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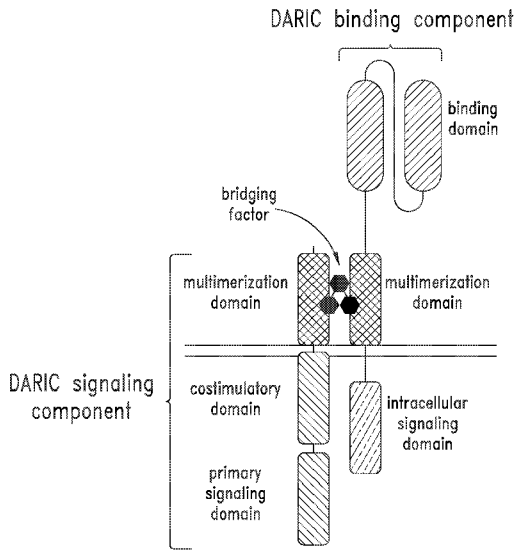

(57) Abstract: The present disclosure provides improved compositions for adoptive T cell therapies for treating, preventing, or ameliorating at least one symptom of a cancer, infectious disease, autoimmune disease, inflammatory disease, and immunodeficiency, or condition associated therewith.

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DIMERIZING AGENT REGULATED IMMUNORECEPTOR COMPLEXES

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 62/835,659, filed April 18, 2019, and U.S. Provisional Application No. 62/779,971, filed December 14, 2018, each of which is incorporated by reference herein in its entirety.

STATEMENT REGARDING SEQUENCE LISTING

The Sequence Listing associated with this application is provided in text format in lieu of a paper copy and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is BLBD_113_02WO_ST25.txt. The text file is 157 KB, was created on December 11, 2019, and is being submitted electronically via EFS-Web, concurrent with the filing of the specification.

BACKGROUND

Technical Field

The present disclosure relates to improved adoptive cell therapies. More particularly, the disclosure relates to improved chemically regulated signaling molecules, cells, and methods of using the same for modulating spatial and temporal control of cellular signal initiation and downstream responses during adoptive immunotherapy.

Description of the Related Art

The global burden of cancer doubled between 1975 and 2000. Cancer is the second leading cause of morbidity and mortality worldwide, with approximately 14.1 million new cases and 8.2 million cancer related deaths in 2012. The most common cancers are breast cancer, lung and bronchus cancer, prostate cancer, colon and rectum cancer, bladder cancer, melanoma of the skin, non-Hodgkin lymphoma, thyroid cancer, kidney and renal

pelvis cancer, endometrial cancer, leukemia, and pancreatic cancer. The number of new cancer cases is projected to rise to 23.6 million by 2030.

Adoptive cellular therapy is emerging as a powerful paradigm for delivering complex biological signals to treat cancer. In contrast to small molecule and biologic drug compositions, adoptive cell therapies have the potential to execute unique therapeutic tasks owing to their myriad sensory and response programs and increasingly defined mechanisms of genetic control. To achieve such therapeutic value, cells need to be outfitted with machinery for sensing and integrating chemical and/or biological information associated with local physiological environments.

BRIEF SUMMARY

The present disclosure generally relates, in part, to dimerizing agent regulated immunoreceptor complex (DARIC) compositions, polynucleotides, polypeptides and methods of making and using the same.

In various embodiments, a non-natural cell comprises a first polypeptide comprising: a first multimerization domain polypeptide or variant thereof; a first transmembrane domain; a first costimulatory domain; and/or a primary signaling domain; and a second polypeptide comprising: an extracellular binding domain; a second multimerization domain polypeptide or variant thereof; a second transmembrane domain; and a second costimulatory domain; wherein a bridging factor promotes the formation of a polypeptide complex on the non-natural cell surface with the bridging factor associated with and disposed between the first and second multimerization domains.

In certain embodiments, the first and second multimerization domains are different.

In particular embodiments, the first and second costimulatory domains are different.

In further embodiments, the first multimerization domain and the second multimerization domain associate with a bridging factor selected from the group consisting of: rapamycin or a rapalog thereof, coumermycin or a derivative thereof, gibberellin or a derivative thereof, abscisic acid (ABA) or a derivative thereof, methotrexate or a derivative thereof, cyclosporin A or a derivative thereof, FK506/cyclosporin A (FKCsA) or a

derivative thereof, and trimethoprim (Tmp)-synthetic ligand for FK506 binding protein (FKBP) (SLF) or a derivative thereof.

In various embodiments, the first multimerization domain and the second multimerization domain are a pair selected from the group consisting of: FKBP and FKBP-rapamycin binding (FRB), FKBP and calcineurin, FKBP and cyclophilin, FKBP and bacterial dihydrofolate reductase (DHFR), calcineurin and cyclophilin, and PYR1-like 1 (PYL1) and abscisic acid insensitive 1 (ABI1).

In some embodiments, the first multimerization domain comprises an FKBP polypeptide or variant thereof, and the second multimerization domain comprises an FRB polypeptide or variant thereof.

In particular embodiments, the first multimerization domain comprises an FRB polypeptide or variant thereof, and the second multimerization domain comprises an FKBP polypeptide or variant thereof.

In additional embodiments, the bridging factor is selected from the group consisting of: AP21967, sirolimus, everolimus, novolimus, pimecrolimus, ridaforolimus, tacrolimus, temsirolimus, umirolimus, and zotarolimus.

In various embodiments, the first and second multimerization domains are selected from FRB T2098L and FKBP12; and the bridging factor is sirolimus or AP21967.

