell surface protein comprises a sequence derived from a CD3 or a fragment thereof. In some embodiments, the intracellular domain is derived from a different protein than the extracellular domain. In some embodiments, the intracellular domain does not comprise a signaling domain of a CD3 zeta chain (e.g., ITAM1, ITAM2, and/or ITAM3). In some embodiments, the intracellular domain comprises one or more costimulatory domains. A costimulatory domain
includes, without limitation, 41BB, CD28, ICOS, OX40, CD27, CD30, CD150, DAP-10, NKG2D, or a combination or fragment thereof. In some embodiments, the chimeric antigen receptors are expressed by effector cells. In additional aspects, provided herein are chimeric antigen receptors and switches, wherein a switch is an entity that binds to the chimeric antigen receptor. For example, the switch binds to the extracellular domain. In some cases, the switch also binds to an antigen on a tumor cell.

[036] A schematic showing non-limiting examples of chimeric antigen receptors comprising a CD3 epsilon extracellular domain is provided in Figure 1. The extracellular domain may comprise a CD3 epsilon domain or a portion or fragment thereof. A portion or fragment thereof includes any consecutive sequence of a protein or peptide, including, as a non-limiting example, at least about 5, 10, 15, 20, 25 or more amino acids. In some cases, the consecutive sequence is derived from the protein or peptide and has fewer than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid variations (e.g., insertions, deletions, substitutions). In some embodiments, a chimeric antigen receptor comprises an extracellular domain of human CD3 epsilon chain (hCD3ε). In some embodiments, a chimeric antigen receptor comprises an amino terminal portion of a human CD3 epsilon chain. For example, the first 27 amino acids (hCD3ε (N-127)). In some embodiments, a chimeric antigen receptor comprises an amino acid sequence at an amino or carboxyl terminus of an extracellular domain. For example, a chimeric antigen receptor comprises an extracellular domain and a spacer. As another example, a chimeric antigen receptor comprises a tag at the amino terminus of an extracellular domain (e.g., GCN4 tag). In some embodiments, a spacer and/or tag comprises from about 1 to about 50 amino acids. In some embodiments, a spacer is derived from human CD8, human CD28, human FcRs, human CD3ε, or a portion or combination thereof. In some embodiments, a chimeric antigen receptor comprises a transmembrane (TM) domain. In some embodiments, a transmembrane domain is derived from human CD8, human CD28, human FcRs, human CD3ε, or a portion or combination thereof. In some embodiments, a chimeric antigen receptor comprises an intracellular domain. In some cases, an intracellular domain comprises a costimulatory domain. A costimulatory domain includes, without limitation, 41BB, CD28, ICOS, OX40, CD27, CD30, CD150, DAP-10, NKG2D, or a portion or combination thereof. In some cases, an intracellular domain comprises a CD3 zeta domain (CD3 zeta or CDE z CY). In some cases, the CD3 zeta does not comprise an ITAM1, ITAM2, ITAM3, or a portion or combination thereof.

[037] Further disclosed herein, in various embodiments, are chimeric antigen receptors expressing effector cells (CAR-ECs) comprising an extracellular domain recognized by an antibody that targets a cell surface protein of a T cell. In some cases, the antibody is an anti-CD3 antibody that interacts
with a CD3, or fragment thereof, of the extracellular domain. The CD3, or fragment thereof, may comprise an extracellular domain of the CD3 ("chimeric CD3 receptor"). The CD3 fragment may comprise an epsilon domain of the CD3 ("chimeric CD3 epsilon receptor" or "e-CAR"). The anti-CD3 antibody may be a bispecific antibody. The bispecific antibody may comprise a targeting region. The targeting region may comprise a small molecule. The targeting region may comprise a peptide. The targeting region may comprise an antibody. The targeting region may comprise a monoclonal T cell receptor. The targeting region may recognize a tumor associated antigen. In some embodiments, the chimeric antigen receptors expressed by the CAR-ECs further comprise a transmembrane domain and an intracellular domain. In some embodiments, the intracellular domain comprises one or more costimulatory domains. A costimulatory domain includes, without limitation, 41BB, CD28, ICOS, OX40, CD27, CD30, CD150, DAP-10, NKG2D, or a combination or fragment thereof. In some embodiments, the intracellular domain is derived from a different protein than the extracellular domain. In some embodiments, the intracellular domain does not comprise a signaling domain of a CD3 zeta chain (e.g., ITAM1, ITAM2, and/or ITAM3). In additional aspects, provided herein are CAR-ECs and switches, wherein a switch is an entity that binds to the chimeric antigen receptor. For example, the switch is a bispecific antibody. In some cases, the switch also binds to an antigen on a tumor cell.

[038] Further disclosed herein, in various embodiments, are CAR-ECs comprising an extracellular domain comprising a cell surface protein of a T cell, or a fragment thereof. In some cases, the cell surface protein comprises a sequence of an endogenous T cell surface protein. In some cases, the cell surface protein comprises a recombinant or engineered sequence that is not endogenous to a T cell surface protein. In some cases, the cell surface protein comprises a sequence derived from a CD3 or a fragment thereof. In some embodiments, the chimeric antigen receptors expressed by the CAR-ECs further comprise a transmembrane domain and an intracellular domain. In some embodiments, the intracellular domain comprises one or more costimulatory domains. A costimulatory domain includes, without limitation, 41BB, CD28, ICOS, OX40, CD27, CD30, CD150, DAP-10, NKG2D, or a combination or fragment thereof. In some embodiments, the intracellular domain is derived from a different protein than the extracellular domain. In some embodiments, the intracellular domain does not comprise a signaling domain of a CD3 zeta chain (e.g., ITAMI, ITAM2, and/or ITAM3). In additional aspects, provided herein are CAR-ECs and switches, wherein a switch is an entity that binds to the chimeric antigen receptor. In some cases, the switch also binds to an antigen on a tumor cell.
Further disclosed herein, in various embodiments, are CAR-ECs comprising an extracellular domain recognized by an antibody that targets a cell surface protein of a T cell, or a portion thereof; wherein the effector cells are T cells comprising a targeting region that recognizes an antigen presented by a major histocompatibility complex (MHC). In some cases, the CAR expressed by the CAR-ECs further comprises a transmembrane domain and an intracellular domain. In some cases, the targeting region of the T cell is part of a T cell receptor or T cell receptor complex. In some cases, the T cell receptor is an endogenous T cell receptor. In some cases, the T cell receptor is an engineered T cell receptor. In some cases, the antibody that targets a cell surface protein of a T cell comprises a CD3 antigen binding region. In some cases, the antibody that targets a cell surface protein of a T cell is a bispecific antibody that also targets an antigen of a tumor cell. In some embodiments, the intracellular domain is derived from a different protein than the extracellular domain. In some embodiments, the intracellular domain does not comprise a signaling domain of a CD3 zeta chain (e.g., ITAM1, ITAM2, and/or ITAM3). In some embodiments, the intracellular domain comprises one or more costimulatory domains. A costimulatory domain includes, without limitation, 41BB, CD28, ICOS, OX40, CD27, CD30, CD150, DAP-10, NKG2D, or a combination or fragment thereof. In some embodiments, a CAR-EC comprises an extracellular domain comprising a CD3 or fragment thereof, a transmembrane domain, and one or more intracellular domains. In additional aspects, provided herein are CAR-ECs and switches, wherein a switch is an entity that binds to the chimeric antigen receptor. For example, the switch is an antibody binds to the extracellular domain. In some cases, the switch also binds to an antigen on a tumor cell.

Further disclosed herein are CAR-ECs comprising an extracellular domain comprising a cell surface protein of a T cell, or a fragment thereof; wherein the effector cells are T cells comprising a targeting region that recognizes an antigen presented by a MHC. In some cases, the CAR expressed by the CAR-ECs further comprises a transmembrane domain and an intracellular domain. In some cases, the targeting region is part of a T cell receptor or T cell receptor complex. In some cases, the T cell receptor is an endogenous T cell receptor. In some cases, the T cell receptor is an engineered T cell receptor. In some embodiments, the intracellular domain comprises one or more costimulatory domains. A costimulatory domain includes, without limitation, 41BB, CD28, ICOS, OX40, CD27, CD30, CD150, DAP-10, NKG2D, or a combination or fragment thereof. In some embodiments, the intracellular domain is derived from a different protein than the extracellular domain. In some embodiments, the intracellular domain does not comprise a signaling domain of a CD3 zeta chain (e.g., ITAM1, ITAM2, and/or ITAM3). In additional aspects, provided herein are CAR-ECs and switches, wherein a switch is an entity that binds to the chimeric antigen receptor. For example, the
switch binds to the extracellular domain. In some cases, the switch also binds to an antigen on a tumor cell.

[041] The CARs disclosed herein offer several distinct advantages over current CARs. For example, CAR-EC switches used in this approach may recruit endogenous T cells in addition to the chimeric CD3 receptor effector cells disclosed herein to yield synergistic anti-tumor activity. Additional endogenous T-cell receptor signaling domains may enable a more robust response compared to previous CAR-T therapy approaches which only rely on CAR signaling. Furthermore, a large number of existing bispecific antibodies that are being clinically tested or have already been clinically tested may be directly tested as potential switches with the CAR-ECs disclosed herein. Additionally, the CARs disclosed herein may be engineered without a CD3 zeta intracellular domain (which takes up about 50% length in 2nd generation CAR gene), thus allowing for the incorporation of other components (i.e. multiple costimulatory domains).

[042] Further disclosed herein are chimeric antigen receptors expressing effector cells (CAR-ECs) useful for the treatment of a variety of diseases and conditions. Generally, the effector cell is a T cell. The CAR-EC may be a T cell that expresses a CAR comprising an extracellular domain of a CD3 ("chimeric CD3 receptor- engineered T cell"). The CAR-EC may be a T cell that expresses a CAR comprising an extracellular domain of a CD3. The extracellular domain of the CD3 may be an epsilon domain. Such CARs may be referred to herein as "chimeric CD3 epsilon receptor or "eCAR". Effector cells expressing eCAR may be referred to herein as "chimeric CD3 epsilon receptor- expressing effector cells" (eCAR-ECs) or "chimeric CD3 epsilon receptor- expressing T cells" (eCART) cells.

[043] Disclosed herein are methods of treating a disease or condition comprising administering the CAR-ECs disclosed herein, and one or more CAR-EC switches, wherein the CAR-EC switch comprises an anti-CD3 antibody and a targeting region. The CAR-EC switch may provide for a titratable response, improved safety and/or cessation of CAR-EC cell activity by ceasing switch administration after treatment. The CAR-EC switches may control CAR-EC activity, by "turning off" CAR-EC activity or "turning on" CAR-EC activity. The targeting region may comprise an antibody (e.g., the CAR-EC switch is a bispecific antibody). The targeting region may comprise a small molecule. The small molecule may bind a prostate specific membrane antigen (e.g., DUPA). The small molecule may bind a folate receptor. The small molecule may be folate.

[044] Further disclosed herein are CAR-EC platforms comprising CAR-EC switches and effector cells, wherein the CAR comprises an extracellular domain that may be recognized by an anti-CD3 antibody that interacts with a cluster of differentiation protein 3 (CD3) or fragment thereof. The CD3
fragment may be an extracellular domain of the CD3 ("chimeric CD3 receptor"). The CD3 fragment may be an epsilon domain of the CD3 ("chimeric CD3 epsilon receptor" or "e-CAR"). The CAR may provide for a "universal" chimeric antigen receptor that can bind multiple CAR-EC switches, providing for sequential or simultaneous targeting of one or more types of target cells (e.g., treatment of heterogeneous or mutating tumors). For example, the CARs disclosed herein may bind any conjugate, such as, by non-limiting example, a bispecific antibody, an antibody-small molecule conjugate and/or an antibody drug conjugate, wherein in at least a part of the conjugate interacts with the extracellular CD3 domain (e.g., CD3 epsilon).

Figures 2-5 provide schematics of T cell activation using chimeric antigen receptors and/or switches as described herein. In Figure 2, a bispecific antibody having specificity for a tumor associated antigen and CD3 (anti-CD3/anti-TAA antibody) activates a native T cell by binding to the tumor cell and the T cell. In this platform, cytotoxic T cells from a patient's own immune system are recruited to target and kill the tumor cell. The bispecific antibody or switch can be any bispecific antibody known in the art that targets both a T cell and a tumor cell and should not be limited to the examples shown herein. In Figures 3 and 4, a bispecific antibody having specificity for a tumor antigen acts as a switch to activate a CAR-EC cell that expresses a CAR comprising an extracellular domain recognized by the bispecific antibody, a transmembrane domain, and an intracellular domain comprising a costimulatory domain. In Figure 3, the intracellular domain further comprises a CD3 zeta domain. In Figure 4, the intracellular domain further comprises an additional costimulatory domain. The systems exemplified in Figures 3 and 4 are unlike conventional bispecific antibody therapy in that ligation of e-CARs on T cells by bispecific antibodies, in the presence of target cells, will benefit from additional co-stimulatory signals and thus, result in enhanced in vivo persistency and proliferation of engineered T cell similar to what has been observed in conventional CAR-T cells. Importantly, the activity of the engineered T cells now is dependent on the dose of bispecific antibodies, which will significantly improve the safety profile of CAR-T therapy.

Figure 5 shows activation of a CAR-EC through binding of a targeting region of the cell to a MHC-peptide complex presented on a tumor cell. In the example shown in Figure 5, the targeting region of the T cell is a T cell receptor or T cell receptor complex. In some embodiments, a T cell comprising a targeting region that targets a tumor cell is a tumor-infiltrating lymphocyte (TIL) cell. The TIL shown in Figure 5 is engineered with a CAR comprising an intracellular domain comprising one or more costimulatory domains. A distinctive feature of this platform is that it utilizes the specificity of endogenous T cell receptors of engineered T cells, as opposed to the artificially introduced tumor targeting moieties such as antibody- or monoclonal TCR-based therapies in
conventional CAR-T approaches, for the recognition of the target tumor cells. Thus, this exemplified platform combines beneficial features of two immunotherapeutic platforms, CAR-T and TIL-based therapies.

[047] Further disclosed herein are chimeric antigen receptors comprising an extracellular domain comprising a sequence of an extracellular portion of a cell surface protein; a transmembrane domain; and an intracellular domain; wherein the intracellular domain comprises a sequence that is not based on or derived from an extracellular portion of the cell surface protein. In some embodiments, the extracellular domain does not comprise an antibody or fragment thereof. In some embodiments, the cell is a T cell. In some embodiments, the cell surface protein is CD3. In some embodiments, the extracellular portion of the cell surface protein comprises CD3 epsilon. In some embodiments, the extracellular domain comprises a sequence from any of SEQ ID NOs: 1-3. In some embodiments, the extracellular domain comprises at least about 5, 10, 20, 25, 50, or 100 consecutive amino acids derived from SEQ ID NO. 1. In some embodiments, the consecutive amino acids of the extracellular domain derived from SEQ ID NO. 1 differs from a sequence of SEQ ID NO. 1 by less than about 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acids. In some embodiments, the intracellular domain comprises a costimulatory domain. In some embodiments, the intracellular domain comprises a sequence derived from a 4IBB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a combination thereof. In some embodiments, the intracellular domain comprises a sequence of a CD3 zeta chain. In some embodiments, the intracellular domain does not comprise a sequence of a CD3 zeta chain selected from ITAM1, ITAM2, ITAM3, or a combination thereof. In some embodiments, the transmembrane domain comprises a sequence derived from a CD8 transmembrane domain, a CD28 transmembrane domain, an FcR transmembrane domain, a CD3 epsilon transmembrane domain, or a combination thereof. In some embodiments, the chimeric antigen receptor further comprises a spacer between the extracellular domain and the transmembrane domain, wherein if the spacer comprises an amino acid sequence, the amino acid sequence comprises from about 1 to about 50 amino acids. In some embodiments, the chimeric antigen receptor comprises an amino acid sequence about or at least about 80%, 85%, 90%, 95%, 98%, 99% or identical to an amino acid sequence from any of SEQ ID NOs: 31-35, or a portion or combination thereof. In some embodiments, further provided herein is a nucleic acid sequence encoding for a chimeric antigen receptor as described herein. In some embodiments, further provided herein is an effector cell expressing the chimeric antigen receptor as described herein. In some embodiments, further provided herein is a platform comprising the effector
cell as described herein and an antibody comprising an antigen binding domain that binds to the extracellular domain of the chimeric antigen receptor. In some embodiments, the antibody is a bispecific antibody that further comprises an antigen binding domain that binds to an antigen on a target cell. In some embodiments, the target cell is a tumor cell. In some embodiments, the effector cell is a tumor-infiltrating lymphocyte. In some embodiments, the effector cell expresses a T cell receptor that binds to a MHC-peptide complexed presented on a target cell. In some embodiments, the target cell is a tumor cell.

[048] Further disclosed herein are effector cells expressing a chimeric antigen receptor, wherein the chimeric antigen receptor comprises an extracellular domain comprising a sequence derived from an extracellular portion of a cell surface protein; a transmembrane domain; and an intracellular domain; wherein the intracellular domain comprises a sequence that is not based on or derived from an intracellular portion of the cell surface protein. In some embodiments, the extracellular domain does not comprise an antibody or fragment thereof. In some embodiments, the effector cell is a T cell. In some embodiments, the cell surface protein is CD3. In some embodiments, the extracellular portion of the cell surface protein comprises CD3 epsilon. In some embodiments, the extracellular domain comprises a sequence of any of SEQ ID NOs: 1-3. In some embodiments, the extracellular domain comprises at least about 5, 10, 20, 25, 50, or 100 consecutive amino acids derived from SEQ ID NO. 1. In some embodiments, the consecutive amino acids of the extracellular domain derived from SEQ ID NO. 1 differs from a sequence of SEQ ID NO. 1 by less than about 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acids. In some embodiments, the intracellular domain comprises a costimulatory domain. In some embodiments, the intracellular domain comprises a sequence derived from a 4IBB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a combination thereof. In some embodiments, the intracellular domain comprises a sequence of a CD3 zeta chain. In some embodiments, the intracellular domain does not comprise a sequence of a CD3 zeta chain selected from ITAM1, ITAM2, ITAM3, or a combination thereof. In some embodiments, the transmembrane domain comprises a sequence derived from a CD8 transmembrane domain, a CD28 transmembrane domain, an FcR transmembrane domain, a CD3 epsilon transmembrane domain, or a combination thereof. In some embodiments, the effector cell is a tumor-infiltrating lymphocyte. In some embodiments, the effector cell expresses a T cell receptor that binds to a MHC-peptide complexed presented on a target cell. In some embodiments, the target cell is a tumor cell. In some embodiments, the chimeric antigen receptor comprises an amino acid sequence about or at least about 80%, 85%, 90%, 95%,
98%, 99% or identical to an amino acid sequence from any of SEQ ID NOs: 31-35, or a portion or combination thereof. In some embodiments, further provided herein is a platform comprising the effector cell as described herein and an antibody comprising an antigen binding domain that binds to the extracellular domain of the chimeric antigen receptor. In some embodiments, the antibody is a bispecific antibody that further comprises an antigen binding domain that binds to an antigen on a target cell. In some embodiments, the target cell is a tumor cell.

