

T CELL-ANTIGEN COUPLER WITH VARIOUS CONSTRUCT OPTIMIZATIONS

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/699,173, filed on July 17, 2018, U.S. Provisional Application No. 62/703,037, filed on July 25, 2018, U.S. Provisional Application No. 62/773,120, filed on November 29, 2018, U.S. Provisional Application No. 62/826,853, filed on March 29, 2019, U.S. Provisional Application No. 62/828,879, filed on April 03, 2019, U.S. Provisional Application No. 62/839,235, filed on April 26, 2019, U.S. Non-Provisional Application No. 16/442,274, filed on June 14, 2019, and U.S. Provisional Application No. 62/874,426, filed on July 15, 2019, each of which are incorporated herein by reference in their entireties.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on July 17, 2019, is named 55247704601_SL.txt and is 131,072 bytes in size.

SUMMARY

[0002a] In one aspect, the present disclosure refers to a nucleic acid sequence encoding a CD19 Trifunctional T cell-antigen coupler (CD19-TAC) comprising: (a) a first polynucleotide encoding a ligand that selectively binds a CD19 antigen, optionally wherein the ligand that selectively binds the CD19 antigen is a single chain variable fragment (scFv); (b) a second polynucleotide encoding a UCHT1 ligand that binds CD3, optionally wherein the UCHT1 ligand is a single chain antibody; and (c) a third polynucleotide encoding a TCR signaling domain polypeptide comprising a cytosolic domain and a transmembrane domain, optionally wherein the cytosolic domain is a CD4 cytosolic domain and the transmembrane domain is a CD4 transmembrane domain; wherein components encoded by (a), components encoded by (b), and components encoded by (c) are fused directly to each other, or joined by at least one linker and the nucleic acid sequence does not encode a co-stimulatory domain.

[0003] Disclosed herein, in certain examples, are nucleic acid sequences encoding a CD19 Trifunctional T cell-antigen coupler (CD19-TAC). In some examples, the nucleic acid sequence encoding a CD19 Trifunctional T cell-antigen coupler (CD19-TAC) comprises: (a) a first polynucleotide encoding a ligand that selectively binds a CD19 antigen. In some examples, the nucleic acid sequence encoding a CD19 Trifunctional T cell-

antigen coupler (CD19-TAC) comprises: (b) a second polynucleotide encoding a UCHT1 ligand that binds CD3. In some examples, the nucleic acid sequence encoding a CD19 Trifunctional T cell-antigen coupler (CD19-TAC) comprise: (c) a third polynucleotide encoding a TCR signaling domain polypeptide comprising a cytosolic domain and a transmembrane domain. In some examples, the components encoded by the first, second, and/or third polynucleotides are connected in any suitable manner, such as in any suitable order and/or comprising any suitable linker(s). In some examples, the components encoded by (a), components encoded by (b), and components encoded by (c) are fused directly to each other, or joined by at least one linker. In some examples, the ligand that selectively binds the CD19 antigen is a single chain variable fragment (scFv). In some examples, the ligand that selectively binds the CD19 antigen comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at

least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 36. In some examples, the UCHT1 ligand is a single chain antibody. In some examples, the UCHT1 ligand comprises a Y182T mutation (SEQ ID NO: 72). In some examples, the UCHT1 ligand is a humanized variant of UCHT1 (huUCHT1) ligand (SEQ ID NO: 44). In some examples, the UCHT1 ligand is a humanized variant of UCHT1 comprising a Y177T mutation (huUCHT1 (Y177T)) (SEQ ID NO: 46). In some examples, the UCHT1 ligand comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 14, SEQ ID NO: 72, SEQ ID NO: 44, or SEQ ID NO: 46. In some examples, the cytosolic domain is a CD4 cytosolic domain and the transmembrane domain is a CD4 transmembrane domain. In some examples, the third polynucleotide encodes a polypeptide comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 18. In some examples, the component encoded by (a) and the component encoded by (c) are fused to the component encoded by (b). In some examples, the component encoded by (b) and the component encoded by (c) are fused to the component encoded by (a). In some examples, at least one linker joins the component encoded by (a) to the component encoded by (b). In some examples, the at least one linker is a G₄S flexible linker (SEQ ID NO: 73), a large protein domain, a long helix structure, or a short helix structure. In some examples, the at least one linker comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 12 (G₄S flexible linker ("G₄S" disclosed as SEQ ID NO: 73)), SEQ ID NO: 32 (large protein domain), SEQ ID NO: 30 (long helix structure), or SEQ ID NO: 28 (short helix structure). In some examples, the CD3 is of a TCR complex on a cell expressing the second polynucleotide. In some examples, the binding of the CD3 induces activation of a cell expressing the second polynucleotide. In some examples, the CD19-TAC comprises a nucleic acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 63. In some examples, the CD19-TAC comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 64. In some examples, the nucleic acid sequence does not encode a co-stimulatory domain. In some examples, the nucleic acid sequence does not encode an activation domain.