In certain embodiments, the first transmembrane domain and the second transmembrane domain are independently selected from a polypeptide selected from the group consisting of: alpha, beta, gamma, or delta chain of the T-cell receptor, CD3δ, CD3ε, CD3γ, CD3ζ, CD4, CD5, CD8α, CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD 134, CD137, CD152, CD154, CD278, amnionless (AMN), and programmed cell death 1 (PDCD1).

In further embodiments, the first transmembrane domain and the second transmembrane domain are independently selected from the group consisting of: a CD4 transmembrane domain, a CD8α transmembrane domain, and an AMN transmembrane domain.

In particular embodiments, the first transmembrane domain and the second transmembrane domain are different.

In some embodiments, the costimulatory domain and/or the primary signaling domain comprise an immunoreceptor tyrosine activation motif (ITAM).

In additional embodiments, the first and second costimulatory domain are independently selected from a costimulatory molecule selected from the group consisting of: Toll-like receptor 1 (TLR1), TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, caspase recruitment domain family member 11 (CARD11), CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD94, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), DNAX-Activation Protein 10 (DAP10), Linker for activation of T-cells family member 1 (LAT), SH2 Domain-Containing Leukocyte Protein Of 76 kD (SLP76), T cell receptor associated transmembrane adaptor 1 (TRAT1), TNFR2, TNFRS14, TNFRS18, TNFRS25, and zeta chain of T cell receptor associated protein kinase 70 (ZAP70).

In various embodiments, the first costimulatory domain is isolated from a costimulatory molecule selected from the group consisting of: CD28, CD134, and CD137 and the second costimulatory domain is isolated from CD28, CD278, TNFRS14, TNFRS18, TNFRS25, OX40 or TNFR2.

In particular embodiments, the first costimulatory domain is isolated from CD137 and the second costimulatory domain is isolated from OX40 or TNFR2.

In further embodiments, the first costimulatory domain is isolated from CD137 and the second costimulatory domain is isolated from OX40.

In various embodiments, the first costimulatory domain is isolated from CD137 and the second costimulatory domain is isolated from TNFR2.

In certain embodiments, the primary signaling domain isolated from a polypeptide selected from the group consisting of: FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d.

In some embodiments, the primary signaling domain is isolated from a CD3ζ polypeptide.

In additional embodiments, the extracellular binding domain comprises an antibody or antigen binding fragment thereof, a receptor ectodomain, or a ligand.

In various embodiments, the extracellular binding domain comprises an antibody or antigen binding fragment thereof selected from the group consisting of: a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a F(ab')2 fragment, a bispecific Fab dimer (Fab2), a trispecific Fab trimer (Fab3), an Fv, an single chain Fv protein ("scFv"), a bis-scFv, (scFv)2, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody).

In further embodiments, the extracellular binding domain comprises a humanized antibody or antigen binding fragment thereof.

In particular embodiments, the extracellular binding domain comprises a human antibody or antigen binding fragment thereof.

In additional embodiments, the extracellular binding domain comprises an scFv.

In particular embodiments, the extracellular binding domain comprises one or more camelid VHH antibodies.

In certain embodiments, the extracellular binding domain binds an antigen selected from the group consisting of: tumor associated antigens (TAA), tumor specific antigens (TSA), NKG2D ligands, γδ T cell receptor (TCR) ligands, and αβ TCR ligands.

In various embodiments, the extracellular binding domain binds an antigen selected from the group consisting of: alpha folate receptor (FRα), $\alpha_v\beta_6$ integrin, B cell maturation antigen (BCMA), B7-H3 (CD276), B7-H6, carbonic anhydrase IX (CAIX), CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, carcinoembryonic antigen (CEA), claudin 6, (CLDN6), claudin 18 isoform 2 (CLDN18.2), C-type lectin-like molecule-1 (CLL-1), CD2 subset 1 (CS-1), chondroitin sulfate proteoglycan 4 (CSPG4), cutaneous T cell lymphoma-associated antigen 1 (CTAGE1), delta like canonical Notch ligand 3 (DLL3), epidermal growth factor receptor (EGFR), epidermal growth factor receptor variant III (EGFRvIII), epithelial glycoprotein 2 (EGP2), epithelial glycoprotein 40 (EGP40), epithelial cell adhesion molecule (EPCAM), ephrin type-A receptor 2 (EPHA2), erb-b2 receptor tyrosine

kinase 4 (ERBB4), fibroblast activation protein (FAP), Fc Receptor Like 5 (FCRL5), fetal acetylcholinesterase receptor (AchR), ganglioside G2 (GD2), ganglioside G3 (GD3), Glypican-3 (GPC3), EGFR family including ErbB2 (HER2), HER2 p95, IL-10Rα, IL-13Rα2, Kappa, cancer/testis antigen 2 (LAGE-1A), Lambda, Lewis-Y (LeY), L1 cell adhesion molecule (L1-CAM), melanoma antigen gene (MAGE)-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGEA10, melanoma antigen recognized by T cells 1 (MelanA or MART1), Mesothelin (MSLN), MUC1, MUC16, MHC class I chain related proteins A (MICA), MHC class I chain related proteins B (MICB), neural cell adhesion molecule (NCAM), cancer/testis antigen 1 (NY-ESO-1), polysialic acid; placenta-specific 1 (PLAC1), preferentially expressed antigen in melanoma (PRAME), prostate stem cell antigen (PSCA), prostate-specific membrane antigen (PSMA), receptor tyrosine kinase-like orphan receptor 1 (ROR1), synovial sarcoma, X breakpoint 2 (SSX2), Survivin, tumor associated glycoprotein 72 (TAG72), tumor endothelial marker 1 (TEM1/CD248), tumor endothelial marker 7-related (TEM7R), trophoblast glycoprotein (TPBG), UL16-binding protein (ULBP) 1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, vascular endothelial growth factor receptor 2 (VEGFR2), and Wilms tumor 1 (WT-1).