Further disclosed herein are platforms comprising an effector cell expressing a chimeric antigen receptor, wherein the chimeric antigen receptor comprises an extracellular domain comprising a sequence derived from an extracellular portion of a cell surface protein, wherein the extracellular domain does not comprise an antibody or fragment thereof, a transmembrane domain, and an intracellular domain; and a chimeric antigen receptor effector cell switch comprising an antigen binding domain that binds to the extracellular domain of the chimeric antigen receptor. In some embodiments, the intracellular domain comprises a sequence that is not based on or derived from an intracellular portion of the cell surface protein. In some embodiments, the effector cell switch further comprises an antigen binding domain that binds to an antigen on a target cell. In some embodiments, the target cell is a tumor cell. In some embodiments, the effector cell is a T cell. In some embodiments, the cell surface protein is CD3. In some embodiments, the extracellular portion of the cell surface protein comprises CD3 epsilon. In some embodiments, the extracellular domain comprises a sequence of any of SEQ ID NOs: 1-3. In some embodiments, the extracellular domain comprises at least about 5, 10, 20, 25, 50, or 100 consecutive amino acids derived from SEQ ID NO. 1. In some embodiments, the consecutive amino acids of the extracellular domain derived from SEQ ID NO. 1 differs from a sequence of SEQ ID NO. 1 by less than about 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acids. In some embodiments, the intracellular domain comprises a costimulatory domain. In some embodiments, the intracellular domain comprises a sequence derived from a 41BB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a combination thereof. In some embodiments, the intracellular domain comprises a sequence of a CD3 zeta chain. In some embodiments, the intracellular domain does not comprise a sequence of a CD3 zeta chain selected from ITAM1, ITAM2, ITAM3, or a combination thereof. In some embodiments, the transmembrane domain comprises a sequence derived from a CD8 transmembrane domain, a CD28 transmembrane domain, an FcR transmembrane domain, a CD3 epsilon transmembrane domain, or a combination thereof. In some embodiments, the effector cell is a tumor-infiltrating lymphocyte. In some embodiments, the
effector cell expresses a T cell receptor that binds to a MHC-peptide complexed presented on a target cell. In some embodiments, the target cell is a tumor cell. In some embodiments, the chimeric antigen receptor comprises an amino acid sequence about or at least about 80%, 85%, 90%, 95%, 98%, 99% or identical to an amino acid sequence from any of SEQ ID NOs: 31-35, or a portion or combination thereof.

[050] Further disclosed herein is an effector cell comprising an engineered component of a TCR complex, wherein the engineered component comprises a modification that improves an immune response. In some embodiments, the engineered component comprises an extracellular domain of CD3. In some embodiments, the modification comprises an addition of a costimulatory domain to the engineered component. In some embodiments, the costimulatory domain comprises a sequence derived from a 4IBB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a combination thereof. In some embodiments, the modification does not comprise an addition of an antibody or antibody fragment to the component of the TCR complex. In some embodiments, the modification does not comprise an addition of a CD3 zeta chain to the component of the TCR complex. In some embodiments, the effector cell is a tumor-infiltrating lymphocyte. In some embodiments, the TCR complex binds to a MHC-peptide complex presented on a target cell. In some embodiments, the target cell is a tumor cell. In some embodiments, further provided is a platform comprising the effector cell as described and a switch comprising an antigen binding domain configured to bind to the TCR complex. In some embodiments, the switch further comprises an antigen binding domain that binds to an antigen of a target cell. In some embodiments, the target cell is a tumor cell.

[051] Further disclosed herein is a method of treating a condition comprising administering to a subject in need thereof (a) an effector cell that expresses a chimeric antigen receptor comprising: an extracellular domain comprising a sequence derived from an extracellular portion of a cell surface protein, a transmembrane domain, and an intracellular domain; wherein the intracellular domain comprises a sequence that is not based on or derived from an intracellular portion of the cell surface protein; wherein the extracellular domain does not comprise an antibody or fragment thereof; and (b) a chimeric antigen receptor effector cell switch comprising: a first antigen binding region that binds to the extracellular domain of the chimeric antigen receptor, and a second antigen binding region that binds to an antigen on a target cell. In some embodiments, the condition is an infection. In some embodiments, the condition is a cancer. In some embodiments, the cancer is selected from a relapsed cancer, a refractory cancer, a glioblastoma, an ovarian cancer, a prostate cancer, a hormone
refractory prostate cancer and a breast cancer. In some embodiments, the cancer comprises a tumor selected from a liquid tumor or a solid tumor. In some embodiments, the cancer comprises a heterogeneous tumor. In some embodiments, the effector cell is selected from a T cell, an effector B cell, a natural killer cell, a macrophage and a progenitor thereof. In some embodiments, the effector cell is a T cell. In some embodiments, the 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. In some embodiments, the extracellular domain is selected from a CD3 extracellular domain, a homolog thereof and a portion thereof. In some embodiments, the extracellular domain is selected from a CD3 epsilon domain. In some embodiments, the intracellular domain comprises one or more signaling domains selected from signaling domains of 41BB, CD28, ICOS, OX40, CD27, CD30, CD150, DAP-10 and NKG2D. In some embodiments, the method further comprises administering a first chimeric antigen receptor effector cell switch and a second chimeric antigen receptor effector cell switch, wherein the first chimeric antigen receptor effector cell switch binding region binds to a first cell surface marker on a first target and the second chimeric antigen receptor effector cell switch binding region binds to a second cell surface marker on a second target. In some embodiments, the first chimeric antigen receptor effector cell switch and the second chimeric antigen receptor effector cell switch are administered simultaneously. In some embodiments, the first chimeric antigen receptor effector cell switch and the second chimeric antigen receptor effector cell switch are administered sequentially.

[052] Further disclosed herein is a method of treating a condition in a subject in need thereof comprising administering an effector cell that expresses a chimeric antigen receptor (CAR-EC), wherein the chimeric antigen receptor: (a) comprises an extracellular domain comprising a sequence derived from an extracellular portion of a cell surface protein, a transmembrane domain, and an intracellular domain, wherein the intracellular domain comprises a sequence that is not based on or derived from an intracellular portion of the cell surface protein; and (b) associates with a T cell receptor (TCR), wherein the TCR comprises a TCR extracellular domain that interacts with an antigen on a target cell. In some embodiments, the method further comprises adoptive cell transfer, wherein a lymphocyte is isolated from the subject and modified to express the chimeric antigen receptor, thereby producing the CAR-EC. In some embodiments, the lymphocyte is a tumor infiltrating lymphocyte (TIL). In some embodiments, the antigen is a tumor-associated antigen. In some embodiments, the antigen comprises a major histocompatibility-peptide complex. In some embodiments, the target cell is a cancer cell. In some embodiments, the condition is a cancer.
some embodiments, the chimeric antigen receptor effector cell is a T regulatory cell. In some embodiments, the condition is an autoimmune disease. In some embodiments, the condition is selected from type I diabetes and rheumatoid arthritis. In some embodiments, the extracellular domain is selected from a CD3 extracellular domain, a homolog thereof and a portion thereof. In some embodiments, the extracellular domain is selected from a CD3 epsilon domain. In some embodiments, the extracellular domain does not comprise an antibody or fragment thereof. In some embodiments, the intracellular domain comprises one or more signaling domains selected from signaling domains of 41BB, CD28, ICOS, OX40, CD27, CD30, CD150, DAP-10 and NKG2D.

[053] Unless otherwise specified, the terms "CAR" and "chimeric antigen receptor," as used herein, may be used interchangeably and may refer to "chimeric CD3 receptor" and "chimeric CD3 epsilon receptor" ("e-CAR"). Also, unless otherwise specified, the terms "switch" and "CAR-EC switch," as used herein, are used interchangeably and may refer to a small molecule-antibody switch, an antibody drug conjugate and/or a bispecific antibody switch. The antibody portion(s) of the switch and/or hapten- may comprise at least a portion of an antibody or an entire antibody. For example, the antibody portion(s) 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(s) of the switch may comprise at least a portion of the Fc (fragment, crystallizable) region. The antibody portion(s) may comprise at least a portion of the complementarity determining region (e.g., CDR1, CDR2, CDR3). The antibody portion(s) of the switch may comprise may comprise at least a portion of the Fab (fragment, antigen-binding) region. As used herein, in some instances, an antibody that "recognizes" or "targets" an antigen, (e.g., an antigen comprising an extracellular domain of a CAR), is an antibody that binds to the antigen.

[054] In some embodiments, as used herein a sequence "derived from" and/or "based on" a protein sequence refers to contiguous amino acids comprising an identical or modified sequence of the protein sequence from which it is derived. In some cases, the derived sequence comprises at least about 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90 or 100 contiguous amino acids that are identical or modified from the protein sequence from which it is derived. Modification may comprise a deletion, addition and/or substitution of one or more amino acids. In some cases, amino acids modified in the derived sequence comprise less than about 30%, 20%, 10%, 5%, or 1% of the total sequence derived from or based on the protein sequence. In some cases, about or less than about 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acids are modified in a derived sequence.
In some embodiments, an antibody or a fragment thereof refers to an entire immunoglobulin molecule or a polypeptide comprising fragment of an immunoglobulin including, but not limited to, heavy chain, light chain, variable domain, constant domain, complementarity determining region (CDR), framework region, fragment antigen binding (Fab) region, Fab', F(ab')2, F(ab')3, Fab', fragment crystallizable (Fc) region, single chain variable fragment (scFv), di-scFv, single domain immunoglobulin, trifunctional immunoglobulin, chemically linked F(ab')2, and any combination thereof. In some embodiments, a polypeptide comprising fragment of an immunoglobulin comprises at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 400, or 500 amino acids of an immunoglobulin. In some embodiments, a sequence free of an antibody or a fragment thereof refers to a sequence that does not comprise a contiguous sequence of an antibody or fragment thereof, wherein the contiguous sequence comprises at least about 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 100 or 250 amino acids of an immunoglobulin molecule or a polypeptide comprising fragment of an immunoglobulin. In some embodiments, a sequence free of an antibody of fragment thereof refers to a sequence that does not comprise a single chain variable fragment.

In some embodiments, a costimulatory domain is a domain of a protein that provides a costimulatory signal during T cell activation. In some cases, a costimulatory domain interacts with another costimulatory domain containing protein within a T cell. In some embodiments, a costimulatory domain is derived from 41BB, CD28, ICOS, OX40, CD27, CD30, CD150, DAP-10, NKG2D, or a portion or combination thereof.

Before the present methods, platforms and compositions are described in greater detail, it is to be understood that this invention is not limited to particular method, platform 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.

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.

[059] 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.

[060] 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.

[061] 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.

[062] 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.

[063] Methods, platforms and compositions are provided for producing CARs, CAR-ECs and CAR-EC platforms used to bring an effector cell together with a target in a subject. These methods, platforms and compositions find therapeutic use in a number of diseases. For example, immune
disorders, cancers, tumors and blood cell malignancies may be more effectively treated with a CAR-EC platform when the CAR provides a more robust response and CAR-EC switches of the CAR-EC platform additionally recruit endogenous T-cells for synergistic enhanced activity. Heterogeneous tumors may also be more effectively treated with multiple CAR-EC switches that target more than one tumor antigen. These and other objects, 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.

[064] 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.

I. Engineered Cell Surface Receptors (Chimeric Antigen Receptor)

[065] Disclosed herein are engineered cell surface proteins comprising an extracellular domain comprising a sequence of an extracellular portion of a cell surface protein; a transmembrane domain; and an intracellular domain comprising a sequence of an intracellular signaling protein; wherein the engineered cell surface protein is engineered by combining sequences from at least two different protein sources. In some embodiments, a first protein source is the cell surface protein and a second protein source is the intracellular signaling protein. In some embodiments, the extracellular domain does not comprise an antibody or fragment thereof. In some embodiments, the intracellular signaling protein is a costimulatory domain. In some embodiments, the costimulatory domain comprises a 41BB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a portion or combination thereof. In some embodiments, the costimulatory domain comprises a sequence of any of SEQ ID NOs: 6-15. In some embodiments, the intracellular domain further comprises a T cell receptor zeta chain. In some embodiments, the intracellular signaling protein is a T cell receptor zeta chain. In some embodiments, the T cell receptor zeta chain does not comprise an ITAMI domain, ITAM2 domain, ITAM3 domain, or a portion or combination thereof. In some embodiments, the cell surface protein is a protein expressed on the surface of a T cell. In some embodiments, the cell surface protein is CD3 epsilon. In some embodiments, the extracellular domain comprises a sequence from of any of
SEQ ID NOs: 1-3. In some embodiments, the transmembrane domain comprises a sequence from a CD8 transmembrane domain, a CD28 transmembrane domain, an FcR transmembrane domain, a CD3 epsilon transmembrane domain, or a combination thereof. In some embodiments, the extracellular domain comprises a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence selected from any of SEQ ID NOs: 1-3. In some embodiments, the extracellular domain comprises at least about 5 consecutive amino acids of SEQ ID NO. 1. In some embodiments, the extracellular domain comprises at least about 20, 25, 27, 30, 35 or 50 consecutive amino acids of SEQ ID NO. 1. In some embodiments, the consecutive amino acids of the extracellular domain derived from SEQ ID NO. 1 differ from a sequence of SEQ ID NO. 1 by less than about 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acids. In some embodiments, the transmembrane domain comprises a sequence from SEQ ID NO. 4. In some embodiments, the transmembrane domain comprises a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to SEQ ID NO. 4. In some embodiments, the intracellular domain comprises one or more sequences selected from any of SEQ ID NOs: 5-15. In some embodiments, the intracellular domain comprises a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence selected from any of SEQ ID NOs: 5-15. In some embodiments, the intracellular domain comprises at least about 20, 25, 27, 30, 35 or 50 consecutive amino acids from any of SEQ ID NOs: 5-15. In some embodiments, the consecutive amino acids of the intracellular domain derived from any of SEQ ID NOs: 5-15 differ from a sequence of SEQ ID NOs: 5-15 by less than about 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acids. In some embodiments, provided herein is a nucleic acid encoding for an engineered cell surface protein as described herein. In some embodiments, provided herein is an effector cell expressing an engineered cell surface protein as described herein. In some embodiments, the effector cell is a tumor-infiltrating lymphocyte. In some embodiments, provided herein are kits comprising an effector cell as described herein and an antibody comprising an antigen binding domain that binds to the extracellular domain of the engineered cell surface protein. In some embodiments, the antibody is a bispecific antibody that further comprises an antigen binding domain that binds to an antigen on a target cell.

[066] Disclosed herein are engineered cell surface proteins comprising an extracellular domain comprising a CD3 epitope; a transmembrane domain; and an intracellular domain comprising a sequence of an intracellular signaling protein; wherein the engineered cell surface protein is engineered by combining the CD3 epitope with a protein other than CD3. In some embodiments, the protein other than CD3 is the intracellular signaling protein, the transmembrane domain, or both the intracellular signaling protein and the transmembrane domain. In some embodiments, the
extracellular domain does not comprise an antibody or fragment thereof. In some embodiments, the CD3 epitope is from a CD3 epsilon domain. In some embodiments, the CD3 epitope comprises a sequence from the amino terminal portion of the CD3 epsilon domain. In some embodiments, the amino terminal portion of the CD3 epsilon domain comprises the first 30 amino acids of the CD3 epsilon domain. In some embodiments, the CD3 epitope comprises a sequence from any of SEQ ID NOs: 1-3. In some embodiments, the intracellular signaling protein is a costimulatory domain. In some embodiments, the costimulatory domain comprises a 4IBB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a portion or combination thereof. In some embodiments, the costimulatory domain comprises a sequence from any of SEQ ID NOs: 6-15. In some embodiments, the intracellular domain further comprises a T cell receptor zeta chain. In some embodiments, the intracellular signaling protein is a T cell receptor zeta chain. In some embodiments, the T cell receptor zeta chain does not comprise an ITAMI domain, ITAM2 domain, ITAM3 domain, or a portion or combination thereof. In some embodiments, the transmembrane domain comprises a sequence derived from a CD8 transmembrane domain, a CD28 transmembrane domain, an FcR transmembrane domain, a CD3 epsilon transmembrane domain, or a combination thereof. In some embodiments, the extracellular domain comprises a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence selected from any of SEQ ID NOs: 1-3. In some embodiments, the extracellular domain comprises at least about 5 consecutive amino acids of SEQ ID NO. 1. In some embodiments, the extracellular domain comprises at least about 20, 25, 27, 30, 35 or 50 consecutive amino acids of SEQ ID NO. 1. In some embodiments, the consecutive amino acids of the extracellular domain derived from SEQ ID NO. 1 differ from a sequence of SEQ ID NO. 1 by less than about 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acids. In some embodiments, the transmembrane domain comprises a sequence from SEQ ID NO. 4. In some embodiments, the transmembrane domain comprises a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to SEQ ID NO. 4. In some embodiments, the intracellular domain comprises one or more sequences selected from any of SEQ ID NOs: 5-15. In some embodiments, the intracellular domain comprises a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99%, identical to a sequence selected from any of SEQ ID NOs: 5-15. In some embodiments, the intracellular domain comprises at least about 20, 25, 27, 30, 35 or 50 consecutive amino acids from any of SEQ ID NOs: 5-15. In some embodiments, the consecutive amino acids of the intracellular domain derived from any of SEQ ID NOs: 5-15 differ from a sequence of SEQ ID NOs:
5-15 by less than about 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acids. In some embodiments, provided herein is a nucleic acid encoding for an engineered cell surface protein as described herein. In some embodiments, provided herein is an effector cell expressing an engineered cell surface protein as described herein. In some embodiments, the effector cell is a tumor-infiltrating lymphocyte. In some embodiments, the extracellular domain derived from SEQ ID NO. 1 differs from a protein thereof.