[0003a] In another aspect, the present disclosure refers to a polypeptide encoded by the nucleic acid sequence as disclosed herein.

[0004] In yet another aspect, the present disclosure refers to a vector construct comprising: (a) a nucleic acid sequence disclosed herein (e.g., a nucleic acid sequence encoding a CD19-TAC); and (b) a promoter functional in a mammalian cell.

[0005] In yet another aspect, the present disclosure refers to a T cell expressing the nucleic acid sequence as disclosed herein. Disclosed herein, in certain examples, are T cells comprising a nucleic acid sequence disclosed herein (e.g., a nucleic acid sequence encoding a CD19-TAC).

[0006] In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising the T cell disclosed herein, and a pharmaceutically acceptable excipient.

[0006a] In yet another aspect, the present disclosure refers to the pharmaceutical composition as disclosed herein, for use in a method of treating a cancer in an individual in need thereof.

[0007] [0006b] In yet another aspect, the present disclosure refers to use of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for the treatment of cancer. Disclosed herein, in certain examples, are methods of treating cancer expressing CD19 in an individual in need thereof, comprising administering to the individual a pharmaceutical composition disclosed herein. (e.g., a pharmaceutical composition comprising a T cell comprising any nucleic acid sequence described herein, such as any nucleic acid sequence or sequences described herein as encoding a CD19 Trifunctional T cell-antigen coupler (CD19-TAC)). In some examples, the cancer is a B cell malignancy. In some examples, the cancer is B cell lymphoma, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), or Non-Hodgkins Lymphoma. In some examples, the pharmaceutical composition is administered transarterially, subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, intravenously or intraperitoneally.

[0007a] In yet another aspect, the present disclosure refers to a nucleic acid sequence encoding a Human Epidermal Growth Factor Receptor 2 (HER2) Trifunctional T cell antigen coupler (Tri-TAC), wherein the Tri-TAC comprises an amino acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 66, SEQ ID NO: 68, or SEQ ID NO: 76.

[0008] Disclosed herein, in certain examples, are nucleic acid sequences encoding a Trifunctional T cell-antigen coupler (Tri-TAC) comprising: (a) a first polynucleotide encoding a target-specific ligand; (b) a second polynucleotide encoding a ligand that binds a protein associated with a TCR complex; and (c) a third polynucleotide encoding a T cell receptor signaling domain polypeptide; wherein the ligand that binds the protein associated with the TCR complex is selected from OKT3, F6A or L2K. In some examples, component encoded by (a), component encoded by (b), and component encoded by (c) are fused directly to each other, or joined by at least one linker. In some examples, the component encoded by (a) and the component encoded by (b) are directly fused and joined to the component encoded by (c) by a linker. In some examples, the component encoded by (b) and the component encoded by (c) are directly fused and joined to the component encoded by (a) by a linker. In some examples, the at least one linker is a G4S flexible linker (SEQ ID NO: 73), a large protein domain, a long helix structure, or a short helix structure. In some examples, the at least one linker has an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 12 (G4S flexible linker ("G4S" disclosed as SEQ ID NO: 73)), SEQ ID NO: 32 (large protein domain), SEQ ID NO: 30 (long helix structure), or SEQ ID NO: 28 (short helix structure). In some examples, the ligand that binds the protein associated with the TCR