In further embodiments, a non-natural cell comprises a first polypeptide comprising: an FK506 binding protein (FKBP) multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain; and a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP-rapamycin binding (FRB) multimerization domain polypeptide or variant thereof; a second transmembrane domain; and an OX40 costimulatory domain; wherein a bridging factor promotes the formation of a polypeptide complex on the non-natural cell surface with the bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

In various embodiments, a non-natural cell comprises a first polypeptide comprising: an FKBP multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain; and a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FRB

multimerization domain polypeptide or variant thereof; a second transmembrane domain; and a TNFR2 costimulatory domain; wherein a bridging factor promotes the formation of a polypeptide complex on the non-natural cell surface with the bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

In certain embodiments, a non-natural cell comprises a first polypeptide comprising: an FRB multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain; and a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP multimerization domain polypeptide or variant thereof; a second transmembrane domain; and an OX40 costimulatory domain; wherein a bridging factor promotes the formation of a polypeptide complex on the non-natural cell surface with the bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

In various embodiments, a non-natural cell comprises a first polypeptide comprising: an FRB multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain; and a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP multimerization domain polypeptide or variant thereof; a second transmembrane domain; and a TNFR2 costimulatory domain; wherein a bridging factor promotes the formation of a polypeptide complex on the non-natural cell surface with the bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

In additional embodiments, the bridging factor is selected from the group consisting of: AP21967, sirolimus, everolimus, novolimus, pimecrolimus, ridaforolimus, tacrolimus, temsirolimus, umirolimus, and zotarolimus.

In further embodiments, the FRB multimerization domain is FRB T2098L; the FKBP multimerization domain is FKBP12; and the bridging factor is sirolimus or AP21967.

In particular embodiments, the first transmembrane domain and the second transmembrane domain are independently selected from a polypeptide selected from the group consisting of: alpha, beta, gamma, or delta chain of the T-cell receptor, CD3δ, CD3ε, CD3γ, CD3ζ, CD4, CD5, CD8α, CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD 134, CD137, CD152, CD154, AMN, and PDCD1.

In various embodiments, the first transmembrane domain and the second transmembrane domain are independently selected from a polypeptide selected from the group consisting of: a CD4 transmembrane domain and a CD8α transmembrane domain.

In some embodiments, the costimulatory domain and/or the primary signaling domain comprise an immunoreceptor tyrosine activation motif (ITAM).

In certain embodiments, the costimulatory domain is isolated from a costimulatory molecule selected from the group consisting of: Toll-like receptor 1 (TLR1), TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, caspase recruitment domain family member 11 (CARD11), CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD94, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), DNAX-Activation Protein 10 (DAP10), Linker for activation of T-cells family member 1 (LAT), SH2 Domain-Containing Leukocyte Protein Of 76 kD (SLP76), T cell receptor associated transmembrane adaptor 1 (TRAT1), TNFR2, TNFRS14, TNFRS18, TNFRS25, and zeta chain of T cell receptor associated protein kinase 70 (ZAP70).

In additional embodiments, the costimulatory domain is isolated from a costimulatory molecule selected from the group consisting of: CD28, CD134, and CD137.

In various embodiments, the costimulatory domain is isolated from a CD137 costimulatory molecule.

In certain embodiments, the primary signaling domain isolated from a polypeptide selected from the group consisting of: FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d.

In particular embodiments, the primary signaling domain is isolated from a CD3ζ polypeptide.

In some embodiments, the antibody or antigen binding fragment thereof selected is from the group consisting of: a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a F(ab')2 fragment, a bispecific Fab dimer (Fab2), a trispecific Fab trimer (Fab3), an Fv, an single chain Fv protein ("scFv"), a bis-scFv, (scFv)2, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody).

In various embodiments, the antibody or antigen binding fragment thereof is human or humanized.

In various embodiments, the antibody or antigen binding fragment thereof comprises an scFv or one or more camelid VHH antibodies.

In further embodiments, the antibody or antigen binding fragment thereof binds an antigen selected from the group consisting of: tumor associated antigens (TAA), tumor specific antigens (TSA), NKG2D ligands, γδ T cell receptor (TCR) ligands, and αβ TCR ligands.

In certain embodiments, the antibody or antigen binding fragment thereof binds an antigen selected from the group consisting of: FRα, $\alpha_v\beta_6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, CEA, CLDN6, CLDN18.2, CLL-1, CS-1, CSPG4, CTAGE1, DLL3, EGFR, EGFRvIII, EGP2, EGP40, EPCAM, EPHA2, ERBB4, FAP, FCRL5, AchR, GD2, GD3, GPC3, HER2, HER2 p95, IL-10Rα, IL-13Rα2, Kappa, LAGE-1A, Lambda, LeY, L1-CAM, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGEA10, MelanA or MART1, SLN), MUC1, MUC16, MICA, MICB, NCAM, NY-ESO-1, PLAC1, PRAME, PSCA, PSMA, ROR1, SSX2, Survivin, TAG72, TEM1/CD248, TEM7R, TPBG, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, VEGFR2, and WT-1.

In additional embodiments, the antibody or antigen binding fragment thereof binds BCMA, B7-H3, CLDN6, CLDN18.2, DLL3, ERBB4, HER2, HER2 p95, MUC16, MICA, MICB, TAG72, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and ULBP6.

In additional embodiments, the antibody or antigen binding fragment thereof binds BCMA, B7-H3, CD19, CD20, CD22, CD33, CD79A, CD79B, and/or EGFRvIII.

In some embodiments, the antibody or antigen binding fragment thereof binds BCMA.

In various embodiments, the antibody or antigen binding fragment thereof binds CD19.