In some embodiments, provided herein is a protein comprising an extracellular domain comprising a sequence of an extracellular portion of a cell surface protein; a transmembrane domain; and an intracellular domain comprising a sequence of a costimulatory domain; wherein the engineered cell surface protein is engineered by combining sequences from at least two different protein sources. In some embodiments, a first protein source is the cell surface protein and a second protein source is a protein comprising the costimulatory domain. In some embodiments, the extracellular domain does not comprise an antibody or fragment thereof. In some embodiments, the costimulatory domain comprises a 4IBB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a portion or combination thereof. In some embodiments, the costimulatory domain comprises a sequence from any of SEQ ID NOs: 6-15. In some embodiments, the cell surface protein is a protein expressed on the surface of a T cell. In some embodiments, the cell surface protein is CD3 epsilon. In some embodiments, the extracellular domain comprises a sequence from any of SEQ ID NOs: 1-3. In some embodiments, the intracellular domain further comprises a T cell receptor zeta chain. In some embodiments, the T cell receptor zeta chain does not comprise an ITAMI domain, ITAM2 domain, ITAM3 domain, or a portion or combination thereof. In some embodiments, the transmembrane domain comprises a sequence derived from a CD8 transmembrane domain, a CD28 transmembrane domain, an FcR transmembrane domain, a CD3 epsilon transmembrane domain, or a combination thereof. In some embodiments, the extracellular domain comprises a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence selected from any of SEQ ID NOs: 1-3. In some embodiments, the extracellular domain comprises at least about 5 consecutive amino acids of SEQ ID NO. 1. In some embodiments, the extracellular domain comprises at least about 20, 25, 27, 30, 35 or 50 consecutive amino acids of SEQ ID NO. 1. In some embodiments, the consecutive amino acids of the extracellular domain derived from SEQ ID NO. 1 differ from a
sequence of SEQ ID NO. 1 by less than about 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acids. In some embodiments, the transmembrane domain comprises a sequence from SEQ ID NO. 4. In some embodiments, the transmembrane domain comprises a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to SEQ ID NO. 4. In some embodiments, the intracellular domain comprises one or more sequences selected from any of SEQ ID NOs: 5-15. In some embodiments, the intracellular domain comprises a sequence from any of SEQ ID NOs: 6-15. In some embodiments, the intracellular domain comprises a sequence from any of SEQ ID NOs: 5-15. In some embodiments, the intracellular domain comprises a sequence that is at least about 50%-, 60%-, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence selected from any of SEQ ID NOs: 5-15. In some embodiments, the intracellular domain comprises at least about 20, 25, 27, 30, 35 or 50 consecutive amino acids from any of SEQ ID NOs: 5-15. In some embodiments, the consecutive amino acids of the intracellular domain derived from any of SEQ ID NOs: 5-15 differ from a sequence of SEQ ID NOs: 5-15 by less than about 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acids. In some embodiments, provided herein is a nucleic acid encoding for an engineered cell surface protein as described herein. In some embodiments, provided herein is an effector cell expressing an engineered cell surface protein as described herein. In some embodiments, the effector cell is a tumor-infiltrating lymphocyte. In some embodiments, provided herein are kits comprising an effector cell as described herein and an antibody comprising an antigen binding domain that binds to the extracellular domain of the engineered cell surface protein. In some embodiments, the antibody is a bispecific antibody that further comprises an antigen binding domain that binds to an antigen on a target cell.

[068] Disclosed herein are engineered cell surface proteins comprising an extracellular domain comprising a CD3 epitope; a transmembrane domain; and an intracellular domain comprising a sequence of a costimulatory domain; wherein the engineered cell surface protein is engineered by combining the CD3 epitope with a protein other than CD3. In some embodiments, the protein other than CD3 comprises the costimulatory domain. In some embodiments, the extracellular domain does not comprise an antibody or fragment thereof. In some embodiments, the CD3 epitope is from a CD3 epsilon domain. In some embodiments, the CD3 epitope comprises a sequence from the amino terminal portion of the CD3 epsilon domain. In some embodiments, the amino terminal portion of the CD3 epsilon domain comprises the first 30 amino acids of the CD3 epsilon domain. In some embodiments, the CD3 epitope comprises a sequence from any of SEQ ID NOs: 1-3. In some embodiments, the costimulatory domain comprises a 41BB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a portion or combination thereof. In some embodiments, the costimulatory domain comprises a sequence from any of SEQ ID NOs: 6-15. In some embodiments, the intracellular
domain further comprises a T cell receptor zeta chain. In some embodiments, the T cell receptor zeta chain does not comprise an ITAMI domain, ITAM2 domain, ITAM3 domain, or a portion or combination thereof. In some embodiments, the transmembrane domain comprises a sequence derived from a CD8 transmembrane domain, a CD28 transmembrane domain, an FcR transmembrane domain, a CD3 epsilon transmembrane domain, or a combination thereof. In some embodiments, the extracellular domain comprises a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence selected from any of SEQ ID NOs: 1-3. In some embodiments, the extracellular domain comprises at least about 5 consecutive amino acids of SEQ ID NO. 1. In some embodiments, the extracellular domain comprises at least about 20, 25, 27, 30, 35 or 50 consecutive amino acids of SEQ ID NO. 1. In some embodiments, the consecutive amino acids of the extracellular domain derived from SEQ ID NO. 1 differ from a sequence of SEQ ID NO. 1 by less than about 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acids. In some embodiments, the transmembrane domain comprises a sequence from SEQ ID NO. 4. In some embodiments, the transmembrane domain comprises a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to SEQ ID NO. 4. In some embodiments, the intracellular domain comprises one or more sequences selected from any of SEQ ID NOs: 5-15. In some embodiments, the intracellular domain comprises a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99%, identical to a sequence selected from any of SEQ ID NOs: 5-15. In some embodiments, the intracellular domain comprises at least about 20, 25, 27, 30, 35 or 50 consecutive amino acids from any of SEQ ID NOs: 5-15. In some embodiments, the consecutive amino acids of the intracellular domain derived from any of SEQ ID NOs: 5-15 differ from a sequence of SEQ ID NOs: 5-15 by less than about 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acids. In some embodiments, provided herein is a nucleic acid encoding for an engineered cell surface protein as described herein. In some embodiments, provided herein is an effector cell expressing an engineered cell surface protein as described herein. In some embodiments, the effector cell is a tumor-infiltrating lymphocyte. In some embodiments, provided herein are kits comprising an effector cell as described herein and an antibody comprising an antigen binding domain that binds to the CD3 epitope of the engineered cell surface protein. In some embodiments, the antibody is a bispecific antibody that further comprises an antigen binding domain that binds to an antigen on a target cell.

[069] In some aspects, an engineered cell surface protein is a chimeric antigen receptor.

[070] Further disclosed herein are chimeric antigen receptors comprising an extracellular domain that interacts with an anti-CD3 antibody or fragment thereof; a transmembrane domain; and an intracellular domain, wherein at least a portion of the transmembrane domain or at least a portion of
the intracellular domain is not based on or derived from a CD3 protein. The extracellular domain
may comprise a CD3 epsilon domain.

[071] Further disclosed herein are chimeric antigen receptors comprising an extracellular domain, a
transmembrane domain, and an intracellular domain, wherein at least a portion of the transmembrane
domain or at least a portion of the intracellular domain is not based on or derived from a CD3
protein; and wherein the chimeric antigen receptor associates with a T cell receptor (TCR), wherein
the TCR comprises a TCR extracellular domain that interacts with an antigen on a target cell (Figure
5). The extracellular domain may comprise a CD3 epsilon domain. The chimeric antigen receptor
may be a co-receptor of the TCR. The chimeric antigen receptor may be a co-receptor in a TCR
complex. The intracellular domain of the chimeric antigen receptor may comprise a signaling
domain. The signaling domain may activate the T cell, when the TCR extracellular domain interacts
with the antigen. The signaling domain may enhance a TCR activity when the TCR extracellular
domain interacts the antigen. The TCR activity may be an intracellular signaling activity mediated by
an intracellular domain of the TCR.

[072] Further disclosed herein are chimeric antigen receptors comprising a T cell receptor (TCR)
complex component or portion thereof, wherein the TCR complex component comprises an
extracellular domain that interacts with a target molecule; a transmembrane domain; and an
intracellular signaling domain, wherein the T cell receptor complex component or portion thereof
and the intracellular domain are a single protein. The TCR complex component may be a T cell
receptor co-receptor. The T cell receptor co-receptor may be CD3 epsilon.

[073] The chimeric antigen receptor may comprise an extracellular domain, a transmembrane
domain and an intracellular domain. The chimeric antigen receptor may comprise a plurality of
extracellular domains, a plurality of transmembrane domains and/or a plurality of intracellular
domains. The chimeric antigen receptor may comprise one or more spacers. The spacer may link the
extracellular domain to the transmembrane domain. The spacer may be flexible enough to allow the
extracellular domain to orient in different directions to facilitate binding a CAR-EC switch. The
spacer may comprise about 1 amino acid, about 5 amino acids, about 10 amino acids, about 20 amino
acids, about 50 amino acids or about 100 amino acids. The extracellular domain may interact with
the anti-CD3 antibody or fragment thereof of the CAR-EC switch. The extracellular domain may
comprise at least a portion of a CD3. The extracellular domain may comprise an epsilon domain of a
CD3. The extracellular domain may comprise about 105, about 100, about 95, 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 or about 5 amino acids of the epsilon domain of
CD3. The extracellular domain may comprise about 27 amino acids of the epsilon domain of CD3. The extracellular domain may consist of the N-terminal 27 amino acids of the epsilon domain of CD3. The extracellular domain may comprise about 5 amino acids of the epsilon domain of CD3. The extracellular domain may consist of the N-terminal 5 amino acids of the epsilon domain of CD3. The extracellular domain may comprise an amino acid sequence selected from any of SEQ ID NOs: 1-3. The extracellular domain may be at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% homologous or identical to an amino acid sequence selected from SEQ ID NOs: 1-3. The extracellular domain may be encoded by a nucleic acid sequence comprising a nucleic acid sequence selected from any of SEQ ID NOs: 16-18. The extracellular domain may be encoded by a nucleic acid sequence comprising a sequence that is at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% homologous or identical to an amino acid sequence selected from any of SEQ ID NOs: 16-18.

[074] The CAR may comprise a transmembrane domain. The transmembrane domain may comprise a transmembrane domain selected from a CD8 transmembrane domain, a CD28 transmembrane domain, an FcR transmembrane domain, and a CD3 epsilon transmembrane domain. The transmembrane domain may comprise at least a portion of a cytoplasmic signaling domain. The transmembrane domain may comprise a sequence selected from SEQ ID NO. 4. The transmembrane domain may comprise a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence of SEQ ID NO. 4. The transmembrane domain may be encoded by a sequence selected from SEQ ID NO. 19. The transmembrane domain may be encoded by a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence of SEQ ID NO. 19.

[075] Unless otherwise specified, the terms "signaling domain," "stimulatory domain," "co-stimulatory domain" and "intracellular domain," as used herein, may be used interchangeably.

[076] The intracellular domain may comprise a stimulatory domain. The intracellular domain may comprise one or more co-stimulatory domains. The intracellular domain may comprise two co-stimulatory domains. The stimulatory or co-stimulatory domains may be selected from at least a portion of 41BB, CD28, ICOS, OX40, CD27, CD30, CD150, DAP-10 and NKG2D. 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 signaling molecule selected from the group comprising CD3 zeta, CD28, and 41BB. 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 CD3 zeta. The signaling molecule may comprise CD28. The signaling molecule may comprise 41BB. The intracellular domain may comprise at least a portion of CD3 zeta. The intracellular domain may comprise at least a portion of CD28. The intracellular domain may comprise at least a portion of 41BB. 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 ligand that binds to CD83. The intracellular domain may comprise a sequence selected from any of SEQ ID NOs: 5-15. The intracellular domain may comprise a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence from any of SEQ ID NOs: 5-15. The intracellular domain may be encoded by a sequence selected from any of SEQ ID NOs: 20-30. The intracellular domain may be encoded by a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence from any of SEQ ID NOs: 20-30.

[077] The CAR may comprise a sequence selected from any of SEQ ID NOs: 31-35. The CAR may comprise a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence from any of SEQ ID NOs: 31-35. The CAR may be encoded by a sequence selected from any of SEQ ID NOs: 36-40. The CAR may encoded by a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence from any of SEQ ID NOs: 36-40.

II. Chimeric Antigen Receptor Effector Cells (CAR-EC)

[078] Disclosed herein are effector cells expressing an engineered cell surface protein comprising an extracellular domain comprising a sequence of an extracellular portion of the cell surface protein; a transmembrane domain; and an intracellular domain comprising a sequence of an intracellular signaling protein; wherein the engineered cell surface protein is engineered by combining sequences
from at least two different protein sources. In some embodiments, a first protein source is the cell surface protein and a second protein source is the intracellular signaling protein. In some embodiments, the extracellular domain does not comprise an antibody or fragment thereof. In some embodiments, the intracellular signaling protein is a costimulatory domain. In some embodiments, the costimulatory domain comprises a 41BB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a portion or combination thereof. In some embodiments, the extracellular domain further comprises a T cell receptor zeta chain. In some embodiments, the intracellular signaling protein is a T cell receptor zeta chain. In some embodiments, the T cell receptor zeta chain does not comprise an ITAMI domain, ITAM2 domain, ITAM3 domain, or a portion or combination thereof. In some embodiments, the cell surface protein is a protein expressed on the surface of a T cell. In some embodiments, the cell surface protein is CD3 epsilon. In some embodiments, the extracellular domain comprises a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence selected from any of SEQ ID NOs: 1-3. In some embodiments, the extracellular domain comprises at least about 5 consecutive amino acids of SEQ ID NO. 1. In some embodiments, the extracellular domain comprises at least about 20, 25, 27, 30, 35 or 50 consecutive amino acids of SEQ ID NO. 1. In some embodiments, the consecutive amino acids of the extracellular domain derived from SEQ ID NO. 1 differ from a sequence of SEQ ID NO. 1 by less than about 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acids. In some embodiments, the transmembrane domain comprises a sequence from SEQ ID NO. 4. In some embodiments, the transmembrane domain comprises a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to SEQ ID NO. 4. In some embodiments, the intracellular domain comprises one or more sequences selected from any of SEQ ID NOs: 5-15. In some embodiments, the intracellular domain comprises a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence selected from any of SEQ ID NOs: 5-15. In some embodiments, the intracellular domain comprises at least about 20, 25, 27, 30, 35 or 50 consecutive amino acids from any of SEQ ID NOs: 5-15. In some embodiments, the consecutive amino acids of the intracellular domain derived from any of SEQ ID NOs: 5-15 differ from a sequence of SEQ ID NOs: 5-15 by less than about 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acids. In some embodiments, the effector cell is a tumor-infiltrating lymphocyte. In some embodiments, provided herein are kits comprising an effector cell as described herein and an antibody comprising an antigen binding domain that binds to the extracellular domain.
of the engineered cell surface protein. In some embodiments, the antibody is a bispecific antibody that further comprises an antigen binding domain that binds to an antigen on a target cell.

[079] Disclosed herein are effector cells comprising an engineered component of a TCR complex comprising a modification that improves an immune response. In some embodiments, the component of the TCR complex comprises an extracellular domain of CD3. In some embodiments, the modification comprises an addition of a costimulatory domain to the component of the TCR complex. In some embodiments, the costimulatory domain comprises a sequence derived from a 41BB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a combination thereof. In some embodiments, the modification does not comprise an addition of an antibody or antibody fragment to the component of the TCR complex. In some embodiments, the modification does not comprise an addition of a CD3 zeta chain to the component of the TCR complex. In some embodiments, the effector cell is a tumor-infiltrating lymphocyte.

[080] In some aspects, effector cells express an engineered cell surface receptor or chimeric antigen receptor as described herein.

[081] Disclosed herein are chimeric antigen receptor effector cells (CAR-EC) that express a chimeric antigen receptor, wherein the chimeric antigen receptor comprises chimeric antigen receptor comprising an extracellular domain that interacts with an anti-CD3 antibody or fragment thereof; a transmembrane domain; and an intracellular domain, wherein at least a portion of the transmembrane domain or at least a portion of the intracellular domain is not based on or derived from a CD3 protein.

[082] While preferred embodiments of the present disclosure describe chimeric antigen receptor 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 have an effect on a target or target cell when brought into proximity of the target or target cell. The effector cell may have a cytotoxic effect on a target or target cell when brought into proximity of the target or target cell.

[083] 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 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<sub>MSC</sub>). The effector cell may be a central memory T cell (T<sub>C</sub>). 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 αβ T cell. The effector cell may be a γδ T cell. The effector cell may be a natural killer T cell. The effector cell may be a helper T cell. The T cell may express forkhead box P3 (FoxP3). The T cell may express interleukin-2 receptor alpha chain (IL-2Ra, also known as CD25).

The effector cell may be a regulatory T cell (T<sub>Reg</sub>). The T<sub>Reg</sub> cell may be a CD4+CD25+ T<sub>Reg</sub> cell. The T<sub>Reg</sub> cell may be a FOXP3+ T<sub>Reg</sub> cell. T<sub>Reg</sub> cells may comprise induced Regulatory T (iTreg) cells (CD4+ CD25+ Foxp3+). iTreg cells have been shown to suppress T cell proliferation and experimental autoimmune diseases. iTreg cells include Treg<sub>17</sub> cells. iTreg cells may develop from mature CD4+ conventional T cells outside of the thymus: a defining distinction between natural regulatory T (nTreg) cells and iTreg cells.

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. The effector cell may be a tumor-infiltrating lymphocyte (TIL). The TIL may be a T cell.

Adopted cells

The effector cell may be an adopted cell, resulting from an adoptive cell transfer. Adoptive cell transfer refers to the transfer of cells, most commonly immune-derived cells, back into the same subject or into a new recipient host with the goal of transferring the immunologic functionality and characteristics into the new host. Use of autologous cells may help the recipient by minimizing graft versus host disease (GVHD) issues. To produce immune cells for adoptive transfer, blood cells are drawn and expanded, most often in vitro, using cell culture methods which may require exposure to an interleukin (e.g., IL-2). Anti-CD3 antibody is commonly used to promote the proliferation of T cells in culture. Cells are returned to the subject in large numbers intravenously in an activated state. Subsets of cells may be selected to be returned to the subject (e.g., T cells, stem cells, tumor infiltrating lymphocytes). Alternatively or additionally, subsets of cells may be removed (e.g., T<sub>reg</sub> cells in the case of targeting cancer cells). Clinically, this approach may be exploited to transfer either immune-promoting or tolerogenic cells (often lymphocytes) to a subject to either enhance immunity against viruses and cancer or to promote tolerance in the setting of autoimmune disease, such as Type I diabetes or rheumatoid arthritis.
Broadly speaking, engineering T cells with an eCAR provides a way to link the main TCR signaling with other desired co-signaling pathways under the same TCR specificity, resulting in the enhanced activity and proliferation of the T cells. Therefore, this approach can be expanded to the other T cell-based therapies such as the adoptive transfer for regulatory T cells (T\textsubscript{reg} cells). While removing T\textsubscript{reg} cells may be advantageous in some instances (e.g., adoptive cell transfer for cancer treatment), in other instances, adoptive cell transfer with T\textsubscript{reg} cells is desirable (e.g., treatment of an autoimmune disease, type I diabetes, rheumatoid arthritis). Some diseases may be caused or exacerbated by a lack of T\textsubscript{reg} activity, which is generally anti-inflammatory, keeping the inflammatory activity of conventional T cells under control or balanced. In addition, similar to the cytotoxic effector T cells, TCR specificity is also known to govern the function of T\textsubscript{reg} cells. The methods disclosed herein, comprise treating a condition in a subject with T\textsubscript{reg} cells, wherein the T\textsubscript{reg} cells express a chimeric antigen receptor that generates, stimulates and/or enhances activity of the T\textsubscript{reg} cells. The chimeric antigen receptor may comprise a transmembrane domain and an intracellular domain, wherein at least a portion of the transmembrane domain or at least a portion of the intracellular domain is not based on or derived from a CD3 protein. The chimeric antigen receptor may comprise a CD3 epsilon domain.