complex is OKT3. In some examples, the ligand that binds a protein associated with the TCR complex comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 22. In some examples, the ligand that binds the protein associated with the TCR complex is F6A. In some examples, the ligand that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 24. In some examples, the ligand that binds the protein associated with the TCR complex is L2K. In some examples, the ligand that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 26. In some examples, the protein associated with the TCR complex is CD3. In some examples, the target-specific ligand selectively binds a tumor antigen. In some examples, the target-specific ligand is a designed ankyrin repeat (DARPin) polypeptide, or a single chain variable fragment (scFv). In some examples, the target-specific ligand selectively binds a CD19 antigen, a HER2 antigen, or a BCMA antigen. In some examples, the target-specific ligand selectively binds a HER-2 antigen comprises an antigen binding domain of an antibody selected from Trastuzumab, Pertuzumab, Lapatinib, Neratinib, Ado-trastuzumab Emtansine, Gancotamab, Margetuximab, Timigutuzumab, and Ertumaxomab. In some examples, the target-specific ligand selectively binds a BCMA antigen comprises an antigen binding domain of an antibody selected from Belantamab mafodotin, and GSK2857916. In some examples, the target-specific ligand comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 36, SEQ ID NO: 8 or SEQ ID NO: 34. In some examples, the T cell receptor signaling domain polypeptide comprises a cytosolic domain and a transmembrane domain. In some examples, the cytosolic domain is a CD4 cytosolic domain and the transmembrane domain is a CD4 transmembrane domain, or wherein the cytosolic domain is a CD8 cytosolic domain and the transmembrane domain is a CD8 transmembrane domain. In some examples, the nucleic acid sequences further comprise a leader sequence. In some examples, the leader sequence comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 6, SEQ ID NO: 48, or SEQ ID NO: 50. In some examples, the CD3 is of a TCR complex on a cell expressing the second polynucleotide. In some examples, the binding of the CD3 induces activation of a cell expressing the second polynucleotide. In some

examples, the Tri-TAC comprises a nucleic acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 75, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, or SEQ ID NO: 61. In some examples, the Tri-TAC comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 76, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, or SEQ ID NO: 62. In some examples, the nucleic acid sequence does not encode a co-stimulatory domain. In some examples, the nucleic acid sequence does not encode an activation domain.

[0009] Disclosed herein, in certain examples, are nucleic acid sequences encoding a Trifunctional T cell-antigen coupler (Tri-TAC) comprising: (a) a first polynucleotide encoding a target-specific ligand; (b) a second polynucleotide encoding a ligand that binds a protein associated with a TCR complex; and (c) a third polynucleotide encoding a T cell receptor signaling domain polypeptide; wherein the nucleic acid sequence further comprises a leader sequence, and wherein component encoded by (a), component encoded by (b), and component encoded by (c) are fused directly to each other, or joined by at least one linker. In some examples, the target-specific ligand selectively binds a tumor antigen. In some examples, the target-specific ligand is a designed ankyrin repeat (DARPin) polypeptide, or a single chain variable fragment (scFv). In some examples, the target-specific ligand selectively binds a CD19 antigen, a HER2 antigen, or a BCMA antigen. In some examples, the target-specific ligand selectively binds a HER-2 antigen comprises an antigen binding domain of an antibody selected from Trastuzumab, Pertuzumab, Lapatinib, Neratinib, Ado-trastuzumab Emtansine, Gancotamab, Margetuximab, Timigutuzumab, and Ertumaxomab. In some examples, the target-specific ligand selectively binds a BCMA antigen comprises an antigen binding domain of an antibody selected from Belantamab mafodotin, and GSK2857916. In some examples, the target-specific ligand comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 36, SEQ ID NO: 8, SEQ ID NO: 34, SEQ ID NO: 52, or SEQ ID NO: 54. In some examples, the ligand that binds the protein associated with the TCR complex is selected from UCHT1, UCHT1 (Y182T), huUCHT1, huUCHT1 (Y177T), OKT3, F6A, or L2K. In some examples, the ligand that binds a protein associated with the TCR complex has an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 14, SEQ ID NO: 72, SEQ ID NO: 44, SEQ ID NO: 46, SEQ