In particular embodiments, the antibody or antigen binding fragment thereof binds CD20 or CD22.

In various embodiments, the antibody or antigen binding fragment thereof binds B7-H3.

In further embodiments, the antibody or antigen binding fragment thereof binds CD33.

In additional embodiments, the antibody or antigen binding fragment thereof binds CD79A.

In various embodiments, the antibody or antigen binding fragment thereof binds CD79B.

In particular embodiments, the antibody or antigen binding fragment thereof binds EGFRvIII.

In some embodiments, a non-natural cell comprises a first polypeptide comprising: an FKBP12 multimerization domain polypeptide or variant thereof; a CD8α transmembrane domain; a CD137 costimulatory domain; and a CD3ζ primary signaling domain; and a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FRB T2098L multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and a TNFR2 costimulatory domain; wherein a bridging factor promotes the formation of a polypeptide complex on the non-natural cell surface with the bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

In various embodiments, a non-natural cell comprises a first polypeptide comprising: an FKBP12 multimerization domain polypeptide or variant thereof; a CD8α

transmembrane domain; a CD137 costimulatory domain; and a CD3ζ primary signaling domain; and a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FRB T2098L multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and an OX40 costimulatory domain; wherein a bridging factor promotes the formation of a polypeptide complex on the non-natural cell surface with the bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

In certain embodiments, a non-natural cell comprises a first polypeptide comprising: an FRB T2098L multimerization domain polypeptide or variant thereof; a CD8α transmembrane domain; a CD137 costimulatory domain; and a CD3ζ primary signaling domain; and a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP12 multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and a TNFR2 costimulatory domain; wherein a bridging factor promotes the formation of a polypeptide complex on the non-natural cell surface with the bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

In various embodiments, a non-natural cell comprises a first polypeptide comprising: an FRB T2098L multimerization domain polypeptide or variant thereof; a CD8α transmembrane domain; a CD137 costimulatory domain; and a CD3ζ primary signaling domain; and a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP12 multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and an OX40 costimulatory domain; wherein a bridging factor promotes the formation of a polypeptide complex on the non-natural cell surface with the bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

In further embodiments, the bridging factor is AP21967 or sirolimus.

In some embodiments, the antibody or antigen binding fragment thereof selected is from the group consisting of: a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a F(ab')2 fragment, a bispecific Fab dimer (Fab2), a trispecific Fab trimer (Fab3),

an Fv, an single chain Fv protein ("scFv"), a bis-scFv, (scFv)2, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody).

In particular embodiments, the antibody or antigen binding fragment thereof is human or humanized.

In additional embodiments, the antibody or antigen binding fragment thereof comprises an scFv or one or more camelid VHH antibodies.

In various embodiments, the antibody or antigen binding fragment thereof binds an antigen selected from the group consisting of: tumor associated antigens (TAA), tumor specific antigens (TSA), NKG2D ligands, γδ T cell receptor (TCR) ligands, and αβ TCR ligands.

In certain embodiments, the antibody or antigen binding fragment thereof binds an antigen selected from the group consisting of: FRα, $\alpha_v\beta_6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, CEA, CLDN6, CLDN18.2, CLL-1, CS-1, CSPG4, CTAGE1, DLL3, EGFR, EGFRvIII, EGP2, EGP40, EPCAM, EPHA2, ERBB4, FAP, FCRL5, AchR, GD2, GD3, GPC3, HER2, HER2 p95, IL-10Rα, IL-13Rα2, Kappa, LAGE-1A, Lambda, LeY, L1-CAM, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGEA10, MelanA or MART1, SLN), MUC1, MUC16, MICA, MICB, NCAM, NY-ESO-1, PLAC1, PRAME, PSCA, PSMA, ROR1, SSX2, Survivin, TAG72, TEM1/CD248, TEM7R, TPBG, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, VEGFR2, and WT-1.

In particular embodiments, the antibody or antigen binding fragment thereof binds BCMA, B7-H3, CLDN6, CLDN18.2, DLL3, ERBB4, HER2, HER2 p95, MUC16, MICA, MICB, TAG72, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and ULBP6.

In particular embodiments, the antibody or antigen binding fragment thereof binds BCMA, B7-H3, CD19, CD20, CD22, CD33, CD79A, CD79B, and/or EGFRvIII.

In particular embodiments, the antibody or antigen binding fragment thereof binds BCMA.

In further embodiments, the antibody or antigen binding fragment thereof binds CD19.

In various embodiments, the antibody or antigen binding fragment thereof binds CD20 or CD22.

In some embodiments, the antibody or antigen binding fragment thereof binds B7H3.

In additional embodiments, the antibody or antigen binding fragment thereof binds CD33.

In various embodiments, the antibody or antigen binding fragment thereof binds CD79A.

In some embodiments, the antibody or antigen binding fragment thereof binds CD79B.

In further embodiments, the antibody or antigen binding fragment thereof binds EGFRvIII, optionally wherein the antibody is EGFR806 or an antigen binding fragment thereof.

In particular embodiments, the multimerization domains localize extracellularly when of the first polypeptide and the second polypeptide are expressed.

In various embodiments, the cell is a hematopoietic cell.

In additional embodiments, the cell is a T cell.

In various embodiments, the cell is a CD3+, CD4+, and/or CD8+ cell.

In particular embodiments, the cell is an immune effector cell.

In further embodiments, the cell is a cytotoxic T lymphocyte (CTL), a tumor infiltrating lymphocyte (TIL), or a helper T cell.

In certain embodiments, the cell is a natural killer (NK) cell or natural killer T (NKT) cell.

In additional embodiments, the source of the cell is peripheral blood mononuclear cells, bone marrow, lymph nodes tissue, cord blood, thymus issue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, or tumors.