Tumor infiltrating lymphocytes (TILs)

The effector cell may be a tumor-infiltrating lymphocyte (TIL). TILs are a type of white blood cell found in tumors. TILs are implicated in killing tumor cells, and the presence of lymphocytes in tumors is often associated with better clinical outcomes. To obtain TILs, autologous lymphocytes may be isolated from patients’ tumors and grown to very large numbers of cells in vitro. Prior to TIL treatment, the subject may be given nonmyeloablative chemotherapy to deplete native lymphocytes ("lymphodepletion") that can suppress tumor killing. Once lymphodepletion is complete, the subject may be infused with the TILs. TILs may be administered in combination with interleukin 2 (IL-2).

The present application provides for TILs that are modified to express a CAR and applications thereof (e.g., CAR-TIL therapy). disclosed herein are T cells (e.g., TILs) modified to express a chimeric antigen receptor. Further disclosed herein are methods for treating a condition in a subject in need thereof, comprising administering a TIL, wherein the TIL expresses a CAR. The CAR may be a co-receptor of a T cell receptor (TCR) expressed by the TIL. The CAR may associate with a TCR of the TIL. The CAR may enhance TCR activation. The CAR may have intracellular signaling domains that are activated upon association and/or interaction with a TCR, wherein the TCR is bound to an antigen on a target cell. These methods may be referred to as chimeric antigen
receptor tumor infiltrating lymphocyte therapy (CAR-TIL therapy). An advantage of this application is to utilize the specificity of endogenous T cell receptors (TCRs) of the engineered T cells (e.g., antigen specific MHC), circumventing the need to introduce artificial tumor targeting moieties (e.g., antibody-based switches) used in conventional CAR-T approaches, for the recognition of the target tumor cells. The endogenous TCRs expressed on tumor specific T cells are heterogeneous, but may be pre-selected for specifically targeting tumor-associated peptide antigens bound to major histocompatibility complexes (MHCs) on tumor cells. Moreover, the diverse repertoire of the endogenous, tumor specific TCRs are suitable to target heterogeneous tumors.

The methods may further comprise administering a therapy to immunosuppress the subject before, during and/or after CAR-TIL therapy. The methods may not require administering a therapy to immunosuppress the subject before, during and/or after CAR-TIL therapy.

The CAR-TILs disclosed herein may express a CAR, wherein the CAR comprises an extracellular CD3 epsilon domain. The resulting cells and associated therapies may be referred to herein as epsilon chimeric antigen receptor expressing tumor infiltrating lymphocytes (eCAR-TILs) and epsilon chimeric antigen receptor expressing tumor infiltrating lymphocyte therapy (eCAR-TIL therapy), respectively. There are several advantages specific to the eCAR-TIL therapy approach. First, the engineered signaling domains in eCAR provide costimulatory signals to circumvent immune-suppressive tumor cells and microenvironments. Second, the use of the TCRs with pre-selected specificity alleviates the potentials for the manifest of autoimmune responses by eCAR-engineered T cells. Furthermore, the enhanced in vivo proliferation of eCAR-engineered TILs can significantly reduce the T cell doses used in current TIL therapy and can eliminate the necessity of the co-injection of interleukin-2 (IL-2) which support T cell survival and proliferation but also causes serious systemic toxicities in patients.

The effector cell may comprise one or more peptides selected from any of SEQ ID NOs: 1-15 and 31-35. The T cell may comprise a peptide that is 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 a peptide selected from any of SEQ ID NOs: 1-15 and 31-35. The effector cell may comprise a peptide that is at least about 70%> identical to a peptide selected from any of SEQ ID NOs: 1-15 and 31-35.

The effector cell may comprise one or more polynucleotides selected from any of SEQ ID NOs: 16-30 and 36-40. The effector cell may comprise a polynucleotide that is 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 polynucleotides selected from any of SEQ ID NOs: 16-30 and 36-40. The effector cell may comprise a polynucleotide that is at least about 70% identical to one or more
polynucleotides selected from any of SEQ ID NOs: 16-30 and 36-40. The T cell may express a polypeptide encoded by one or more polynucleotides selected from any of SEQ ID NOs: 16-30 and 36-40. The effector cell may express a polypeptide encoded by a polynucleotide that is 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 polynucleotides selected from any of SEQ ID NOs: 16-30 and 36-40. The polynucleotide may be constitutively expressed. The polynucleotide may be conditionally expressed.

[097] Methods of producing CAR-ECs

[098] Disclosed herein is a method of producing a chimeric antigen receptor effector cell comprising introducing a chimeric antigen receptor encoding polynucleotide into an effector cell, wherein the chimeric antigen receptor encoding polynucleotide encodes a chimeric antigen receptor comprising an extracellular domain that interacts with an anti-CD3 antibody or fragment thereof; a transmembrane domain; and an intracellular domain, wherein at least a portion of the transmembrane domain or at least a portion of the intracellular domain is not based on or derived from a CD3 protein. The chimeric antigen receptor effector cell may be a T cell. The method may comprise transfecting the effector cell with the chimeric antigen receptor encoding polynucleotide. The method may comprise virally infecting the effector cell with the chimeric antigen receptor encoding polynucleotide. The method may comprise transducing the effector cell with one or more viruses comprising the chimeric antigen receptor encoding polynucleotide. The virus may be a lentivirus. The virus may be an adenovirus. The virus may be a retrovirus. The virus may be an adeno-associated virus. The virus may be a self-complementary adeno-associated virus (scAAV). The virus may be a modified human immunodeficiency (HIV) virus. The virus may be a modified herpes simplex virus (HSV) virus.

[099] The method may further comprise cloning the chimeric antigen receptor encoding polynucleotide into a vector. Cloning the chimeric antigen receptor encoding polynucleotide into a vector may comprise any standard method required for cloning known in the art (e.g., PCR, restriction digest, ligation, transformation, drug resistance selection, sequencing validation, etc.) to combine the various domains in a preferred sequence required to produce the desired chimeric antigen receptor.

III. CAR-EC switches

[0100] Disclosed herein are chimeric antigen receptor effector cell (CAR-EC) switches comprising: a chimeric antigen receptor binding region and a targeting region, wherein the CAR binding region binds an extracellular domain of a CD3, homolog thereof, or portion thereof. The CD3 may be a
human CD3. The CAR binding region may comprise a peptide. The CAR binding region may
comprise an anti-CD3 antibody or fragment thereof. The anti-CD3 antibody or fragment thereof may
comprise at least a portion of an antibody or an entire antibody. For example, the anti-CD3 antibody
or fragment thereof 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 anti-CD3 antibody or fragment thereof
may comprise at least a portion of an Fc (fragment, crystallizable) region. The anti-CD3 antibody or
fragment thereof may comprise at least a portion of the complementarity determining region (e.g.,
CDR1, CDR2, CDR3). The anti-CD3 antibody or fragment thereof may comprise at least a portion
of the Fab (fragment, antigen-binding) region. The anti-CD3 antibody or fragment thereof may
comprise a human antibody, a humanized antibody, a chimeric antibody, a murine antibody or a
fragment thereof.

[0101] The targeting region may comprise at least a portion of an antibody or an entire antibody. For
example, the anti-CD3 antibody or fragment thereof 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 anti-CD3
antibody or fragment thereof may comprise at least a portion of an Fc (fragment, crystallizable)
region. The anti-CD3 antibody or fragment thereof may comprise at least a portion of the
complementarity determining region (e.g., CDR1, CDR2, CDR3). The targeting antibody or
fragment thereof may comprise at least a portion of the Fab (fragment, antigen-binding) region. The
targeting antibody or fragment thereof may comprise a human antibody, a humanized antibody, a
chimeric antibody, a murine antibody or a fragment thereof.

[0102] The CAR-binding region may have a binding affinity for the extracellular domain of the CAR
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 12 nM, 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.
[0103] The targeting region may comprise a targeting antibody or fragment thereof. For example, the CAR-EC switch may comprise a bispecific antibody. The bispecific antibody may be any available bispecific antibody with a region that interacts with a CD3 or portion thereof. Examples of such antibodies that have been developed and/or clinically tested include, but are not limited to, an anti-CD3/anti-CD19 bispecific antibody, an anti-CD3/anti-PSMA bispecific antibody, an anti-CD3/anti-epCAM bispecific antibody, an anti-CD3/anti-CEA bispecific antibody, an anti-CD3/anti-gpl00 bispecific antibody, an anti-CD3/anti-CD20 bispecific antibody, an anti-CD3/anti-Her2 bispecific antibody, an anti-CD3/anti-BCL1 bispecific antibody, an anti-CD3/anti-EGFR bispecific antibody, an anti-CD3/anti-EphA2 bispecific antibody, an anti-CD3/anti-MCSP bispecific antibody, an anti-CD3/anti-FAPalpha bispecific antibody, an anti-CD3/anti-HLA-A2 bispecific antibody, and an anti-CD3/anti-Her3 bispecific antibody. Additional examples of targeting antibodies or fragments thereof include, but are not limited to, an anti-EGFRvIII antibody, an anti-Her2 antibody, an anti-CS1 antibody, an anti-CLL-1 antibody, an anti-CD33 antibody, an anti-CD20 antibody, an anti-RORl antibody, an anti-CD44v6 antibody, an anti-PVRL4 antibody, an anti-IL13Ra2 antibody and a bscWuel antibody.

[0104] In some embodiments, a targeting region of a bispecific antibody may be selected from a small molecule, a ligand, a protein, a peptide, a peptoid, a DNA aptamer, a peptide nucleic acid, a vitamin a substrate or a substrate analog. The targeting region may interact with a cell surface protein. The targeting region may interact with a cell surface marker. The cell surface protein or cell surface marker may be selected from an antigen, a receptor, a co-receptor, a trans-membrane protein, a lipid moiety, a carbohydrate moiety and combinations thereof. The cell surface protein may be selected from a cholecystokinin B receptor, a gonadotropin-releasing hormone receptor, a somatostatin receptor 2, an avb3 integrin, a gastrin-releasing peptide receptor, a neurokinin 1 receptor, a melanocortin 1 receptor, a neuropeptide Y receptor, a neurotensin receptor, a neuropeptide Y receptor and C-type lectin like molecule 1. The antigen may comprise a prostate specific membrane antigen. The targeting region may comprise an octreotide. The targeting region may comprise an octreotate. The targeting region may comprise a somatostatin analog. The targeting region may comprise a CD38 NAD+ glycohydrolase inhibitor. The targeting region may comprise a pentagastrin. The targeting region may comprise a gonadotropin releasing hormone. The targeting region may comprise a CCKB antagonist. The targeting region may comprise a cRGD. The targeting region may comprise a bombesin. The targeting region may comprise 2-[3-(1,3-dicarboxypropy)ureidol] pentanedioic acid (DUPA). The targeting region may be sufficiently small to penetrate a tumor. The targeting agent antibody conjugate may further comprise a second targeting agent. The CAR-EC switch may
comprise 1, 2, 3, 4, or more targeting regions. The targeting region may be a small molecule (e.g., the CAR-EC switch comprises an anti-CD3 antibody- small molecule conjugate). The target region may be a toxin (e.g., the CAR-EC switch comprises an immunotoxin). The targeting region may be a drug (e.g., the CAR-EC switch comprises an anti-CD3 antibody-drug conjugate).

[0105] Bispecific antibodies useful with the chimeric antigen receptors, effector cells and/or platforms described herein include, without limitation, Triomab® family, knobs into holes (kih) IgG common LC, CrossMab, ortho-Fab IgG, dual variable domain immunoglobulins (DVD-Ig), 2 in 1-IgG, IgG-scFv, scFv₂-Fc, bi-Nanobody, bispecific T cell engager (BiTE), tandAbs, Dual affinity retargeting (DART), DART-Fc, scFv-HSA-scFv and DNL-Fab3. Provided herein, in various embodiments, are platforms comprising an engineered cell surface protein (e.g., a chimeric antigen receptor) as described herein and a bispecific antibody. Further provided herein, in various embodiments, are effector cells comprising an engineered cell surface protein (e.g., a chimeric antigen receptor) as described herein and a bispecific antibody. In some embodiments, the bispecific antibody is an anti-angiopoietin2/anti-VEGF bispecific antibody. In some cases, the anti-angiopoietin2/anti-VEGF bispecific antibody is RG7221. In some cases, the anti-angiopoietin2/anti-VEGF bispecific antibody is RG7716. In some embodiments, the bispecific antibody is an anti-FXI/anti-FX bispecific antibody. In some cases, the anti-FXI/anti-FX bispecific antibody is RG6013. In some embodiments, the bispecific antibody is an anti-Her1/anti-Her3 bispecific antibody. In some cases, the anti-Her1/anti-Her3 bispecific antibody is RG7597. In some embodiments, the bispecific antibody is an anti-Her2/anti-Her3 bispecific antibody. In some cases, the anti-Her2/anti-Her3 bispecific antibody is MM1 11. In some embodiments, the bispecific antibody is an anti-IGFIR/anti-Her3 bispecific antibody. In some cases, the anti-IGFIR/anti-Her3 bispecific antibody is MM 141. In some embodiments, the bispecific antibody is an anti-TNFalpha/anti-IL17 bispecific antibody. In some cases, the anti-TNFalpha/anti-IL17 bispecific antibody is ABT122. In some embodiments, the bispecific antibody is an anti-IL1a/anti-IL1b bispecific antibody. In some cases, the anti-IL1a/anti-IL1b bispecific antibody is ABT981. In some embodiments, the bispecific antibody targets two beta amyloid epitopes. In some cases, the bispecific antibody that targets two beta amyloid epitopes is BI1034020. In some embodiments, the bispecific antibody is an anti-IL17A/anti-IL17F bispecific antibody. In some cases, the anti-IL17A/anti-IL17F bispecific antibody is ALX0761. In some embodiments, the bispecific antibody is an anti-IL4/anti-IL13 bispecific antibody. In some cases, the anti-IL4/anti-IL13 bispecific antibody is SAR156597. In some embodiments, the bispecific antibody is an anti-CEA/anti-hapten bispecific antibody. In some cases, the anti-CEA/anti-hapten bispecific antibody is TF2. In some embodiments, the bispecific antibody is an anti-IL23/anti-IL17 bispecific
antibody. In some cases, the anti-IL23/anti-IL17 bispecific antibody is IL-17/IL-34 biAb. In some embodiments, the bispecific antibody is an anti-CD30/anti-CD16 bispecific antibody. In some cases, the anti-CD30/anti-CD16 bispecific antibody is AFM13. In some embodiments, the bispecific antibody is an anti-Herl/anti-cMET bispecific antibody. In some cases, the anti-Herl/anti-cMET bispecific antibody is LY3164530.

[0106] Provided herein, in various embodiments, are platforms comprising a chimeric antigen receptor as described herein and an anti-CD3 bispecific antibody. Further provided herein, in various embodiments, are effector cells comprising a chimeric antigen receptor as described herein and an anti-CD3 bispecific antibody. In some embodiments, the anti-CD3 bispecific antibody is an anti-CD3/anti-CD19 bispecific antibody. In some cases, the anti-CD3/anti-CD19 bispecific antibody is blinatumomab (AMG103, MT103). In some cases, the anti-CD3/anti-CD19 bispecific antibody is AFM11. In some embodiments, the anti-CD3 bispecific antibody is an anti-CD3/anti-EpCAM bispecific antibody. In some cases, the anti-CD3/anti-EpCAM bispecific antibody is catumaxomab. In some cases, the anti-CD3/anti-EpCAM bispecific antibody is MT110 (AMB 110). In some embodiments, the anti-CD3 bispecific antibody is an anti-CD3/anti-CD20 bispecific antibody. In some cases, the anti-CD3/anti-CD20 bispecific antibody is lymphomun FBTA05. In some embodiments, the anti-CD3 bispecific antibody is an anti-CD3/anti-Her2 bispecific antibody. In some cases, the anti-CD3/Her2 bispecific antibody is ertumaxomab. In some embodiments, the anti-CD3 bispecific antibody is an anti-CD3/anti-CEA bispecific antibody. In some cases, the anti-CD3/anti-CEA bispecific antibody is MT111. In some embodiments, the anti-CD3 bispecific antibody is an anti-CD3/anti-PSMA bispecific antibody. In some cases, the anti-CD3/anti-PSMA bispecific antibody is MT112 (BAY20101 12). In some embodiments, the anti-CD3 bispecific antibody is an anti-CD3/anti-CD123 bispecific antibody. In some cases, the anti-CD3/anti-CD123 bispecific antibody is MGD006. In some embodiments, the anti-CD3 bispecific antibody is an anti-CD3/anti-GPA33 bispecific antibody. In some cases, the anti-CD3/anti-GPA33 bispecific antibody is MGD007.

[0107] The bispecific antibody may be an IgG fusion, an Fc fusion or a Fab fusion. The bispecific antibody may comprise a heterodimeric IgG. The heterodimeric IgG may be selected from a quadroma triomab, an in vitro assembled knobs-into holes antibody, a common light chain, a crossMab, (SEED) body or LUZ-Y. The bispecific antibody may comprise a Fab, Fab₂, Fab₃, Bis-scFv, minibody (bivalent), scFv, triabody (trivalent), diabody or a tetrabody. The bispecific antibody may comprise a tandem scFv BiTE, a dual targeting antibody, a triomab, a humanized IgG, a
nanobody, a tetravalent bispecific tandem antibody, a TCR-scFv, a TandAb, an IgGl, an IgG2 or a DVD-Ig™.

IV. Target Cells

[0108] Disclosed herein are methods and platforms comprising CAR-ECs and CAR-EC switches that interact with a target. The target may be a target cell. The target cell may comprise a cell from a subject suffering from a disease or condition. The target cell may comprise an infected cell. The target cell may comprise a pathogenically infected cell. The target cell may comprise 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, fungus). The target may be one or more cells. The target may be a virus or a portion thereof. The target may be one or more dividing cells. The target may be a fragment of a cell.

[0109] The target may be an antigen on a target cell. The antigen may be at least a portion of a surface antigen or a cell surface marker on a target cell. The antigen may be a receptor or a co-receptor on a target cell. Generally, binding of the T cell and the target cell to the CAR-EC switch construct brings the target cell into a proximity with the T cell that is sufficiently close for an activity of the T cell to have an effect on the target cell. For example, when the T cell and the target cell are bound to the CAR-EC switch, the T cell may release cytokines that bind to cytokine receptors on the target cell.