ID NO: 22, SEQ ID NO: 24, or SEQ ID NO: 26. In some examples, the protein associated with the TCR complex is CD3. In some examples, the T cell receptor signaling domain polypeptide comprises a cytosolic domain and a transmembrane domain. In some examples, the cytosolic domain is a CD4 cytosolic domain and the transmembrane domain is a CD4 transmembrane domain, or wherein the cytosolic domain is a CD8 cytosolic domain and the transmembrane domain is a CD8 transmembrane domain. In some examples, the leader sequence comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 6, SEQ ID NO: 48, or SEQ ID NO: 50. In some examples, the component encoded by (a) and the component encoded by (b) are directly fused and joined to the component encoded by (c) by a linker. In some examples, the component encoded by (b) and the component encoded by (c) are directly fused and joined to the component encoded by (a) by a linker. In some examples, the at least one linker is a G₄S flexible linker (SEQ ID NO: 73), a large protein domain, a long helix structure, or a short helix structure. In some examples, the at least one linker has an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 12 (G₄S flexible linker ("G₄S" disclosed as SEQ ID NO: 73)), SEQ ID NO: 32 (large protein domain), SEQ ID NO: 30 (long helix structure), or SEQ ID NO: 28 (short helix structure). In some examples, the CD3 is of a TCR complex on a cell expressing the second polynucleotide. In some examples, the binding of the CD3 induces activation of a cell expressing the second polynucleotide. In some examples, the Tri-TAC comprises a nucleic acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 75, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, or SEQ ID NO: 61. In some examples, the Tri-TAC comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 76, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, or SEQ ID NO: 62. In some examples, the nucleic acid sequence does not encode a co-stimulatory domain. In some examples, the nucleic acid sequence does not encode an activation domain.

[0010] In yet another aspect, the present discloser refers to a polypeptide encoded by the nucleic acid sequence disclosed herein.

[0011] In yet another aspect, the present discloser refers to a vector construct comprising: (a) a nucleic acid sequence disclosed herein; and (b) a promoter functional in a mammalian cell.

[0012] In yet another aspect, the present discloser refers to a T cell expressing the nucleic acid sequence as disclosed herein. Disclosed herein, in certain examples, are T cells comprising the nucleic acid sequence disclosed herein.

[0013] In yet another aspect, the present discloser refers to a pharmaceutical composition comprising the T cell disclosed herein, and a pharmaceutically acceptable excipient.

[0013a] In yet another aspect, the present disclosure refers to the pharmaceutical composition as disclosed herein, for use in a method of treating a cancer in an individual in need thereof.

[0013b] In yet another aspect, the present disclosure refers to use of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for the treatment of cancer.

[0014] Disclosed herein, in certain examples, are methods of treating a cancer in an individual in need thereof, comprising administering to the individual a pharmaceutical composition disclosed herein. In some examples, the subject is a mammal. In some examples, the cancer is a solid cancer or a liquid cancer. In some examples, the cancer is a lung cancer, a breast cancer, multiple myeloma, glioblastoma, gastric cancer, ovarian cancer, stomach cancer, colorectal cancer, urothelial cancer, endometrial cancer, or a colon cancer. In some examples, the cancer comprises a CD19 expressing cancer cell. In some examples, the cancer is a B cell malignancy. In some examples, the cancer is B cell lymphoma, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), or Non-Hodgkins Lymphoma. In some examples, the cancer comprises a HER-2 expressing cancer cell. In some examples, the cancer is breast cancer, bladder cancer, pancreatic cancer, ovarian cancer, or stomach cancer. In some examples, the cancer comprises a BCMA expressing cancer cell. In some examples, the cancer is leukemia, lymphoma, or multiple myeloma. In some examples, the pharmaceutical composition is administered to the individual transarterially, subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, intravenously or intraperitoneally. In some examples, the pharmaceutical composition is in a unit dose form. In some examples, the pharmaceutical composition comprises about $0.5 - 2 \times 10^9$ T cells. In some examples, the pharmaceutical composition is administered daily, weekly, bi-weekly, monthly, bi-month or yearly.

[0014a] In yet another aspect, the present disclosure refers to a nucleic acid sequence encoding a B-cell maturation antigen (BCMA) Trifunctional T cell antigen coupler (Tri-TAC), wherein the Tri-TAC comprises an amino acid sequence having at least 95%, at least 96%, at

least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, or SEQ ID NO: 62.