In various embodiments, a fusion polypeptide comprises a first polypeptide comprising: a first multimerization domain polypeptide or variant thereof; a first transmembrane domain; a first costimulatory domain; and/or a primary signaling domain; and a polypeptide cleavage signal; and a second polypeptide comprising: an extracellular binding domain; a second multimerization domain polypeptide or variant thereof; a second transmembrane domain; and a second costimulatory domain.

In some embodiments, the first and second multimerization domains are different.

In some embodiments, the first and second costimulatory domains are different.

In particular embodiments, the first multimerization domain and the second multimerization domain associate with a bridging factor selected from the group consisting of: rapamycin or a rapalog thereof, coumermycin or a derivative thereof, gibberellin or a derivative thereof, abscisic acid (ABA) or a derivative thereof, methotrexate or a derivative thereof, cyclosporin A or a derivative thereof, FK506/cyclosporin A (FKCsA) or a derivative thereof, and trimethoprim (Tmp)-synthetic ligand for FK506 binding protein (FKBP) (SLF) or a derivative thereof.

In further embodiments, the first multimerization domain and the second multimerization domain are a pair selected from the group consisting of: FKBP and FKBP-rapamycin binding (FRB), FKBP and calcineurin, FKBP and cyclophilin, FKBP and bacterial dihydrofolate reductase (DHFR), calcineurin and cyclophilin, and PYR1-like 1 (PYL1) and abscisic acid insensitive 1 (ABI1).

In various embodiments, the first multimerization domain comprises an FKBP polypeptide or variant thereof, and the second multimerization domain comprises an FRB polypeptide or variant thereof.

In additional embodiments, the first multimerization domain comprises an FRB polypeptide or variant thereof, and the second multimerization domain comprises an FKBP polypeptide or variant thereof.

In certain embodiments, the bridging factor is selected from the group consisting of: AP21967, sirolimus, everolimus, novolimus, pimecrolimus, ridaforolimus, tacrolimus, temsirolimus, umirolimus, and zotarolimus.

In various embodiments, the first and second multimerization domains are selected from FRB T2098L and FKBP12; and the bridging factor is sirolimus or AP21967.

In further embodiments, the first transmembrane domain and the second transmembrane domain are independently selected from a polypeptide selected from the group consisting of: alpha, beta, gamma, or delta chain of the T-cell receptor, CD3δ, CD3ε, CD3γ, CD3ζ, CD4, CD5, CD8α, CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD 134, CD137, CD152, CD154, CD278, amnionless (AMN), and programmed cell death 1 (PDCD1).

In particular embodiments, the first transmembrane domain and the second transmembrane domain are independently selected from the group consisting of: a CD4 transmembrane domain, a CD8α transmembrane domain, and an AMN transmembrane domain.

In additional embodiments, the first transmembrane domain and the second transmembrane domain are different.

In certain embodiments, the costimulatory domain and/or the primary signaling domain comprise an immunoreceptor tyrosine activation motif (ITAM).

In various embodiments, the first and second costimulatory domain are independently selected from a costimulatory molecule selected from the group consisting of: Toll-like receptor 1 (TLR1), TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, caspase recruitment domain family member 11 (CARD11), CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD94, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), DNAX-Activation Protein 10 (DAP10), Linker for activation of T-cells family member 1 (LAT), SH2 Domain-Containing Leukocyte Protein Of 76 kD (SLP76), T cell receptor associated transmembrane adaptor 1 (TRAT1), TNFR2, TNFRS14, TNFRS18, TNFRS25, and zeta chain of T cell receptor associated protein kinase 70 (ZAP70).

In particular embodiments, the first costimulatory domain is isolated from a costimulatory molecule selected from the group consisting of: CD28, CD134, and CD137

and the second costimulatory domain is isolated from CD28, CD278, TNFRS14, TNFRS18, TNFRS25, OX40 or TNFR2.

In some embodiments, the first costimulatory domain is isolated from CD137 and the second costimulatory domain is isolated from OX40 or TNFR2.

In further embodiments, the first costimulatory domain is isolated from CD137 and the second costimulatory domain is isolated from OX40.

In various embodiments, the first costimulatory domain is isolated from CD137 and the second costimulatory domain is isolated from TNFR2.

In additional embodiments, the primary signaling domain isolated from a polypeptide selected from the group consisting of: FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d.

In particular embodiments, the primary signaling domain is isolated from a CD3ζ polypeptide.

In various embodiments, the extracellular binding domain comprises an antibody or antigen binding fragment thereof, a receptor ectodomain, or a ligand.

In additional embodiments, the extracellular binding domain comprises an antibody or antigen binding fragment thereof selected from the group consisting of: a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a F(ab')2 fragment, a bispecific Fab dimer (Fab2), a trispecific Fab trimer (Fab3), an Fv, an single chain Fv protein ("scFv"), a bis-scFv, (scFv)2, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody).

In further embodiments, the extracellular binding domain comprises a humanized antibody or antigen binding fragment thereof.

In various embodiments, the extracellular binding domain comprises a human antibody or antigen binding fragment thereof.

In some embodiments, the extracellular binding domain comprises an scFv.

In certain embodiments, the extracellular binding domain comprises one or more camelid VHH antibodies.

In additional embodiments, the extracellular binding domain binds an antigen selected from the group consisting of: tumor associated antigens (TAA), tumor specific antigens (TSA), NKG2D ligands, γδ T cell receptor (TCR) ligands, and αβ TCR ligands.