[0110] The target cell may be selected from a stem cell, a cancer stem cell, a pluripotent cell, a hematopoietic stem cell or an endothelial progenitor cell. 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. Alternatively, or additionally, 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.

[0111] The target cell may comprise a cancerous cell. The cancerous 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 and skin. The cancerous cell may be derived from bone. The cancerous cell may be derived from blood. The cancerous 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 cancerous cell may be derived from a CD19-positive B lymphocyte. The cancerous cell may be derived from a stem cell. The cancerous cell may be derived from a pluripotent cell. The cancerous 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.

V. CAR-EC platform

[0112] Disclosed herein are chimeric antigen receptor effector cell (CAR-EC) platforms comprising an effector cell expressing a chimeric antigen receptor (CAR), wherein the CAR comprises chimeric antigen receptor comprising an extracellular domain that interacts with an anti-CD3 antibody or fragment thereof; a transmembrane domain; and an intracellular domain, wherein at least a portion of the transmembrane domain or at least a portion of the intracellular domain is not based on or derived from a CD3 protein. The CAR-EC platform may 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.

[0113] The CAR-EC may express a CAR, wherein the extracellular domain may comprise an extracellular domain of CD3 or a fragment thereof. The extracellular domain may comprise an epsilon domain of CD3 or a fragment thereof. A chimeric antigen receptor comprising an extracellular domain that interacts with an anti-CD3 antibody or fragment thereof. The extracellular domain may comprise a sequence selected from any of SEQ ID NOs: 1-3. The extracellular domain may consist essentially of a sequence selected from any of SEQ ID NOs: 1-3. The extracellular domain may comprise at least about 5 consecutive amino acids of SEQ ID NO. 1. The extracellular domain may comprise at least about 20, 25, 27, 30, 35 or 50 consecutive amino acids of SEQ ID NO. 1. The extracellular domain may comprise a sequence that is at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% homologous or identical to a sequence selected from any of SEQ ID NOs: 1-3. The extracellular domain may be encoded by a sequence selected from any of SEQ ID NOs: 16-18. The extracellular domain may be encoded by a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98% or 99% identical to a sequence selected from any of SEQ ID NOs: 16-18. The extracellular domain may not comprise a CD3 zeta chain signaling domain selected form ITAM1, ITAM2 and ITAM.

[0114] The CAR-EC may express a CAR, wherein the transmembrane domain may comprise a transmembrane domain of CD3 or a fragment thereof. The transmembrane domain may comprise a sequence selected from SEQ ID NO. 4. The transmembrane domain may consist essentially of a sequence selected from SEQ ID NO. 4. The transmembrane domain may comprise at least about 5
consecutive amino acids of SEQ ID NO. 4. The transmembrane domain may comprise at least about 10 consecutive amino acids of SEQ ID NO. 4. The transmembrane domain may comprise a sequence that is at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence selected from SEQ ID NO. 4. The transmembrane domain may be encoded by a sequence selected from SEQ ID NO. 19. The transmembrane domain may be encoded by a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98% or 99% identical to a sequence selected from SEQ ID NO. 19.

[0115] The CAR-EC may express a CAR, wherein the intracellular domain may comprise an intracellular costimulatory domain of 41BB, CD28, ICOS, OX40, CD27, CD30, CD150, DAP-10, NKG2D or a combination or fragment thereof. The CAR-EC may express a CAR, wherein the intracellular domain may comprise one or more costimulatory domains. The CAR-EC may express a CAR, wherein the intracellular domain comprises a CD3 zeta domain or a fragment thereof. The CAR-EC may express a CAR, wherein the intracellular domain comprises a CD3 zeta domain without an ITAM1, ITAM2, and/or ITAM3 domain. The intracellular domain may comprise a sequence selected from any of SEQ ID NOs: 5-15. The intracellular domain may consist essentially of a sequence selected from any of SEQ ID NOs: 5-15. The intracellular domain may comprise at least about 5 consecutive amino acids from any of SEQ ID NOs: 5-15. The intracellular domain may comprise at least about 10 consecutive amino acids from any of SEQ ID NOs: 5-15. The intracellular domain may comprise a sequence that is at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence selected from any of SEQ ID NOs: 5-15. The intracellular domain may be encoded by a sequence selected from any of SEQ ID NOs: 20-30. The intracellular domain may be encoded by a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98% or 99% identical to a sequence selected from any of SEQ ID NOs: 20-30.

[0116] The CAR-EC may express a CAR comprising a sequence selected from any of SEQ ID NOs: 31-35. The CAR-EC may express a CAR comprising at least about 20, 50, 75, 100, 125, or 150 consecutive amino acids of any of SEQ ID NOs: 31-35, wherein the amino acid sequence may differ by 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid residues anywhere within the consecutive amino acid sequence. The CAR-EC may express a CAR comprising an amino acid sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to an amino acid sequence of any of SEQ ID NOs: 31-35. The CAR-EC may express a CAR encoded by a sequence selected from any of SEQ ID NOs: 36-40. The CAR-EC may express a CAR encoded by a sequence comprising at least about 20, 50, 75, 100, 125, or 150 consecutive nucleic acids of any of SEQ ID NOs: 36-40, wherein the nucleic acid sequence may differ by 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleic acid residues anywhere within the consecutive nucleic acid sequence. The CAR-EC may express a CAR encoded by a
sequence comprising a nucleic acid sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a nucleic acid sequence from any of SEQ ID NOs: 36-40.

The CAR-EC platform may comprise a CAR-EC switch, wherein the targeting region of the CAR-EC switch may be selected from a peptide, an antibody, an antibody fragment and a small molecule. The targeting region may bind a cell surface antigen on a cell. The cell surface antigen may be a tumor associated antigen. The cell may be a cancer cell.

The CAR-EC platform may comprise a plurality of CAR-EC switches, wherein at least one of the CAR-EC switches comprises an anti-CD3 antibody. The plurality of CAR-EC switches may comprise two or more CAR-EC switches. The plurality of CAR-EC switches may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more CAR-EC switches. The plurality of CAR-EC switches 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 CAR-EC switches may be selected from one or more CAR-EC switches disclosed herein or a combination thereof.

The CAR-EC platforms disclosed herein may further comprise two or more CAR-EC switches. Two or more CAR-EC switches may comprise two or more identical CAR binding regions. Two or more CAR-EC switches may comprise two or more different CAR binding regions. Two or more CAR-EC switches may comprise two or more similar CAR binding regions. The two or more CAR binding regions may comprise similar amino acid sequences. The amino acid sequences of the two or more CAR binding regions 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% identical. The amino acid sequences of the two or more CAR binding regions may be about 75% identical.

Two or more CAR-EC switches may comprise two or more different targeting regions. Two or more CAR-EC switches may comprise two or more identical targeting regions. The two or more CAR-EC switches may comprise two or more similar targeting regions. The two or more targeting regions may comprise similar amino acid sequences. The amino acid sequences of the two or more targeting regions 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% identical. The amino acid sequences of the two or more targeting regions may be about 75% identical.
VI. Kits, Vectors and Polynucleotides

[0121] Disclosed herein are kits comprising: (a) an effector cell expressing an engineered cell surface protein comprising an extracellular domain comprising a sequence of an extracellular portion of a cell surface protein, a transmembrane domain, and an intracellular domain comprising a sequence of an intracellular signaling protein; wherein the engineered cell surface protein is engineered by combining sequences from at least two different protein sources; and (b) a bispecific antibody comprising an antigen binding domain that binds to the extracellular domain of the engineered cell surface protein. In some embodiments, a first protein source is the cell surface protein and a second protein source is the intracellular signaling protein. In some embodiments, the extracellular domain does not comprise an antibody or fragment thereof. In some embodiments, the intracellular signaling protein is a costimulatory domain. In some embodiments, the costimulatory domain comprises a 41BB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a portion or combination thereof. In some embodiments, the intracellular domain further comprises a T cell receptor zeta chain. In some embodiments, the intracellular signaling protein is a T cell receptor zeta chain. In some embodiments, the T cell receptor zeta chain does not comprise an ITAMI domain, ITAM2 domain, ITAM3 domain, or a portion or combination thereof. In some embodiments, the cell surface protein is CD3 epsilon. In some embodiments, the extracellular domain comprises a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence selected from any of SEQ ID NOs: 1-3. In some embodiments, the extracellular domain comprises at least about 5 consecutive amino acids of SEQ ID NO. 1. In some embodiments, the extracellular domain comprises at least about 20, 25, 27, 30, 35 or 50 consecutive amino acids of SEQ ID NO. 1. In some embodiments, the consecutive amino acids of the extracellular domain derived from SEQ ID NO. 1 differ from a sequence of SEQ ID NO. 1 by less than about 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acids. In some embodiments, the transmembrane domain comprises a sequence from SEQ ID NO. 4. In some embodiments, the transmembrane domain comprises a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to SEQ ID NO. 4. In some embodiments, the intracellular domain comprises one or more sequences selected from any of SEQ ID NOs: 5-15. In some embodiments, the intracellular domain comprises a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence selected from any of SEQ ID NOs: 5-15. In some embodiments, the intracellular domain comprises at least about 20, 25, 27, 30, 35 or 50 consecutive amino acids from any of SEQ ID NOs: 5-15. In some embodiments, the consecutive
amino acids of the intracellular domain derived from any of SEQ ID NOs: 5-15 differ from a sequence of SEQ ID NOs: 5-15 by less than about 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acids. In some embodiments, the effector cell is a T cell. In some embodiments, the effector cell is a tumor-infiltrating lymphocyte. In some embodiments, the bispecific antibody comprises an antigen binding domain that binds to an antigen on a target cell. In some embodiments, the target cell is a tumor cell.

[0122] Disclosed herein are polynucleotides and vectors encoding one or more chimeric antigen receptors, wherein the chimeric antigen receptor comprises an extracellular domain that interacts with an anti-CD3 antibody or fragment thereof; a transmembrane domain; and an intracellular domain, wherein at least a portion of the transmembrane domain or at least a portion of the intracellular domain is not based on or derived from a CD3 protein. The extracellular domain may comprise an extracellular domain of CD3 or a fragment thereof. The extracellular domain may comprise an epsilon domain of CD3 or a fragment thereof. A chimeric antigen receptor comprising an extracellular domain that interacts with an anti-CD3 antibody or fragment thereof. The extracellular domain may comprise a sequence selected from any of SEQ ID NOs: 1-3. The extracellular domain may consist essentially of a sequence selected from any of SEQ ID NOs: 1-3. The extracellular domain may comprise at least about 5 consecutive amino acids of SEQ ID NO. 1. The extracellular domain may comprise at least about 20, 25, 27, 30, 40 or 50 consecutive amino acids of SEQ ID NO. 1. The extracellular domain may comprise a sequence that is at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% homologous or identical to a sequence selected from any of SEQ ID NOs: 1-3. The extracellular domain may be encoded by a sequence selected from any of SEQ ID NOs: 9-11. The extracellular domain may be encoded by a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98% or 99% identical to a sequence selected from any of SEQ ID NOs: 16-18. The extracellular domain may not comprise a CD3 zeta chain signaling domain selected from ITAM1, ITAM2 and ITAM. The transmembrane domain may comprise a sequence selected from SEQ ID NO. 4. The transmembrane domain may consist essentially of a sequence selected from SEQ ID NO. 4. The transmembrane domain may comprise at least about 5 consecutive amino acids of SEQ ID NO. 4. The transmembrane domain may comprise at least about 10 consecutive amino acids of SEQ ID NO. 4. The transmembrane domain may comprise a sequence that is at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence selected from SEQ ID NO. 4. The transmembrane domain may be encoded by a sequence selected from SEQ ID NO. 19. The transmembrane domain may be encoded by a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98% or 99% identical to a sequence selected from SEQ ID NO. 19. The intracellular domain may comprise an intracellular costimulatory domain of 41BB, CD28, ICOS, OX40, CD27,
CD30, CD150, DAP-10, NKG2D or a combination or fragment thereof. The intracellular domain may comprise one or more costimulatory domains. The intracellular domain may comprise a CD3 zeta domain or a fragment thereof. The intracellular domain may comprise a CD3 zeta domain without an ITAM1, ITAM2, and/or ITAM3 domain. The intracellular domain may comprise a sequence selected from any of SEQ ID NOs: 5-15. The intracellular domain may consist essentially of a sequence selected from any of SEQ ID NOs: 5-15. The intracellular domain may comprise at least about 5 consecutive amino acids of any of SEQ ID NOs: 5-15. The intracellular domain may comprise at least about 10 consecutive amino acids of any of SEQ ID NOs: 5-15. The intracellular domain may comprise a sequence that is at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence selected from any of SEQ ID NOs: 5-15. The intracellular domain may be encoded by a sequence selected from any of SEQ ID NOs: 20-30. The intracellular domain may be encoded by a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98% or 99% identical to a sequence selected from any of SEQ ID NOs: 20-30. The vector may express other peptides or proteins, including, but not limited to peptide tags, fluorescent proteins, cell surface markers and drug resistance genes.

[0123] Disclosed herein are kits comprising one or more chimeric antigen receptors, wherein the chimeric antigen receptor comprises an extracellular domain that interacts with an anti-CD3 antibody or fragment thereof; a transmembrane domain; and an intracellular domain, wherein at least a portion of the transmembrane domain or at least a portion of the intracellular domain is not based on or derived from a CD3 protein. The extracellular domain may comprise an extracellular domain of CD3 or a fragment thereof. The extracellular domain may comprise an epsilon domain of CD3 or a fragment thereof. A chimeric antigen receptor comprising an extracellular domain that interacts with an anti-CD3 antibody or fragment thereof. The extracellular domain may comprise a sequence selected from any of SEQ ID NOs: 1-3. The extracellular domain may consist essentially of a sequence selected from any of SEQ ID NOs: 1-3. The extracellular domain may comprise at least about 5 consecutive amino acids of SEQ ID NO. 1. The extracellular domain may comprise at least about 20, 25, 27, 30, 40 or 50 consecutive amino acids of SEQ ID NO. 1. The extracellular domain may comprise a sequence that is at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% homologous or identical to a sequence selected from any of SEQ ID NOs: 1-3. The extracellular domain may be encoded by a sequence selected from any of SEQ ID NOs: 16-18. The extracellular domain may be encoded by a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98% or 99% identical to a sequence selected from any of SEQ ID NOs: 16-18. The extracellular domain may not comprise a CD3 zeta chain signaling domain selected form ITAMI, ITAM2 and ITAM. The
transmembrane domain may comprise a sequence selected from SEQ ID NO. 4. The transmembrane domain may consist essentially of a sequence selected from SEQ ID NO. 4. The transmembrane domain may comprise at least about 5 consecutive amino acids of SEQ ID NO. 4. The transmembrane domain may comprise at least about 10 consecutive amino acids of SEQ ID NO. 4. The transmembrane domain may comprise a sequence that is at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence selected from SEQ ID NO. 4. The transmembrane domain may be encoded by a sequence selected from SEQ ID NO. 19. The transmembrane domain may be encoded by a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98% or 99% identical to a sequence selected from SEQ ID NO. 19. The intracellular domain may comprise an intracellular costimulatory domain of 41BB, CD28, ICOS, OX40, CD27, CD30, CD150, DAP-10, NKG2D or a combination or fragment thereof. The intracellular domain may comprise one or more costimulatory domains. The intracellular domain may comprise a CD3 zeta domain or a fragment thereof. The intracellular domain may comprise a CD3 zeta domain without an ITAM1, ITAM2, and/or ITAM3 domain. The intracellular domain may comprise a sequence selected from any of SEQ ID NOs: 5-15. The intracellular domain may consist essentially of a sequence selected from any of SEQ ID NOs: 5-15. The intracellular domain may comprise at least about 5 consecutive amino acids from any of SEQ ID NOs: 5-15. The intracellular domain may comprise at least about 10 consecutive amino acids from any of SEQ ID NOs: 5-15. The intracellular domain may comprise a sequence that is at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence selected from any of SEQ ID NOs: 5-15. The intracellular domain may be encoded by a sequence selected from any of SEQ ID NOs: 20-30. The intracellular domain may be encoded by a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98% or 99% identical to a sequence selected from any of SEQ ID NOs: 20-30. The kit may comprise one or more effector cells. The effector cell may be a T cell. The effector cell may express one or more CARs disclosed herein. The kit may further comprise one or more 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.

**VII. Indications**

[0124] Disclosed herein are uses of CARs comprising an extracellular domain, a transmembrane domain; and an intracellular domain, wherein at least a portion of the transmembrane domain or at least a portion of the intracellular domain is not based on or derived from a CD3 protein; and wherein the CAR associates with a T cell receptor (TCR), wherein the TCR comprises a TCR extracellular domain that interacts with an antigen on a target cell. The extracellular domain may be a CD3 epsilon domain or portion thereof.
Further disclosed herein are uses of CARs comprising an extracellular domain that interacts with an anti-CD3 antibody and CAR-EC switches comprising an anti-CD3 antibody and a targeting region to treat a disease or condition in a subject in need thereof. The extracellular domain may comprise an extracellular domain of CD3 or a fragment thereof. The extracellular domain may comprise an epsilon domain of CD3 or a fragment thereof. A chimeric antigen receptor comprising an extracellular domain that interacts with an anti-CD3 antibody or fragment thereof. The extracellular domain may comprise a sequence selected from any of SEQ ID NOs: 1-3. The extracellular domain may consist essentially of a sequence selected from any of SEQ ID NOs: 1-3. The extracellular domain may comprise at least 5 consecutive amino acids of SEQ ID NO. 1. The extracellular domain may comprise at least 27 consecutive amino acids of SEQ ID NO. 1. The extracellular domain may comprise a sequence that is at least 50%, 60%, 70%>, 80%>, 90%>, 95%, 98%, or 99% homologous or identical to a sequence selected from any of SEQ ID NOs: 1-3. The extracellular domain may be encoded by a sequence selected from any of SEQ ID NOs: 16-18. The extracellular domain may be encoded by a sequence that is at least about 50%>, 60%>, 70%>, 80%>, 90%, 95%, 98% or 99% identical to a sequence selected from any of SEQ ID NOs: 16-18. The extracellular domain may not comprise a CD3 zeta chain signaling domain selected form ITAM1, ITAM2 and ITAM. The transmembrane domain may comprise a sequence selected from SEQ ID NO. 4. The transmembrane domain may consist essentially of a sequence selected from SEQ ID NO. 4. The transmembrane domain may comprise at least about 5 consecutive amino acids of SEQ ID NO. 4. The transmembrane domain may comprise at least about 10 consecutive amino acids of SEQ ID NO. 4. The transmembrane domain may comprise a sequence that is at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence selected from SEQ ID NO. 4. The transmembrane domain may be encoded by a sequence selected from SEQ ID NO. 19. The transmembrane domain may be encoded by a sequence that is at least about 50%>, 60%>, 70%>, 80%>, 90%, 95%, 98% or 99% identical to a sequence selected from SEQ ID NO. 19. The intracellular domain may comprise an intracellular costimulatory domain of 4BB, CD28, ICOS, OX40, CD27, CD30, CD150, DAP-10, NKG2D or a combination or fragment thereof. The intracellular domain may comprise one or more costimulatory domains. The intracellular domain may comprise a CD3 zeta domain or a fragment thereof. The intracellular domain may comprise a CD3 zeta domain without an ITAM1, ITAM2, and/or ITAM3 domain. The intracellular domain may comprise a sequence selected from any of SEQ ID NOs: 5-15. The intracellular domain may consist essentially of a sequence selected from any of SEQ ID NOs: 5-15. The intracellular domain may comprise at least about 5 consecutive amino acids from any of SEQ ID NOs: 5-15. The intracellular domain may
comprise at least about 10 consecutive amino acids from any of SEQ ID NOs: 5-15. The intracellular
domain may comprise a sequence that is at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence selected from any of SEQ ID NOs: 5-15. The intracellular domain may be encoded by a sequence selected from any of SEQ ID NOs: 20-30. The intracellular domain may be encoded by a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98% or 99% identical to a sequence selected from any of SEQ ID NOs: 20-30.