[0014b] In yet another aspect, the present disclosure refers to a polypeptide encoded by the nucleic acid sequence as disclosed herein.

[0014c] In yet another aspect, the present disclosure refers to a vector construct comprising: (a) a nucleic acid sequence as disclosed herein; and (b) a promoter functional in a mammalian cell.

[0014d] In yet another aspect, the present disclosure refers to a T cell expressing the nucleic acid sequence as disclosed herein.

[0014e] In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising the T cell as disclosed herein, and a pharmaceutically acceptable excipient.

[0014f] In yet another aspect, the present disclosure refers to the pharmaceutical composition as disclosed herein, for use in a method of treating a cancer in an individual in need thereof.

[0014g] In yet another aspect, the present disclosure refers to use of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for the treatment of cancer.

[0015] Disclosed herein, in certain examples, are nucleic acid sequences encoding a CD19 Trifunctional T cell-antigen coupler (CD19-TAC) comprising: (a) a first polynucleotide encoding a ligand that selectively binds a CD19 antigen; (b) a second polynucleotide encoding a humanized variant of a UCHT1 (huUCHT1) ligand comprising a Y177T mutation (huUCHT1 (Y177T)) that binds CD3; and (c) a third polynucleotide encoding a polypeptide comprising a CD4 cytosolic domain and a CD4 transmembrane domain; wherein ligand encoded by (a), ligand encoded by (b), and polypeptide encoded by (c) are fused directly to each other, or joined by at least one linker. In some examples, the nucleic acid sequence does not encode a co-stimulatory domain, an activation domain, or both a co-stimulatory domain and an activation domain. In some examples, the ligand that selectively binds the CD19 antigen is a single chain variable fragment (scFv). In some examples, the ligand that selectively binds the CD19 antigen comprises an amino acid sequence having at least 80%, at least 90%, or at least

95% sequence identity with SEQ ID NO: 36. In some examples, the ligand that selectively binds the CD19 antigen comprises an amino acid sequence of SEQ ID NO: 36. In some examples, the huUCHT1 (Y177T) ligand is a single chain antibody. In some examples, the huUCHT1 (Y177T) ligand comprises an amino acid sequence having at least 80%, at least 90%, or at least 95% sequence identity with SEQ ID NO: 46. In some examples, the huUCHT1 (Y177T) ligand comprises an amino acid sequence of SEQ ID NO: 46. In some examples, the third polynucleotide encodes a polypeptide comprising an amino acid sequence having at least 80%, at least 90%, or at least 95% sequence identity with SEQ ID NO: 18.

In some examples, the third polynucleotide encodes a polypeptide comprising an amino acid sequence of SEQ ID NO: 18. In some examples, the at least one linker is a G4S flexible linker, a large protein domain, a long helix structure, or a short helix structure. In some examples, the at least one linker comprises an amino acid sequence having at least 80%, at least 90%, or at least 95% sequence identity with SEQ ID NO: 12, SEQ ID NO: 32, SEQ ID NO: 30, or SEQ ID NO: 28. In some examples, the at least one linker comprises an amino acid sequence of SEQ ID NO: 12, SEQ ID NO: 32, SEQ ID NO: 30, or SEQ ID NO: 28. In some examples, the CD3 is expressed on a cell expressing the second polynucleotide. In some examples, the CD19-TAC comprises a nucleic acid sequence having at least 80%, at least 90%, or at least 95% sequence identity with SEQ ID NO: 63. In some examples, the CD19-TAC comprises an amino acid sequence having at least 80%, at least 90%, or at least 95% sequence identity with SEQ ID NO: 64. In some examples, the CD19-TAC comprises a sequence of SEQ ID NO: 63 or SEQ ID NO: 64. Disclosed herein, in certain examples, are vector constructs comprising: (a) a nucleic acid sequence disclosed herein; and (b) a promoter functional in a mammalian cell. Disclosed herein, in certain examples, are compositions comprising the vector disclosed herein, and an excipient. Disclosed herein, in certain examples, are polypeptides encoded by the nucleic acid sequence disclosed herein.