In various embodiments, the extracellular binding domain binds an antigen selected from the group consisting of: FRα, $\alpha_v\beta_6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, CEA, CLDN6, CLDN18.2, CLL-1, CS-1, CSPG4, CTAGE1, DLL3, EGFR, EGFRvIII, EGP2, EGP40, EPCAM, EPHA2, ERBB4, FAP, FCRL5, AchR, GD2, GD3, GPC3, HER2, HER2 p95, IL-10Rα, IL-13Rα2, Kappa, LAGE-1A, Lambda, LeY, L1-CAM, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGEA10, MelanA or MART1, SLN), MUC1, MUC16, MICA, MICB, NCAM, NY-ESO-1, PLAC1, PRAME, PSCA, PSMA, ROR1, SSX2, Survivin, TAG72, TEM1/CD248, TEM7R, TPBG, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, VEGFR2, and WT-1.

In various embodiments, the extracellular binding domain binds an antigen selected from the group consisting of: BCMA, B7-H3, CLDN6, CLDN18.2, DLL3, ERBB4, HER2, HER2 p95, MUC16, MICA, MICB, TAG72, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and ULBP6.

In various embodiments, the extracellular binding domain binds an antigen selected from the group consisting of: BCMA, B7-H3, CD19, CD20, CD22, CD33, CD79A, CD79B, and/or EGFRvIII.

In additional embodiments, a fusion polypeptide comprises a first polypeptide comprising: a first multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain; a polypeptide cleavage signal; and a second polypeptide comprising: an extracellular binding domain; a second multimerization domain polypeptide or variant thereof; a second transmembrane domain; and an OX40 costimulatory domain.

In particular embodiments, a fusion polypeptide comprises a first polypeptide comprising: a first multimerization domain polypeptide or variant thereof; a first

transmembrane domain; a costimulatory domain; and/or a primary signaling domain; a polypeptide cleavage signal; and a second polypeptide comprising: an extracellular binding domain; a second multimerization domain polypeptide or variant thereof; a second transmembrane domain; and a TNFR2 costimulatory domain.

In further embodiments, the first and second multimerization domains are the same.

In certain embodiments, the first and second multimerization domains are different.

In various embodiments, the first multimerization domain and the second multimerization domain associate with a bridging factor selected from the group consisting of: rapamycin or a rapalog thereof, coumermycin or a derivative thereof, gibberellin or a derivative thereof, ABA or a derivative thereof, methotrexate or a derivative thereof, cyclosporin A or a derivative thereof, FKCsA or a derivative thereof, and SLF or a derivative thereof.

In particular embodiments, the first multimerization domain and the second multimerization domain are a pair selected from the group consisting of: FKBP and FRB, FKBP and calcineurin, FKBP and cyclophilin, FKBP and DHFR, calcineurin and cyclophilin, and PYL1 and ABI1.

In additional embodiments, the first multimerization domain comprises an FKBP polypeptide or variant thereof, and the second multimerization domain comprises an FRB polypeptide or variant thereof.

In particular embodiments, the first multimerization domain comprises an FRB polypeptide or variant thereof, and the second multimerization domain comprises an FKBP polypeptide or variant thereof.

In some embodiments, the first transmembrane domain and the second transmembrane domain are independently selected from a polypeptide selected from the group consisting of: alpha, beta, gamma, or delta chain of the T-cell receptor, CD3δ, CD3ε, CD3γ, CD3ζ, CD4, CD5, CD8α, CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD 134, CD137, CD152, CD154, AMN, and PDCD1.

In further embodiments, the first transmembrane domain and the second transmembrane domain are independently selected from the group consisting of: a CD4 transmembrane domain and a CD8α transmembrane domain.

In particular embodiments, the first transmembrane domain and the second transmembrane domain are the same.

In additional embodiments, the first transmembrane domain and the second transmembrane domain are different.

In various embodiments, the costimulatory domain and/or the primary signaling domain comprise an immunoreceptor tyrosine activation motif (ITAM).

In further embodiments, the costimulatory domain is isolated from a costimulatory molecule selected from the group consisting of: TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, CARD11, CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD94, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), DAP10, LAT, SLP76, TRAT1, TNFR2, TNFRS14, TNFRS18, TNFRS25, and ZAP70.

In particular embodiments, the costimulatory domain is isolated from a costimulatory molecule selected from the group consisting of: CD28, CD134, and CD137.

In some embodiments, the costimulatory domain is isolated from a CD137 costimulatory molecule.

In certain embodiments, the primary signaling domain isolated from a polypeptide selected from the group consisting of: FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d.

In additional embodiments, the primary signaling domain is isolated from a CD3ζ polypeptide.

In various embodiments, the extracellular binding domain comprises an antibody or antigen binding fragment thereof, a receptor ectodomain, or a ligand.

In certain embodiments, the extracellular binding domain comprises an antibody or antigen binding fragment thereof selected from the group consisting of: a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a F(ab')2 fragment, a bispecific Fab dimer (Fab2), a trispecific Fab trimer (Fab3), an Fv, an single chain Fv protein ("scFv"), a

bis-scFv, (scFv)2, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody).

In particular embodiments, the extracellular binding domain comprises a humanized antibody or antigen binding fragment thereof.

In further embodiments, the extracellular binding domain comprises a human antibody or antigen binding fragment thereof.

In particular embodiments, the extracellular binding domain comprises an scFv.

In additional embodiments, the extracellular binding domain comprises one or more camelid VHH antibodies.

In various embodiments, the extracellular binding domain binds an antigen selected from the group consisting of: tumor associated antigens (TAA), tumor specific antigens (TSA), NKG2D ligands, γδ T cell receptor (TCR) ligands, and αβ TCR ligands.