[0126] Further disclosed herein are uses of CARs comprising an extracellular domain that interacts with an anti-CD3 antibody and CAR-EC switches comprising an anti-CD3 antibody and a targeting region in the manufacture of a medicament for the treatment of a disease. The extracellular domain may comprise an extracellular domain of CD3 or a fragment thereof. The extracellular domain may comprise an epsilon domain of CD3 or a fragment thereof. A chimeric antigen receptor comprising an extracellular domain that interacts with an anti-CD3 antibody or fragment thereof. The extracellular domain may comprise a sequence selected from any of SEQ ID NOs: 1-3. The extracellular domain may consist essentially of a sequence selected from any of SEQ ID NOs: 1-3. The extracellular domain may comprise at least about 5 consecutive amino acids of SEQ ID NO. 1. The extracellular domain may comprise at least about 20, 25, 27, 30, 35 or 50 consecutive amino acids of SEQ ID NO. 1. The extracellular domain may comprise a sequence that is at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% homologous to a sequence selected from any of SEQ ID NOs: 1-3. The extracellular domain may be encoded by a sequence selected from any of SEQ ID NOs: 16-18. The extracellular domain may be encoded by a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98% or 99% identical to a sequence selected from any of SEQ ID NOs: 16-18. The extracellular domain may not comprise a CD3 zeta chain signaling domain selected form ITAM1, ITAM2 and ITAM. The transmembrane domain may comprise a sequence selected from SEQ ID NO. 4. The transmembrane domain may consist essentially of a sequence selected from SEQ ID NO. 4. The transmembrane domain may comprise at least about 5 consecutive amino acids of SEQ ID NO. 4. The transmembrane domain may comprise at least about 10 consecutive amino acids of SEQ ID NO. 4. The transmembrane domain may comprise a sequence that is at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence selected from SEQ ID NO. 4. The transmembrane domain may be encoded by a sequence selected from SEQ ID NO. 19. The transmembrane domain may be encoded by a sequence that is at least about 50%>, 60%>, 70%>, 80%>, 90%, 95%, 98% or 99% identical to a sequence selected from SEQ ID NO. 19. The intracellular domain may comprise an intracellular costimulatory domain of 41BB, CD28, ICOS, OX40, CD27, CD30, CD150, DAP-10, NKG2D or a combination or fragment thereof. The intracellular domain
may comprise one or more costimulatory domains. The intracellular domain may comprise a CD3 zeta domain or a fragment thereof. The intracellular domain may comprise a CD3 zeta domain without an ITAM1, ITAM2, and/or ITAM3 domain. The intracellular domain may comprise a sequence selected from any of SEQ ID NOs: 5-15. The intracellular domain may consist essentially of a sequence selected from any of SEQ ID NOs: 5-15. The intracellular domain may comprise at least about 5 consecutive amino acids from any of SEQ ID NOs: 5-15. The intracellular domain may comprise at least about 10 consecutive amino acids from any of SEQ ID NOs: 5-15. The intracellular domain may comprise a sequence that is at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence selected from any of SEQ ID NOs: 5-15. The intracellular domain may be encoded by a sequence selected from any of SEQ ID NOs: 20-30. The intracellular domain may be encoded by a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98% or 99% identical to a sequence selected from any of SEQ ID NOs: 20-30.

[0127] 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, myelodysplasia 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. The cell proliferative disorder may comprise a CD19-positive Burkitt's lymphoma.

[0128] The disease or condition may be a cancer, a pathogenic infection, autoimmune disease, inflammatory disease, or genetic disorder.

[0129] 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.
[0130] 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.

[0131] The cancer may comprise a prostate cancer. The prostate cancer may be a PSMA-positive prostate cancer. PSMA expression may be highly upregulated and restricted to cancer cells in some or all stages of the prostate cancer. The cancer may be hormone-refractory prostate cancer.

[0132] 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.

[0133] The cancer may comprise a skin cancer. The skin cancer may comprise a neo-angiogenic skin cancer. The skin cancer may comprise a melanoma.

[0134] The cancer may comprise a kidney cancer.


[0136] The cancer may comprise a colorectal cancer. The cancer may comprise a gastric cancer. The cancer may comprise a colon cancer.

[0137] 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.

[0138] 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.

[0139] The cancer may comprise a sarcoma. The sarcoma may comprise an Ewing's sarcoma.

[0140] 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).

[0141] 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.

[0142] 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 mesothelioma. 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.

[0143] 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 astrocitoma 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 oligodendrogliomas. Nongliomas include meningiomas, pituitary adenomas, primary CNS lymphomas, and medulloblastomas. In some instances, the cancer is a meningioma.

[0144] 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.

[0145] 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, Waldenstrom 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.

[0146] 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, a prostate cancer and a PSMA-positive 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.

[0147] 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+.

[0148] 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. 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.

[0150] 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.

[0151] 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.
[0152] 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. bienneusi), 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).

[0153] 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.

[0154] The disease or condition may be an alloimmune reaction or response. The alloimmune response may be caused by a transplant of a tissue from a donor to the subject. The tissue may be selected from a cell sample, a blood sample, a bone marrow sample and an organ or portion thereof. The disease or condition may be a graft versus host disease (GVHD). The GVHD may be acute. The GVHD may be chronic. The condition may be a hematopoietic stem cell transplantation (HSCT). The condition may be a post-HSCT response/reaction.

[0155] The disease or condition may be an inflammatory disease. Examples of inflammatory diseases include, but are not limited to, alveolitis, amyloidosis, angitis, 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, spondyloarthropathies, Still's Disease,
systemic lupus erythematosus, Takayasu's (Pulseless) Disease, Tendinitis, tennis elbow/golf elbow,
thyroid associated arthritis, trigger finger, ulcerative colitis, Wegener's Granulomatosis, and
Whipple's Disease.

[0156] 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 lymphopenia,
fatal cytolysis of solid tumor targets and chronic hypogammaglobulinemia for hematological targets.
Methods of treatment and compositions disclosed herein may be used to treat a cancer comprising
CD19-mediated B cell aplasia. The methods and compositions may minimize the CD19-mediated B
cell aplasia. The method may avoid long-term B-cell aplasia.

[0157] The CAR-EC platforms, methods and kits disclosed herein may be used to treat a
heterogeneous tumor or a heterogeneous blood cell malignancy in a subject in need thereof. The
"pan-B cell" marker CD20 is the most prevalently targeted antigen for B cell neoplasms and the
FDA-approved antibody rituximab is a vital component in the treatment of many leukemias and
lymphomas. However, resistance mechanisms related to modulation of CD20 antigen expression
occurs in a significant number of patients. The methods disclosed herein provide administration of
two or more switches with different specificities (e.g., an anti-CD3/anti-CD19 bispecific antibody
CAR-EC switch and an anti-CD3/anti-CD20 antibody CAR-EC switch). One or more CAR-EC
switches may be administered sequentially or simultaneously.
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 antimitobolite, 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, capcetabine, Carmustine, Chlorambucil, cisplatin, cladribine, clofarabine, cyclophosphamide, cytarabine, dacarbazine, daunorubicin, docetaxel, doxorubicin, epirubicin, etoposide, estramustine, flouxuridine, fludarabine, fluorouracil, gemcitbine (Gemzar), hydroxyurea, idarubicin, ifosfamide, irinotecan (Camptosar), ixabepilone, L-asparaginase, lomustine, mechloethamine, melphalan, 6-mercaptopurine, methotrexate, mitomycin-C, paclitaxel (Taxol), pemtrexed, pentostatin, streptozocin, temozolomide, teniposide, thioguanine, thiopeta, totopetan (Hycamitn), vincristine, vinblastine, vinorelbine, retinoids, tretinoin (ATRA or Atralin®), bexarotene (Targretn®) 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.

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, angiotatin, endostatin, sorafenib, sunitinib, pazopanib, everolimus, 2-methoxyestriadiol, tegocalan, tetrathiomolybdate, thalidomide, prolactin, αβ3 inhibitor, linomide, tasquimod, soluble VEGFR-1, soluble NRP-1, angiopoietin 2, vasoostatin, 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, osteoponint, maspin, canstatin, proliferin-related protein and restin.

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) antagonist or analog (e.g., leuprolide (Lupron®), goserelin (Zoladex®)).
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.

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.

VIII. Therapeutic Use

Disclosed herein are methods for treating a condition comprising administering to a subject in need thereof: (a) an effector cell that expresses an engineered cell surface protein, the engineered cell surface protein comprising: an extracellular domain comprising a sequence of an extracellular portion of a cell surface protein, a transmembrane domain, and an intracellular domain comprising a sequence of an intracellular signaling protein; wherein the engineered cell surface protein is engineered by combining sequences from at least two different protein sources; and (b) a bispecific antibody comprising: a first antigen binding region that binds to the extracellular domain of the engineered cell surface protein, and a second antigen binding region that binds to an antigen on a target cell. In some embodiments, a first protein source is the cell surface protein and a second protein source is the intracellular signaling protein. In some embodiments, the extracellular domain does not comprise an antibody or fragment thereof. In some embodiments, the intracellular signaling protein is a costimulatory domain. In some embodiments, the costimulatory domain comprises a 41BB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a portion or combination thereof. In some embodiments, the intracellular domain further comprises a T cell receptor zeta chain. In some embodiments, the intracellular signaling protein is a T cell receptor zeta chain. In some embodiments, the cell surface protein is CD3 epsilon. In some embodiments, the effector cell is selected from a T cell, an effector B cell, a natural killer cell, a macrophage and a progenitor thereof. In some embodiments, the effector cell is a T cell 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. In some embodiments, the effector cell is a tumor-infiltrating lymphocyte. In some embodiments, the target cell is a tumor cell. In some embodiments, the condition is a cancer. In some embodiments, the effector cell and bispecific antibody are administered simultaneously. In some embodiments, the effector cell and bispecific antibody are administered sequentially. In some
embodiments, the extracellular domain comprises a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence selected from any of SEQ ID Nos: 1-3. In some embodiments, the extracellular domain comprises at least about 5 consecutive amino acids of SEQ ID NO. 1. In some embodiments, the extracellular domain comprises at least about 20, 25, 27, 30, 35 or 50 consecutive amino acids of SEQ ID NO. 1. In some embodiments, the consecutive amino acids of the extracellular domain derived from SEQ ID NO. 1 differ from a sequence of SEQ ID NO. 1 by less than about 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acids. In some embodiments, the transmembrane domain comprises a sequence from SEQ ID NO. 4. In some embodiments, the transmembrane domain comprises a sequence that is at least about 50%>, 60%>, 70%>, 80%>, 90%>, 95%, 98%, or 99% identical to SEQ ID NO. 4. In some embodiments, the intracellular domain comprises one or more sequences selected from any of SEQ ID NOs: 5-15. In some embodiments, the intracellular domain comprises a sequence that is at least about 50%>, 60%>, 70%>, 80%>, 90%>, 95%, 98%, or 99%, identical to a sequence selected from any of SEQ ID NOs: 5-15. In some embodiments, the intracellular domain comprises at least about 20, 25, 27, 30, 35 or 50 consecutive amino acids from any of SEQ ID NOs: 5-15. In some embodiments, the consecutive amino acids of the intracellular domain derived from any of SEQ ID NOs: 5-15 differ from a sequence of SEQ ID NOs: 5-15 by less than about 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acids.

[0164] Disclosed herein are methods of treating a disease or condition in a subject in need thereof, the method comprising administering a chimeric antigen receptor comprising an extracellular domain that interacts with an anti-CD3 antibody and a chimeric antigen receptor effector cell (CAR-EC) switch, wherein the CAR-EC switch comprises an anti-CD3 antibody or fragment thereof to the subject. The extracellular domain may comprise an extracellular domain of CD3 or a fragment thereof. The extracellular domain may comprise an epsilon domain of CD3 or a fragment thereof. The extracellular domain may comprise a sequence selected from any of SEQ ID NOs: 1-3. The extracellular domain may consist essentially of a sequence selected from any of SEQ ID NOs: 1-3. The extracellular domain may comprise at least 5 consecutive amino acids of SEQ ID NO. 1. The extracellular domain may comprise at least 27 consecutive amino acids of SEQ ID NO. 1. The extracellular domain may comprise a sequence that is at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99%, homologous or identical to a sequence selected from any of SEQ ID NOs: 1-3. The extracellular domain may be encoded by a sequence selected from SEQ ID NOs: 16-18. The extracellular domain may be encoded by a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98% or 99% identical to a sequence selected from any of SEQ ID NOs: 16-18. The extracellular domain may not comprise a CD3 zeta chain signaling domain selected form ITAM1,
ITAM2 and ITAM. The transmembrane domain may comprise a sequence selected from SEQ ID NO. 4. The transmembrane domain may consist essentially of a sequence selected from SEQ ID NO. 4. The transmembrane domain may comprise at least about 5 consecutive amino acids of SEQ ID NO. 4. The transmembrane domain may comprise at least about 10 consecutive amino acids of SEQ ID NO. 4. The transmembrane domain may comprise a sequence that is at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence selected from SEQ ID NO. 4. The transmembrane domain may be encoded by a sequence selected from SEQ ID NO. 19. The transmembrane domain may be encoded by a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98% or 99% identical to a sequence selected from SEQ ID NO. 19. The intracellular domain may comprise an intracellular costimulatory domain of 41BB, CD28, ICOS, OX40, CD27, CD30, CD150, DAP-10, NKG2D or a combination or fragment thereof. The intracellular domain may comprise one or more costimulatory domains. The intracellular domain may comprise a CD3 zeta domain or a fragment thereof. The intracellular domain may comprise a CD3 zeta domain without an ITAM1, ITAM2, and/or ITAM3 domain. The intracellular domain may comprise a sequence selected from any of SEQ ID NOs: 5-15. The intracellular domain may consist essentially of a sequence selected from any of SEQ ID NOs: 5-15. The intracellular domain may comprise at least about 5 consecutive amino acids from any of SEQ ID NOs: 5-15. The intracellular domain may comprise at least about 10 consecutive amino acids from any of SEQ ID NOs: 5-15. The intracellular domain may comprise a sequence that is at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence selected from any of SEQ ID NOs: 5-15. The intracellular domain may be encoded by a sequence selected from any of SEQ ID NOs: 20-30. The intracellular domain may be encoded by a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98% or 99% identical to a sequence selected from any of SEQ ID NOs: 20-30.

[0165] Further disclosed herein are methods of treating a disease or condition in a subject in need thereof, the method comprising administering a chimeric antigen receptor comprising an extracellular domain that interacts with an anti-CD3 antibody and a chimeric antigen receptor effector cell (CAR-EC) switch, wherein the CAR-EC switch comprises an anti-CD3 antibody or fragment thereof to the subject. The extracellular domain may comprise an extracellular domain of CD3 or a fragment thereof. The extracellular domain may comprise an epsilon domain of CD3 or a fragment thereof. The extracellular domain may comprise a sequence selected from any of SEQ ID NOs: 1-3. The extracellular domain may consist essentially of a sequence selected from any of SEQ ID NOs: 1-3. The extracellular domain may comprise at least 5 consecutive amino acids of SEQ ID NO. 1. The extracellular domain may comprise at least 27 consecutive amino acids of SEQ ID NO.
1. The extracellular domain may comprise a sequence that is at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% homologous to a sequence selected from any of SEQ ID NOs: 1-3. The extracellular domain may be encoded by a sequence selected from any of SEQ ID NOs: 16-18. The extracellular domain may be encoded by a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98% or 99% identical to a sequence selected from any of SEQ ID NOs: 16-18. The extracellular domain may not comprise a CD3 zeta chain signaling domain selected from ITAM1, ITAM2 and ITAM. The transmembrane domain may comprise a sequence selected from SEQ ID NO. 4. The transmembrane domain may consist essentially of a sequence selected from SEQ ID NO. 4. The transmembrane domain may comprise at least about 5 consecutive amino acids of SEQ ID NO. 4. The transmembrane domain may comprise at least about 10 consecutive amino acids of SEQ ID NO. 4. The transmembrane domain may comprise a sequence that is at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence selected from SEQ ID NO. 4. The transmembrane domain may be encoded by a sequence selected from SEQ ID NO. 19. The transmembrane domain may be encoded by a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98% or 99% identical to a sequence selected from SEQ ID NO. 19. The intracellular domain may comprise an intracellular costimulatory domain of 41BB, CD28, ICOS, OX40, CD27, CD30, CD150, DAP-10, NKG2D or a combination or fragment thereof. The intracellular domain may comprise one or more costimulatory domains. The intracellular domain may comprise a CD3 zeta domain or a fragment thereof. The intracellular domain may comprise a CD3 zeta domain without an ITAM1, ITAM2, and/or ITAM3 domain. The intracellular domain may comprise a sequence selected from any of SEQ ID NOs: 5-15. The intracellular domain may consist essentially of a sequence selected from any of SEQ ID NOs: 5-15. The intracellular domain may comprise at least about 5 consecutive amino acids from any of SEQ ID NOs: 5-15. The intracellular domain may comprise at least about 10 consecutive amino acids from any of SEQ ID NOs: 5-15. The intracellular domain may comprise a sequence that is at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence selected from any of SEQ ID NOs: 5-15. The intracellular domain may be encoded by a sequence selected from any of SEQ ID NOs: 20-30. The intracellular domain may be encoded by a sequence that is at least about 50%, 60%, 70%, 80%, 90%, 95%, 98% or 99% identical to a sequence selected from any of SEQ ID NOs: 20-30.