[0016] Disclosed herein, in certain examples, are nucleic acid sequences encoding a HER2 Trifunctional T cell-antigen coupler (HER2-TAC) comprising a nucleic acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 65, SEQ ID NO: 67, or SEQ ID NO: 75. In some examples, the HER2-TAC comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 66, SEQ ID NO: 68, or SEQ ID NO: 76. In some examples, the nucleic acid sequence does not encode a co-stimulatory domain. In some examples, the nucleic acid sequence does not encode an activation domain.

[0017] Disclosed herein, in certain examples, are nucleic acid sequences encoding a BCMA Trifunctional T cell-antigen coupler (BCMA-TAC) comprising a nucleic acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, or SEQ ID NO: 61. In some examples, the BCMA-TAC comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, or SEQ ID NO: 62. In some examples, the nucleic acid sequence does not encode a co-stimulatory domain. In some examples, the nucleic acid sequence does not encode an activation domain.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0019] Fig. 1A is a schematic of natural T-cell activation.

[0020] Fig. 1B is a schematic of CAR based T-cell activation.

[0021] Fig. 1C is a schematic of a trifunctional-T cell-antigen coupler (Tri-TAC) based T cell activation.

[0022] Fig. 1D is a schematic of natural T-cell activation.

[0023] Fig. 1E is a schematic of CAR based T-cell activation.

[0024] Fig. 1F is a schematic of Tri-TAC based T cell activation.

[0025] Fig. 2A is a schematic of a Tri-TAC configuration with the UCHT1 domain being centered between the trans-membrane domain (TM) and the antigen binding domain.

[0026] Fig. 2B is a schematic of a Tri-TAC configuration in which the UCHT1 domain is N-terminal, followed by the antigen binding domain and the trans-membrane domain.

[0027] Fig. 2C is a schematic of a Tri-TAC molecule with a generic antigen binding domain and a UCHT1 domain.

[0028] Fig. 3A is a schematic of a Tri-TAC molecule with a generic antigen binding domain.

[0029] Fig. 3B is a schematic of a Tri-TAC with an anti-HER-2 DARPIn antigen binding domain.

[0030] Fig. 3C is a schematic of a Tri-TAC with an anti-CD19 scFv antigen binding domain.

[0031] Fig. 3D is a schematic of a Tri-TAC with an anti-BCMA scFv antigen binding domain.

CLAIMS

WHAT IS CLAIMED IS:

1. A nucleic acid sequence encoding a CD19 Trifunctional T cell-antigen coupler (CD19-TAC) comprising:
 - (a) a first polynucleotide encoding a ligand that selectively binds a CD19 antigen, optionally wherein the ligand that selectively binds the CD19 antigen is a single chain variable fragment (scFv);
 - (b) a second polynucleotide encoding a UCHT1 ligand that binds CD3, optionally wherein the UCHT1 ligand is a single chain antibody; and
 - (c) a third polynucleotide encoding a TCR signaling domain polypeptide comprising a cytosolic domain and a transmembrane domain, optionally wherein the cytosolic domain is a CD4 cytosolic domain and the transmembrane domain is a CD4 transmembrane domain; wherein components encoded by (a), components encoded by (b), and components encoded by (c) are fused directly to each other, or joined by at least one linker and the nucleic acid sequence does not encode a co-stimulatory domain.
2. The nucleic acid sequence of claim 1, wherein the ligand that selectively binds the CD19 antigen comprises an amino acid sequence having least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 36.
3. The nucleic acid sequence of claim 1 or 2, wherein the UCHT1 ligand is a humanized variant of UCHT1 comprising a Y to T substitution at position 177 of SEQ ID NO: 44 (huUCHT1 (Y177T)).
4. The nucleic acid sequence of any one of claims 1-3, wherein the UCHT1 ligand comprises an amino acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 14, SEQ ID NO: 72, SEQ ID NO: 44, or SEQ ID NO: 46.
5. The nucleic acid sequence of any one of claims 1-4, wherein the third polynucleotide encodes a polypeptide comprises an amino acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 18.
6. The nucleic acid sequence of any one of claims 1-5, wherein the nucleic acid sequence comprises a nucleic acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 63.
7. The nucleic acid sequence of any one of claims 1-6, wherein the CD19-TAC comprises an amino acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 64.