In further embodiments, the extracellular binding domain binds an antigen selected from the group consisting of: FRα, $\alpha_v\beta_6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, CEA, CLDN6, CLDN18.2, CLL-1, CS-1, CSPG4, CTAGE1, DLL3, EGFR, EGFRvIII, EGP2, EGP40, EPCAM, EPHA2, ERBB4, FAP, FCRL5, AchR, GD2, GD3, GPC3, HER2, HER2 p95, IL-10Rα, IL-13Rα2, Kappa, LAGE-1A, Lambda, LeY, L1-CAM, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGEA10, MelanA or MART1, SLN), MUC1, MUC16, MICA, MICB, NCAM, NY-ESO-1, PLAC1, PRAME, PSCA, PSMA, ROR1, SSX2, Survivin, TAG72, TEM1/CD248, TEM7R, TPBG, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, VEGFR2, and WT-1.

In various embodiments, the extracellular binding domain binds an antigen selected from the group consisting of: BCMA, B7-H3, CLDN6, CLDN18.2, DLL3, ERBB4, HER2, HER2 p95, MUC16, MICA, MICB, TAG72, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and ULBP6.

In various embodiments, the extracellular binding domain binds an antigen selected from the group consisting of: BCMA, B7-H3, CD19, CD20, CD22, CD33, CD79A, CD79B, and/or EGFRvIII.

In particular embodiments, a fusion polypeptide comprises a first polypeptide comprising: an FKBP multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain; a polypeptide cleavage signal; and a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FRB multimerization domain polypeptide or variant thereof; a second transmembrane domain; and an OX40 costimulatory domain.

In some embodiments, a fusion polypeptide comprises a first polypeptide comprising: an FKBP multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain; a polypeptide cleavage signal; and a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FRB multimerization domain polypeptide or variant thereof; a second transmembrane domain; and a TNFR2 costimulatory domain.

In various embodiments, a fusion polypeptide comprises a first polypeptide comprising: an FRB multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain; a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP multimerization domain polypeptide or variant thereof; a second transmembrane domain; and an OX40 costimulatory domain.

In additional embodiments, a fusion polypeptide comprises a first polypeptide comprising: an FRB multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain; a polypeptide cleavage signal; and a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP multimerization domain polypeptide or variant thereof; a second transmembrane domain; and a TNFR2 costimulatory domain.

In particular embodiments, the first transmembrane domain and the second transmembrane domain are independently selected from a polypeptide selected from the

group consisting of: alpha, beta, gamma, or delta chain of the T-cell receptor, CD3δ, CD3ε, CD3γ, CD3ζ, CD4, CD5, CD8α, CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD 134, CD137, CD152, CD154, AMN, and PDCD1.

In various embodiments, the first transmembrane domain and the second transmembrane domain are independently selected from a polypeptide selected from the group consisting of: a CD4 transmembrane domain and a CD8α transmembrane domain.

In further embodiments, the costimulatory domain and/or the primary signaling domain comprise an immunoreceptor tyrosine activation motif (ITAM).

In certain embodiments, the costimulatory domain is isolated from a costimulatory molecule selected from the group consisting of: TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, CARD11, CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD94, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), DAP10, LAT, SLP76, TRAT1, TNFR2, TNFRS14, TNFRS18, TNFRS25,and ZAP70.

In various embodiments, the costimulatory domain is isolated from a costimulatory molecule selected from the group consisting of: CD28, CD134, and CD137.

In additional embodiments, the costimulatory domain is isolated from a CD137 costimulatory molecule.

In some embodiments, the primary signaling domain isolated from a polypeptide selected from the group consisting of: FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d.

In further embodiments, the primary signaling domain is isolated from a CD3ζ polypeptide.

In various embodiments, the antibody or antigen binding fragment thereof selected is from the group consisting of: a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a F(ab')2 fragment, a bispecific Fab dimer (Fab2), a trispecific Fab trimer (Fab3), an Fv, an single chain Fv protein ("scFv"), a bis-scFv, (scFv)2, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody).

In particular embodiments, the antibody or antigen binding fragment thereof is human or humanized.

In some embodiments, the antibody or antigen binding fragment thereof comprises an scFv or one or more camelid VHH antibodies.

In certain embodiments, the antibody or antigen binding fragment thereof binds an antigen selected from the group consisting of: tumor associated antigens (TAA), tumor specific antigens (TSA), NKG2D ligands, γδ T cell receptor (TCR) ligands, and αβ TCR ligands.

In various embodiments, the antibody or antigen binding fragment thereof binds an antigen selected from the group consisting of: FRα, $\alpha_v\beta_6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, CEA, CLDN6, CLDN18.2, CLL-1, CS-1, CSPG4, CTAGE1, DLL3, EGFR, EGFRvIII, EGP2, EGP40, EPCAM, EPHA2, ERBB4, FAP, FCRL5, AchR, GD2, GD3, GPC3, HER2, HER2 p95, IL-10Rα, IL-13Rα2, Kappa, LAGE-1A, Lambda, LeY, L1-CAM, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGEA10, MelanA or MART1, SLN), MUC1, MUC16, MICA, MICB, NCAM, NY-ESO-1, PLAC1, PRAME, PSCA, PSMA, ROR1, SSX2, Survivin, TAG72, TEM1/CD248, TEM7R, TPBG, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, VEGFR2, and WT-1.

In additional embodiments, the antibody or antigen binding fragment thereof binds BCMA, B7-H3, CLDN6, CLDN18.2, DLL3, ERBB4, HER2, HER2 p95, MUC16, MICA, MICB, TAG72, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and ULBP6.

In additional embodiments, the antibody or antigen binding fragment thereof binds BCMA, B7-H3, CD19, CD20, CD22, CD33, CD79A, CD79B, and/or EGFRvIII.

In particular embodiments, the antibody or antigen binding fragment thereof binds BCMA.

In various embodiments, the antibody or antigen binding fragment thereof binds CD19.