[0166] The methods may comprise administering one or more CAR-EC switches. 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. The methods may comprise administering two or more CAR-EC switches. The two or
more CAR-EC switches may comprise the same CAR binding region. The two more CAR-EC switches may comprise the same cell targeting regions. The two or more CAR-EC switches may comprise the same linkers. The two or more CAR-EC switches may comprise one or more different CAR binding regions. The two more CAR-EC switches may comprise one or more different cell targeting regions.

[0167] 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.

IX. Method of Clearing Effector cells

[0168] Further disclosed herein are methods of clearing CAR-EC cells in a subject, comprising administering a CAR-EC off switch. The CAR-EC off switch may comprise an anti-CD3 antibody or antibody fragment that targets the CAR on the effector cell. The anti-CD3 antibody or antibody fragment may be conjugated to a drug or a toxin. The drug or toxin may be selected from maytansine (e.g., DM1, DM4), monomethylauristatin E, monomethylauristatin F, Ki-4.dgA, dolastatin 10, calicheamicin, SN-38, duocarmycin, irinotecan, ricin, saporin, gelonin, poke weed antiviral protein, pseudomonas aeruginosa exotoxin A or diphtheria toxin. The toxin may comprise a poison, a bacterial toxin (e.g., bacterial toxins causing tetanus, diphtheria), a plant toxin or animal toxin. The toxin may be a snake venom. The toxin may comprise vinblastine. The toxin may comprise auristatin. The toxin may be contained in a liposome membrane-coated vesicle. Wherein the toxin is contained in a liposome membrane-coated vesicle, the antibody is attached to the vesicle.
Alternatively or additionally, the effector cell expresses a viral protein or fragment thereof. The effector cell expressing a viral protein or fragment thereof may be targeted with a drug. Wherein the effector cell comprises a viral protein or fragment thereof, the drug may be selected from a group comprising abacavir, acyclovir, acyclovir, adefovir, amantadine, amprenavir, amiplogen, arbidol, atazanavir, atripla, balavir, boceprevirmetiv, cidofovir, combivir, darunavir, delavirdine, didanosine, docosanol, edoxudine, efavirenz, emtricitabine, enfuvirtide, entecavir, an entry inhibitor, famciclovir, a fixed dose combination antiretroviral drug, fomivirinsen, fosamprenavir, foscarnet, fosfonet, a fusion inhibitor, ganciclovir, ibacitabine, imunovir, idoxuridine, imiquimod, indinavir, inosine, integrase inhibitor, interferon type III, interferon type II, interferon type I, interferon, lamivudine, lopinavir, loviride, maraviroc, moroxydine, methisazone, nelfinavir, nevirapine, nexavir, nucleoside analogue, oseltamivir, peginterferon alfa-2a, penciclovir, peramivir, pleconaril, podophyllotoxin, protease inhibitor, raltegravir, a reverse transcriptase inhibitor, ribavirin, rimantadine, ritonavir, pyramidine, saquinavir, sofosbuvir, stavudine, a synergistic enhancer retroviral drug, tea tree oil, telaprevir, tenofovir, tenofovir disoproxil, tipranavir, trifluridine, trizivir, tronantadine, truvada, valaciclovir, vicriviroc, vidarabine, viramidine, zacitabine, zanamivir or zidovudine. The drug may be ganciclovir. The drug may be acyclovir.

**EXAMPLES**

The following examples are set forth to illustrate more clearly the principle and practice of embodiments disclosed herein to those skilled in the art and are not to be construed as limiting the scope of any claimed embodiments.

*Example 1. Production of chimeric CD3 receptors*

CAR domains (e.g., extracellular CD3 domain, transmembrane domain, and intracellular stimulatory or co-stimulatory domains) are subcloned into a lentiviral vector in a desired order. T cells are transduced with the resulting lentiviral vector and stably expressing cells are selected. In some instances, the T cells may be from a T cell line. The T cells are generally human T cells. In some instances, the T cells are isolated from a human donor. In some instances, the T cells are isolated from a subject with a condition, wherein the subject is to be treated with a CAR-EC platform and/or method disclosed herein.

*Example 2. Cytotoxicity of chimeric CD3 receptor T cells and anti-CD3 bispecific antibodies*

In vitro cytotoxicity assays are carried out wherein target cells are mixed with chimeric CD3 receptor T cells at a 1:10 ratio, and incubated with different concentrations of anti-CD3 bispecific antibodies. Cytotoxicity is determined by detecting lactate dehydrogenase (LDH) from lysed cells.
LDH assay

[0173] Equal volumes of target cells and effector cells are mixed together to obtain an effector:target ratio of 10:1, and dispensed in 100 µL volumes in a 96-well round-bottom plate. The effector-target mixtures are incubated with different concentrations of BiFabs and unconjugated Fabs single or mixed (ranging from 10 nM down to 50 fM) for 24 hours at 37 °C. Cytotoxicity of each well is measured as levels of LDH (lactate dehydrogenase) in supernatant using Cytotox-96 non-radioactive cytotoxicity assay kit (Promega). Lysis solution, provided in the same kit, is added to wells with only target cells to get the maximum killing, and spontaneous killing is measured from wells with untreated effector and target cells. The absorbance at 490 nm is recorded using EnVision Multilabel Reader (Perkin Elmer). Percent cytotoxicity is calculated using the following formula: % Cytotoxicity = (Absorbance exp - Absorbance spontaneous average)/(Absorbance max killing average - Absorbance spontaneous average).

Example 3. Method of treating a subject with chimeric CD3 receptor T cells and anti-CD3 bispecific antibodies

[0174] A CAR-T with an extracellular CD3 domain (e.g., CD3 epsilon) and a CAR-T switch are administered to a subject in need thereof. In some instances, the subject has an immunological disorder or condition (e.g., infection). In other instances, the subject has a malignancy (e.g., cancer, tumor, leukemia). In some instances, the CAR-T switch is a bispecific antibody with an anti-CD3 CAR targeting antibody and a cancer cell targeting antibody. The cancer cell targeting antibody recognizes a tumor associated antigen. The bispecific antibody provides a proximity between the CAR-T and the cancer cell, such that the CAR-T has a cytotoxic effect on the cancer cell. In some instances, the CAR-T has a more robust cytotoxic effect on the cancer cell than traditional CAR-T cells because of its engineered additional co-stimulatory domains. In some instances, additional endogenous CAR-T cells are also recruited to the cancer cell to provide a synergistic cytotoxic effect toward the cancer cell. In some instances, it is desirable to interrupt or cease CAR-T activity. The administration of the CAR-T switch may be reduced or arrested. In some instances, a CAR-T "off switch" is administered to clear CAR-T cells. In some instances, the CAR-T "off switch" is an immunotoxin. The immunotoxin comprises an anti-CD3 antibody or fragment thereof and a toxin that kills the CAR-T cell when the immunotoxin binds to the CAR-T cell.

Example 4. Method of treating a subject with heterogeneous malignancy by administering chimeric CD3 receptor T cells and a plurality of anti-CD3 bispecific antibodies

[0175] A CAR-T with an extracellular CD3 domain (e.g., CD3 epsilon) and multiple CAR-T switches are administered to a subject in need thereof. In some instances, the subject has a
heterogeneous tumor or cancer. In some instances, multiple switches are required to bring enough CAR-T cells in proximity of the cancer cells to have a desired cytotoxic effect. In some instances, the CAR-T switches are bispecific antibodies with an anti-CD3 CAR targeting antibody and a cancer cell targeting antibody. A first switch comprises a first cancer cell targeting antibody that recognizes a first antigen on a first target cancer cell that is not expressed on a second target cancer cell. A second switch comprises a second cancer cell targeting antibody that recognizes a second antigen on a second target cancer cell that is not expressed on the first target cancer cell. The bispecific antibodies provide a proximity between the CAR-T and both the first and second target cancer cells, such that the CAR-T has a cytotoxic effect on both cancer cells.

**Example 5. Redirection of CD3e-41bbz and CD3e-41BB T cells by anti-GCN4/anti-PSMA bispecific antibody**

[0176] CAR-T cells comprising extracellular CD3 domains were targeted to cells expressing PSMA for cytolytic activity with an antibody specific for the CAR-T cells and PMSA.

[0177] **Synthesis of anti-GCN4/anti-PSMA bispecific antibody.** Variable light and heavy sequences of anti-GCN4 antibody were inserted into a pBAD vector behind the still signal sequence. The sites for unnatural amino acid incorporation (LC-Ser205 and HC-Lys129) were mutated to amber codon. The pBAD vector was co-transformed with pULTRA-pAcF (a vector containing orthogonal M.jannaschii tRNA and aminoacyl-tRNA synthetase specific for pAcF) in DH10B cells. The cells were grown in LB media (2 L) supplemented with 100 µg/ml ampicillin, 25 µg/ml chloramphenicol, and 1 mM pAcF at 37°C and 250 rpm. At OD600 0.8, cells were induced with 0.2% arabinose and moved to 30°C for 20 hours at 270 rpm. Cells were harvested and proteins were extracted by incubating with 150 mL of periplasmic lysis buffer (20 % sucrose, 30 mM Tris, pH 8, 1 mM EDTA, and 0.2 mg/mL lysozyme) for 30 min at 37°C. Extracts were clarified by centrifugation (18000 rpm, 30 min), filtered through 0.22 micron filter, and loaded onto Protein G column (GE healthcare). The column was washed with 20 bed volumes of 50 mM NaOAc, pH 5.2, and proteins were eluted with 10 bed volumes of 100 mM glycine, pH 2.8. For the conjugation of anti-PSMA small molecule ligand (DUPA), mutant Fab was buffer exchanged into 100 mM NaOAc, pH 4.5, and the concentrations were adjusted to ~2 mg/mL. The oxime ligation was conducted with 50 times molar excess of DUPA, and the reaction was complete within 24 hours (48 hours for double mutant conjugation), as monitored by LC-MS. Excess small molecules were removed by size filtration (Amicon 10K) and buffer exchanged into PBS, pH 7.4.

[0178] **Flow cytometry analysis of anti-GCN4/anti-PSMA bispecific antibody.** LNCaP (PSMA+) or DU145 (PSMA-) cells were trypsinized (0.05 % trypsin/EDTA, Hyclone) and washed
with PBS. Cells (0.2x10^6) were blocked with 3% BSA in PBS (200 ml) for 1 hour at 4°C then incubated with varied concentrations of anti-GCN4/anti-PSMA antibodies for 1 hour at 4°C. Cells were washed (cold PBS 1 mL) twice, resuspended in 0.2 mL of cold PBS, and incubated with secondary goat anti-human kappa PE conjugated antibody (100X, Southern Biotech) for 30 min at 4°C. Cells were washed twice with 1 mL of cold PBS before analysis on a BD Accuri flow cytometer with 10,000 cell events per sample. Data was analyzed using FlowJo software (Tree Star Inc.). Figure 6 shows the specificity of the bispecific antibody for PSMA+ cells in a plot of mean fluorescence intensity (MFI) versus concentration of bispecific antibody ("the switch") in pM. The EC50 was 3653 pM for PSMA+ cells and about 266,139 pM for PSMA- cells.

**[0179] T cell transduction.** To create T cells engineered with chimeric CD3 epsilon receptors (eCARs), CD3 ε -4 IBB z and CD3 ε -4 IBB (shown in the schematic of Figure IB) sequences were subcloned into a lentiviral vector under EF-a promoter. For lentiviral production, the HEK293FT cell line was cultured in Dulbecco’s modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum (FBS, Invitrogen) and 1% penicillin and streptomycin. 5 x 10^6 cells were plated on 100 mm culture dish pre-coated with poly-D-lysine and transfected with lentiviral vectors using lipofectamine 2000 (Invitrogen). Following 48 hr of culture, supernatant containing lentivirus was harvested and cryopreserved at -80°C until ready for use. PBMCs were isolated from healthy human donor blood by conventional Ficoll-Paque density gradient method (GE healthcare). PBMCs were cultured in AIM-V (Invitrogen) supplemented with 5% human AB serum and 300 IU/mL of IL-2 (R&D systems) and stimulated with anti-CD3 and anti-CD28 mAbs coated beads (Invitrogen) as described in the manufacturer's instructions. 24 hr thereafter, 1 x 10^6 cells of PBMCs were transduced with 1.5 mL of lentivirus at a multiplicity of infectivity (MOI) of 10-15 with 1 μg/mL of protamine sulfate (Sigma) and incubated overnight at 37°C, 5% CO2. The next day, cells were initially seeded at 0.125 x 10^6 cells/mL and later maintained at 0.5-1 x 10^6 cells/mL in AIM-V media supplemented with 5% human AB serum and 300 IU/mL IL-2. The expression of CAR was measured by flow cytometry using anti-GCN4-Alexa488. Figure 7 is a flow cytometry histogram showing the expression of eCARs in T cells.

**[0180] In vitro cytotoxicity assays.** Target cells (1x10^4) were mixed with CD3ε-41BBz and CD3ε-41BB T cells (1 x 10^5) at a 10:1 E:T ratio in 100 μl of RPMI media supplemented with 10% FBS and incubated with different concentrations of anti-GCN4/anti-PSMA bispecific Ab for 24 hr at 37°C. Cytolytic activity was determined by the amount of LDH (lactate dehydrogenase) released into cultured media using CytoTox 96 Non-radioactive cytotoxicity assay kit (Promega). The absorbance at 490nm was measured using a SpectraMax 250 plate reader (Molecular Devices Corp.).
Cytotoxicity (experimental - effector spontaneous - target spontaneous) / (target maximum - target spontaneous) x 100. Figure 8 shows that anti-GCN4/anti-PSMA bispecific antibody demonstrated potent lysis of PSMA+ C4-2 cells (Figure 8A) but had no effect on PSMA- DU145 cells (Figure 8B). The EC50 was 14.9 pM for CD3e-41BB and 22.2 pM for CD3e-41BB. Importantly, the cytotoxicity induced by CD3e-41BB indicates that eCAR receptors are associated with the endogenous TCR complex, which also triggers signaling from the endogenous CD3 zeta chain upon the crosslinking of eCAR receptors.

**Example 6. Comparison of CD3e-cytoplasmic domains within chimeric CD3e receptors.**

[0181] Chimeric T cells expressing different constructs of CD3 epsilon receptors were tested for their cytotoxicity against C4-2 (PSMA+) cells when combined with an antibody specific for the CAR-T cells and PMSA.

[0182] **In vitro cytotoxicity and cytokine release assay.** A panel of eCAR constructs with CD3 epsilon cytoplasmic domains (CY) were cloned in the configurations shown in Figure IB: CD3e-e, CD3e-41BB, CD3e-CD28, and CD3e-CD28 41BB. CAR T cells were generating as described in Example 5. CAR transduction efficiency was measured by flow cytometry and is shown in Figure 9. To obtain pure CAR T cells, transduced cells were incubated with Biotin-labeled anti-GCN4 Fab (100 nM) then purified by streptavidin magnetic beads (Miltenyi Biotec). Purified CAR T cells were starved in the absence of IL2 for 24 hrs, and were co-cultured with C4-2 cells at effector to target ratio of 10:1 in the presence of different concentrations of anti-GCN4/anti-PSMA bispecific antibody for 24 hrs at 37°C and 5% C02. Cytotoxic activity was determined by measuring lactate dehydrogenase (LDH) levels in the cultured supernatant using the Cytotox-96 nonradioactive cytotoxicity assay kit (Promega). Percent cytotoxicity was calculated by: % cytotoxicity = [(absorbance experimental - absorbance spontaneous average) / (absorbance maximum killing average - absorbance spontaneous average)] x100. Figure 10 shows the cytotoxic effects of the various CAR T cells as a function of bispecific antibody concentration. Supernatants from the same assay were analyzed for released cytokine levels using bead-based immunoassays (CBA kit, BD bioscience). Figure 11 shows the levels of different cytokines released during the assay as a function of eCAR construct and bispecific antibody ("switch") concentration. IL6 was not detected.

[0183] **In vitro cytokine levels induced by anti-CD3/anti-CD19 bispecific antibody.** Purified T cells (5x10^4) were rested for 24 hrs in the absence of IL-2, then co-cultured with 1x10^4 CD19-positive Nairn 6 cells in 100 ul cultured media per well in 96-well round bottom plates in the presence of varied concentrations of CD19 BiTE (blinatumomab, max 1 nM) for 18 hrs. Cultured supernatants were harvested and assayed for the presence of human cytokines using CBA kit,
according to manufacturer's instructions. Figure 12 shows the levels of cytokines induced in T cells as a function of blinatumomab antibody ("switch") concentration. IL6 was not detected.

[0184] **In vitro proliferation assays.** To test in vitro proliferation, 4 ml of non-purified CAR T cells were seeded at 0.5x10^6 cells/mL density in 6-well tissue culture dish in AIMV media supplemented with 5% human serum and 100 U/ml recombinant IL-2. Cells were counted every other day and maintained in fresh culture media to keep 0.5x10^6 cell/ml density. The percentage of CD4 and CD8 positive T cell subsets in the CAR-positive populations were determined by flow cytometry using anti-GCN4-Alexa488, anti-human CD4-PE (eBioscience), and anti-human CD8-PerCP (eBioscience) antibodies. Figure 13 shows a graph of the number of doublings of CAR-positive populations versus the number of days post transduction. Data from day 6 and day 21 shown in Figure 14 indicate the preferential expansion of CD8-positive cytotoxic T subsets over CD4-positive T cells in CD3e-41BBBe and CD3e-CD28e CAR transduced T cells in comparison with control T cells (CD3e-e).

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Table 1. Cell Surface Receptor Domains—Antigenic Acid Sequences

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Table 2. Cell Surface Receptor Domains—Nucleic Acid Sequences

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- 76 -
### Table 2. Cell Surface Receptor Domains-Nucleic Acid Sequences

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### Table 3. Chimeric (ε) epsilon Kccepors-Amino Acid Sequences

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<td>DGNEEMGITTQTPYKVJISGTTVIILTCPQYPGSEILWQHNDKINGGDEDSDKNISSDEDDHLSLEFKSELEQSGYVYYCYPGRSKPENFYLYLRARVCENCMEVMVSMAVATIVIDICTGGLLLYYWYWSKGRKLLLYIFKQFPMFRRVQTTEDGCSRFFPEEEEDECHELVRKFSRSADAPAYKQGQNQLYNELNLGREREYDVLDKRRGRDPEMGGKPRKNPQEGLYNELQDKMDAEYSEIGMKGERRRKGKGDGLYQGLSTATKDTYDALHMQALPPR</td>
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Table 3. Chimeric (ε)3 epsilon Receptors- Amino Acid Sequences

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<th>NAME</th>
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<th>SEQUENCe</th>
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<tbody>
<tr>
<td>CD3e-41BB</td>
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<td>QDGNEMGGITQTPKYVSIGTTVILTCCQYPGSEILWQHNDKNIG GEDDDKNIGSDEHDLSLEKESELEQSGYVVYCYPGRSPEDEANFYLYLRLARVCENCMEMDVMVSATIVIDICTGGGGLLL VYVWShKGR KLLLYIFKQPFPFMPVQTTQEDGCSRFPEEEDGCCE</td>
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Table 4. Chimeric CD3 epsilon Receptors-Nucleic Acid Sequences

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<tr>
<td>NAME</td>
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<td>s EQUENC(E):</td>
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<td>GCAGGCACCCTGTATTCTGGCCTGAAATCAGAGACGCATC</td>
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</table>
CLAIMS

What is claimed is:

1. An engineered cell surface protein comprising
   a. an extracellular domain comprising a sequence of an extracellular portion of a cell
      surface protein;
   b. a transmembrane domain; and
   c. an intracellular domain comprising a sequence of an intracellular signaling protein;

   wherein the engineered cell surface protein is engineered by combining sequences
   from at least two different protein sources.