8. The nucleic acid sequence of any one of claims 1-7, wherein the nucleic acid sequence does not encode an activation domain.
9. A polypeptide encoded by the nucleic acid sequence of any one of claims 1-8.
10. A vector construct comprising:
 - (a) a nucleic acid sequence of any one of claims 1-8; and
 - (b) a promoter functional in a mammalian cell.
11. A T cell expressing the nucleic acid sequence of any one of claims 1-8.
12. A pharmaceutical composition comprising the T cell of claim 11, and a pharmaceutically acceptable excipient.
13. The pharmaceutical composition of claim 12, for use in a method of treating a cancer in an individual in need thereof.
14. The pharmaceutical composition for use of claim 13, wherein the cancer comprises a CD19 expressing cancer cell.
15. The pharmaceutical composition for use of claim 13 or 14, wherein the cancer is a B cell malignancy.
16. The pharmaceutical composition for use of any one of claims 13-15, wherein the cancer is B cell lymphoma, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), or Non-Hodgkins Lymphoma.
17. Use of the pharmaceutical composition of claim 12 in the manufacture of a medicament for the treatment of cancer.
18. A nucleic acid sequence encoding a Human Epidermal Growth Factor Receptor 2 (HER2) Trifunctional T cell antigen coupler (Tri-TAC), wherein the Tri-TAC comprises an amino acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 66, SEQ ID NO: 68, or SEQ ID NO: 76.
19. The nucleic acid sequence of claim 18, wherein the nucleic acid sequence comprises a nucleic acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 65, SEQ ID NO: 67, or SEQ ID NO: 75 .
20. The nucleic acid sequence of claim 18 or 19, wherein the nucleic acid sequence does not encode a co-stimulatory domain and/or does not encode an activation domain.
21. A polypeptide encoded by the nucleic acid sequence of any one of claims 18-20.
22. A vector construct comprising:
 - (a) a nucleic acid sequence of any one of claims 18-20; and
 - (b) a promoter functional in a mammalian cell.
23. A T cell expressing the nucleic acid sequence of any one of claims 18-20.

24. A pharmaceutical composition comprising the T cell of claim 23, and a pharmaceutically acceptable excipient.
25. The pharmaceutical composition of claim 24, for use in a method of treating a cancer in an individual in need thereof.
26. The pharmaceutical composition for use of claim 25, wherein the cancer comprises a HER-2 expressing cancer cell.
27. The pharmaceutical composition for use of claim 25 or 26, wherein the cancer is breast cancer, bladder cancer, pancreatic cancer, ovarian cancer, or stomach cancer.
28. Use of the pharmaceutical composition of claim 24 in the manufacture of a medicament for the treatment of cancer.
29. A nucleic acid sequence encoding a B-cell maturation antigen (BCMA) Trifunctional T cell antigen coupler (Tri-TAC), wherein the Tri-TAC comprises an amino acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, or SEQ ID NO: 62.
30. The nucleic acid sequence of claim 29, wherein the nucleic acid sequence comprises a nucleic acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, or SEQ ID NO: 61.
31. The nucleic acid sequence of claim 29 or 30, wherein the nucleic acid sequence does not encode a co-stimulatory domain and/or does not encode an activation domain.
32. A polypeptide encoded by the nucleic acid sequence of any one of claims 29-31.
33. A vector construct comprising:
- (a) a nucleic acid sequence of any one of claims 29-31; and
 - (b) a promoter functional in a mammalian cell.
34. A T cell expressing the nucleic acid sequence of any one of claims 29-31.
35. A pharmaceutical composition comprising the T cell of claim 34, and a pharmaceutically acceptable excipient.
36. The pharmaceutical composition of claim 35, for use in a method of treating a cancer in an individual in need thereof.
37. The pharmaceutical composition for use of claim 36, wherein the cancer comprises a BCMA expressing cancer cell.
38. The pharmaceutical composition for use of claim 36 or 37, wherein the cancer is leukemia, lymphoma, or multiple myeloma.
39. Use of the pharmaceutical composition of claim 35 in the manufacture of a medicament for the treatment of cancer.