2. The engineered cell surface protein of claim 1, wherein a first protein source is the cell
   surface protein and a second protein source is the intracellular signaling protein.

3. The engineered cell surface protein of claim 1 or claim 2, wherein the extracellular domain
   does not comprise an antibody or fragment thereof.

4. The engineered cell surface protein of any of claims 1-3, wherein the intracellular signaling
   protein is a costimulatory domain.

5. The engineered cell surface protein of claim 4, wherein the costimulatory domain comprises a
   41BB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40
   signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling
   domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a portion or
   combination thereof.

6. The engineered cell surface protein of claim 5, wherein the costimulatory domain comprises a
   sequence of any of SEQ ID NOs: 6-15.

7. The engineered cell surface protein of any of claims 4-6, wherein the intracellular domain
   further comprises a T cell receptor zeta chain.

8. The engineered cell surface protein of any of claims 1-3, wherein the intracellular signaling
   protein is a T cell receptor zeta chain.

9. The engineered cell surface protein of claim 7 or claim 8, wherein the T cell receptor zeta
   chain does not comprise an ITAMI domain, ITAM2 domain, ITAM3 domain, or a portion or
   combination thereof.

10. The engineered cell surface protein of any of claims 1-9, wherein the cell surface protein is a
    protein expressed on the surface of a T cell.

11. The engineered cell surface protein of claim 10, wherein the cell surface protein is CD3
    epsilon.
12. The engineered cell surface protein of claim 11, wherein the extracellular domain comprises a sequence from any of SEQ ID NOs: 1-3.

13. The engineered cell surface protein of any of claims 1-12, wherein the transmembrane domain comprises a sequence from a CD8 transmembrane domain, a CD28 transmembrane domain, an FcR transmembrane domain, a CD3 epsilon transmembrane domain, or a combination thereof.


16. The effector cell of claim 15, wherein the effector cell is a tumor-infiltrating lymphocyte.

17. A kit comprising the effector cell of claim 15 or claim 16 and an antibody comprising an antigen binding domain that binds to the extracellular domain of the engineered cell surface protein.

18. The kit of claim 17, wherein the antibody is a bispecific antibody that further comprises an antigen binding domain that binds to an antigen on a target cell.

19. An engineered cell surface protein comprising
   a. an extracellular domain comprising a CD3 epitope;
   b. a transmembrane domain; and
   c. an intracellular domain comprising a sequence of an intracellular signaling protein;
      wherein the engineered cell surface protein is engineered by combining the CD3 epitope with a protein other than CD3.

20. The engineered cell surface protein of claim 19, wherein the protein other than CD3 is the intracellular signaling protein, the transmembrane domain, or both the intracellular signaling protein and the transmembrane domain.

21. The engineered cell surface protein of claim 19 or claim 20, wherein the extracellular domain does not comprise an antibody or fragment thereof.

22. The engineered cell surface protein of any of claims 19-21, wherein the CD3 epitope is from a CD3 epsilon domain.

23. The engineered cell surface protein of claim 22, wherein the CD3 epitope comprises a sequence from the amino terminal portion of the CD3 epsilon domain.

24. The engineered cell surface protein of claim 23, wherein the amino terminal portion of the CD3 epsilon domain comprises the first 30 amino acids of the CD3 epsilon domain.
25. The engineered cell surface protein of any of claims 19-24, wherein the CD3 epitope comprises a sequence from any of SEQ ID NOs: 1-3.
26. The engineered cell surface protein of any of claims 19-25, wherein the intracellular signaling protein is a costimulatory domain.
27. The engineered cell surface protein of claim 26, wherein the costimulatory domain comprises a 4IBB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a portion or combination thereof.
28. The engineered cell surface protein of claim 26, wherein the costimulatory domain comprises a sequence from any of SEQ ID NOs: 6-15.
29. The engineered cell surface protein of any of claims 26-28, wherein the intracellular domain further comprises a T cell receptor zeta chain.
30. The engineered cell surface protein of any of claims 19-25, wherein the intracellular signaling protein is a T cell receptor zeta chain.
31. The engineered cell surface protein of claim 29 or claim 30, wherein the T cell receptor zeta chain does not comprise an ITAMI domain, ITAM2 domain, ITAM3 domain, or a portion or combination thereof.
32. The engineered cell surface protein of any of claims 19-31, wherein the transmembrane domain comprises a sequence derived from a CD8 transmembrane domain, a CD28 transmembrane domain, an FcR transmembrane domain, a CD3 epsilon transmembrane domain, or a combination thereof.
33. A nucleic acid sequence encoding for the engineered cell surface protein of any of claims 19-32.
34. An effector cell expressing the engineered cell surface protein of any of claims 19-32.
35. The effector cell of claim 34, wherein the effector cell is a tumor-infiltrating lymphocyte.
36. A kit comprising the effector cell of claim 34 or claim 35 and an antibody comprising an antigen binding domain that binds to the CD3 epitope.
37. The kit of claim 36, wherein the antibody is a bispecific antibody that further comprises an antigen binding domain that binds to an antigen on a target cell.
38. An engineered cell surface protein comprising
   a. an extracellular domain comprising a sequence of an extracellular portion of a cell surface protein;
b. a transmembrane domain; and
c. an intracellular domain comprising a sequence of a costimulatory domain;
wherein the engineered cell surface protein is engineered by combining sequences
from at least two different protein sources.
39. The engineered cell surface protein of claim 38, wherein a first protein source is the cell
surface protein and a second protein source is a protein comprising the costimulatory domain.
40. The engineered cell surface protein of claim 38 or claim 39, wherein the extracellular domain
does not comprise an antibody or fragment thereof.
41. The engineered cell surface protein of any of claims 38-40, wherein the costimulatory domain
comprises a 41BB signaling domain, a CD28 signaling domain, an ICOS signaling domain,
an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150
signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a portion or
combination thereof.
42. The engineered cell surface protein of any of claims 38-41, wherein the costimulatory domain
comprises a sequence from any of SEQ ID NOs: 6-15.
43. The engineered cell surface protein of any of claims 38-42, wherein the cell surface protein is
a protein expressed on the surface of a T cell.
44. The engineered cell surface protein of claim 43, wherein the cell surface protein is CD3
epsilon.
45. The engineered cell surface protein of claim 44, wherein the extracellular domain comprises a
sequence from any of SEQ ID NOs: 1-3.
46. The engineered cell surface protein of any of claims 38-45, wherein the intracellular domain
further comprises a T cell receptor zeta chain.
47. The engineered cell surface protein of claim 46, wherein the T cell receptor zeta chain does
not comprise an ITAM1 domain, ITAM2 domain, ITAM3 domain, or a portion or
combination thereof.
48. The engineered cell surface protein of any of claims 38-47, wherein the transmembrane
domain comprises a sequence derived from a CD8 transmembrane domain, a CD28
transmembrane domain, an FcR transmembrane domain, a CD3 epsilon transmembrane
domain, or a combination thereof.
49. A nucleic acid sequence encoding for the engineered cell surface protein of any of claims 38-
48.
50. An effector cell expressing the engineered cell surface protein of any of claims 38-48.
51. The effector cell of claim 50, wherein the effector cell is a tumor-infiltrating lymphocyte.

52. A kit comprising the effector cell of claim 50 or claim 51 and an antibody comprising an antigen binding domain that binds to the extracellular domain of the engineered cell surface protein.

53. The kit of claim 52, wherein the antibody is a bispecific antibody that further comprises an antigen binding domain that binds to an antigen on a target cell.

54. An engineered cell surface protein comprising
   a. an extracellular domain comprising a CD3 epitope;
   b. a transmembrane domain; and
   c. an intracellular domain comprising a sequence of a costimulatory domain;
      wherein the engineered cell surface protein is engineered by combining the CD3 epitope with a protein other than CD3.

55. The engineered cell surface protein of claim 54, wherein the protein other than CD3 comprises the costimulatory domain.

56. The engineered cell surface protein of claim 54 or claim 55, wherein the extracellular domain does not comprise an antibody or fragment thereof.

57. The engineered cell surface protein of any of claims 54-56, wherein the CD3 epitope is from a CD3 epsilon domain.

58. The engineered cell surface protein of claim 57, wherein the CD3 epitope comprises a sequence from the amino terminal portion of the CD3 epsilon domain.

59. The engineered cell surface protein of claim 58, wherein the amino terminal portion of the CD3 epsilon domain comprises the first 30 amino acids of the CD3 epsilon domain.

60. The engineered cell surface protein of any of claims 54-59, wherein the CD3 epitope comprises a sequence from any of SEQ ID NOs: 1-3.

61. The engineered cell surface protein of any of claims 54-60, wherein the costimulatory domain comprises a 4IBB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a portion or combination thereof.

62. The engineered cell surface protein of any of claims 54-60, wherein the costimulatory domain comprises a sequence from any of SEQ ID NOs: 6-15.

63. The engineered cell surface protein of any of claims 54-62, wherein the intracellular domain further comprises a T cell receptor zeta chain.
64. The engineered cell surface protein of claim 63, wherein the T cell receptor zeta chain does not comprise an ITAMI domain, ITAM2 domain, ITAM3 domain, or a portion or combination thereof.

65. The engineered cell surface protein of any of claims 54-64, wherein the transmembrane domain comprises a sequence derived from a CD8 transmembrane domain, a CD28 transmembrane domain, an FcR transmembrane domain, a CD3 epsilon transmembrane domain, or a combination thereof.

66. A nucleic acid sequence encoding for the engineered cell surface protein of any of claims 54-65.

67. An effector cell expressing the engineered cell surface protein of any of claims 54-65.

68. The effector cell of claim 67, wherein the effector cell is a tumor-infiltrating lymphocyte.

69. A kit comprising the effector cell of claim 67 of claim 68 and an antibody comprising an antigen binding domain that binds to the CD3 epitope of the engineered cell surface protein.

70. The kit of claim 69, wherein the antibody is a bispecific antibody that further comprises an antigen binding domain that binds to an antigen on a target cell.

71. An effector cell expressing an engineered cell surface protein comprising
   a. an extracellular domain comprising a sequence of an extracellular portion of the cell surface protein;
   b. a transmembrane domain; and
   c. an intracellular domain comprising a sequence of an intracellular signaling protein;
      wherein the engineered cell surface protein is engineered by combining sequences from at least two different protein sources.

72. The engineered cell surface protein of claim 71, wherein a first protein source is the cell surface protein and a second protein source is the intracellular signaling protein.

73. The effector cell of claim 71 or claim 72, wherein the extracellular domain does not comprise an antibody or fragment thereof.

74. The effector cell of any of claims 71-73, wherein the intracellular signaling protein is a costimulatory domain.

75. The effector cell of claim 74, wherein the costimulatory domain comprises a 4IBB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a portion or combination thereof.
76. The engineered cell surface protein of claim 74 or claim 75, wherein the intracellular domain further comprises a T cell receptor zeta chain.

77. The effector cell of any of claims 71-73, wherein the intracellular signaling protein is a T cell receptor zeta chain.

78. The effector cell of claim 76 or claim 77, wherein the T cell receptor zeta chain does not comprise an ITAMI domain, ITAM2 domain, ITAM3 domain, or a portion or combination thereof.

79. The effector cell of any of claims 71-78, wherein the cell surface protein is a protein expressed on the surface of a T cell.

80. The effector cell of claim 79, wherein the cell surface protein is CD3 epsilon.

81. The effector cell of any of claims 71-80, wherein the effector cell is a tumor-infiltrating lymphocyte.

82. A kit comprising the effector cell of any of claims 71-81 and an antibody comprising an antigen binding domain that binds to the extracellular domain of the engineered cell surface protein.

83. The kit of claim 82, wherein the antibody is a bispecific antibody that further comprises an antigen binding domain that binds to an antigen on a target cell.

84. An effector cell comprising an engineered component of a TCR complex comprising a modification that improves an immune response.

85. The effector cell of claim 84, wherein the component of the TCR complex comprises an extracellular domain of CD3.

86. The effector cell of claim 84 or claim 85, wherein the modification comprises an addition of a costimulatory domain to the component of the TCR complex.

87. The effector cell of claim 86, wherein the costimulatory domain comprises a sequence derived from a 4IBB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a combination thereof.

88. The effector cell of any of claims 84-87, wherein the modification does not comprise an addition of an antibody or antibody fragment to the component of the TCR complex.

89. The effector cell of any of claims 84-88, wherein the modification does not comprise an addition of a CD3 zeta chain to the component of the TCR complex.
90. The effector cell of any of claims 84-89, wherein the effector cell is a tumor-infiltrating lymphocyte.

91. A kit comprising:
   a. an effector cell expressing an engineered cell surface protein comprising
      i. an extracellular domain comprising a sequence of an extracellular portion of a cell surface protein,
      ii. a transmembrane domain, and
      iii. an intracellular domain comprising a sequence of an intracellular signaling protein; wherein the engineered cell surface protein is engineered by combining sequences from at least two different protein sources; and
   b. a bispecific antibody comprising an antigen binding domain that binds to the extracellular domain of the engineered cell surface protein.

92. The kit of claim 91, wherein a first protein source is the cell surface protein and a second protein source is the intracellular signaling protein.

93. The kit of claim 91 or claim 92, wherein the extracellular domain does not comprise an antibody or fragment thereof.

94. The kit of any of claims 91-93, wherein the intracellular signaling protein is a costimulatory domain.

95. The kit of claim 94, wherein the costimulatory domain comprises a 4IBB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a portion or combination thereof.

96. The kit of claim 94 or claim 95, wherein the intracellular domain further comprises a T cell receptor zeta chain.

97. The kit of any of claims 91-93, wherein the intracellular signaling protein is a T cell receptor zeta chain.

98. The kit of claim 96 or claim 97, wherein the T cell receptor zeta chain does not comprise an ITAMI domain, ITAM2 domain, ITAM3 domain, or a portion or combination thereof.

99. The kit of any of claims 91-98, wherein the cell surface protein is CD3 epsilon.

100. The kit of any of claims 91-99, wherein the effector cell is a T cell.

101. The kit of claim 100, wherein the effector cell is a tumor-infiltrating lymphocyte.

102. The kit of any of claims 91-101, wherein the bispecific antibody comprises an antigen binding domain that binds to an antigen on a target cell.
103. The kit of claim 102, wherein the target cell is a tumor cell.

104. A method of treating a condition comprising administering to a subject in need thereof:
   a. an effector cell that expresses an engineered cell surface protein, the engineered cell surface protein comprising: an extracellular domain comprising a sequence of an extracellular portion of a cell surface protein, a transmembrane domain, and an intracellular domain comprising a sequence of an intracellular signaling protein; wherein the engineered cell surface protein is engineered by combining sequences from at least two different protein sources; and
   b. a bispecific antibody comprising: a first antigen binding region that binds to the extracellular domain of the engineered cell surface protein, and a second antigen binding region that binds to an antigen on a target cell.

105. The method of claim 104, wherein a first protein source is the cell surface protein and a second protein source is the intracellular signaling protein.

106. The method of claim 104 or claim 105, wherein the extracellular domain does not comprise an antibody or fragment thereof.

107. The method of any of claims 104-106, wherein the intracellular signaling protein is a costimulatory domain.

108. The method of claim 107, wherein the costimulatory domain comprises a 41BB signaling domain, a CD28 signaling domain, an ICOS signaling domain, an OX40 signaling domain, a CD27 signaling domain, a CD30 signaling domain, a CD150 signaling domain, a DAP-10 signaling domain, an NKG2D signaling domain, or a portion or combination thereof.

109. The method of claim 107 or claim 108, wherein the intracellular domain further comprises a T cell receptor zeta chain.

110. The method of any of claims 104-106, wherein the intracellular signaling protein is a T cell receptor zeta chain.

111. The method of any of claims 104-110, wherein the cell surface protein is CD3 epsilon.

112. The method of any of claims 104-110, wherein the effector cell is selected from a T cell, an effector B cell, a natural killer cell, a macrophage and a progenitor thereof.

113. The method of claim 112, wherein the effector cell is a T cell 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.

114. The method of claim 112, wherein the effector cell is a tumor-infiltrating lymphocyte.
115. The method of any of claims 104-114, wherein the target cell is a tumor cell.
116. The method of any of claims 104-115, wherein the condition is a cancer.
117. The method of any of claims 104-116, wherein the effector cell and bispecific
    antibody are administered simultaneously.
118. The method of any of claims 104-116, wherein the effector cell and bispecific
    antibody are administered sequentially.
FIGURE 4

Tumor cell

T cell

Co-stimulatory signaling
{41bb, CD28, OX40...}

CD3 zeta signaling
FIGURE 6
(DUPA)$_2$-GCN4 binding to Target cells

![Graph showing binding to Target cells](image)

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</table>

FIGURE 7
CAR expression

![Graph showing CAR expression](image)
FIGURE 8A
DUPA-GCN4 dose-response curve C4-2 cells (PSMA+)

% Target cell death

\[
\begin{array}{|c|c|c|c|}
\hline
& \text{CD3e-41BBz} & \text{CD3e-41BB} & \text{Non-transduced} \\
\hline
\text{EC50 (pM)} & 14.9 & 22.2 & N/A \\
\hline
\end{array}
\]

FIGURE 8B
DUPA-GCN4 dose-response curve DU145 cells (PSMA-)
FIGURE 9
CAR transduction efficiency measured by flow cytometry

CD3e-e

antiGCN4 Alexa488

CD3e-41BB

CD3e-CD28e

CD3e-CD2841BB

ECAR+ cells 64.9%
ECAR41BB CAR-T cells 43.3%
ECOD28 CAR-T 32.5%
ECAR41BBCD28 CAR-T 1.36%
FIGURE 10

C4-2 cytotoxicity

<table>
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FIGURE 11

IFNg

TGF

IL-10

IL-6

IL-4

IL-2