In further embodiments, the antibody or antigen binding fragment thereof binds CD20 or CD22.

In certain embodiments, the antibody or antigen binding fragment thereof binds B7-H3.

In certain embodiments, the antibody or antigen binding fragment thereof binds CD33.

In various embodiments, the antibody or antigen binding fragment thereof binds CD79A.

In additional embodiments, the antibody or antigen binding fragment thereof binds CD79B.

In some embodiments, the antibody or antigen binding fragment thereof binds EGFRvIII.

In various embodiments, a fusion polypeptide comprises a first polypeptide comprising: an FKBP12 multimerization domain polypeptide or variant thereof; a CD8α transmembrane domain; a CD137 costimulatory domain; and a CD3ζ primary signaling domain; a polypeptide cleavage signal; and a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FRB T2098L multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and a TNFR2 costimulatory domain.

In particular embodiments, a fusion polypeptide comprises a first polypeptide comprising: an FKBP12 multimerization domain polypeptide or variant thereof; a CD8α transmembrane domain; a CD137 costimulatory domain; and a CD3ζ primary signaling domain; a polypeptide cleavage signal; and a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FRB T2098L multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and an OX40 costimulatory domain.

In further embodiments, a fusion polypeptide comprises a first polypeptide comprising: an FRB T2098L multimerization domain polypeptide or variant thereof; a CD8α transmembrane domain; a CD137 costimulatory domain; and a CD3ζ primary signaling domain; a polypeptide cleavage signal; and a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP12 multimerization domain

polypeptide or variant thereof; a CD4 transmembrane domain; and a TNFR2 costimulatory domain.

In various embodiments, a fusion polypeptide comprises a first polypeptide comprising: an FRB T2098L multimerization domain polypeptide or variant thereof; a CD8α transmembrane domain; a CD137 costimulatory domain; and a CD3ζ primary signaling domain; a polypeptide cleavage signal; and a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP12 multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and an OX40 costimulatory domain.

In additional embodiments, the antibody or antigen binding fragment thereof selected is from the group consisting of: a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a F(ab')2 fragment, a bispecific Fab dimer (Fab2), a trispecific Fab trimer (Fab3), an Fv, an single chain Fv protein ("scFv"), a bis-scFv, (scFv)2, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody).

In particular embodiments, the antibody or antigen binding fragment thereof is human or humanized.

In further embodiments, the antibody or antigen binding fragment thereof comprises an scFv or one or more camelid VHH antibodies.

In some embodiments, the antibody or antigen binding fragment thereof binds an antigen selected from the group consisting of: tumor associated antigens (TAA), tumor specific antigens (TSA), NKG2D ligands, γδ T cell receptor (TCR) ligands, and αβ TCR ligands.

In various embodiments, the antibody or antigen binding fragment thereof binds an antigen selected from the group consisting of: FRα, $\alpha_v\beta_6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, CEA, CLDN6, CLDN18.2, CLL-1, CS-1, CSPG4, CTAGE1, DLL3, EGFR, EGFRvIII, EGP2, EGP40, EPCAM, EPHA2, ERBB4, FAP, FCRL5, AchR, GD2, GD3, GPC3, HER2, HER2 p95, IL-

10Rα, IL-13Rα2, Kappa, LAGE-1A, Lambda, LeY, L1-CAM, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGEA10, MelanA or MART1, SLN), MUC1, MUC16, MICA, MICB, NCAM, NY-ESO-1, PLAC1, PRAME, PSCA, PSMA, ROR1, SSX2, Survivin, TAG72, TEM1/CD248, TEM7R, TPBG, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, VEGFR2, and WT-1.

In additional embodiments, the antibody or antigen binding fragment thereof binds BCMA, B7-H3, CLDN6, CLDN18.2, DLL3, ERBB4, HER2, HER2 p95, MUC16, MICA, MICB, TAG72, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and ULBP6.

In additional embodiments, the antibody or antigen binding fragment thereof binds BCMA, B7-H3, CD19, CD20, CD22, CD33, CD79A, CD79B, and/or EGFRvIII.

In certain embodiments, the antibody or antigen binding fragment thereof binds BCMA.

In various embodiments, the antibody or antigen binding fragment thereof binds CD19.

In particular embodiments, the antibody or antigen binding fragment thereof binds CD20 or CD22.

In further embodiments, the antibody or antigen binding fragment thereof binds B7-H3.

In various embodiments, the antibody or antigen binding fragment thereof binds CD33.

In additional embodiments, the antibody or antigen binding fragment thereof binds CD79A.

In some embodiments, the antibody or antigen binding fragment thereof binds CD79B.

In various embodiments, the antibody or antigen binding fragment thereof binds EGFRvIII, optionally wherein the antibody is EGFR806 or an antigen binding fragment thereof.

In further embodiments, the multimerization domains localize extracellularly when of the first polypeptide and the second polypeptide are expressed.

In certain embodiments, the polypeptide cleavage signal is a viral self-cleaving polypeptide.

In particular embodiments, the polypeptide cleavage signal is a viral self-cleaving 2A polypeptide.

In various embodiments, the polypeptide cleavage signal is a viral self-cleaving polypeptide selected from the group consisting of: a foot-and-mouth disease virus (FMDV) (F2A) peptide, an equine rhinitis A virus (ERAV) (E2A) peptide, a Thosea asigna virus (TaV) (T2A) peptide, a porcine teschovirus-1 (PTV-1) (P2A) peptide, a Theilovirus 2A peptide, and an encephalomyocarditis virus 2A peptide.

In some embodiments, a polynucleotide encodes a first or a second polypeptide or a fusion polypeptide contemplated herein.

In particular embodiments, a cDNA encodes a first or a second polypeptide or a fusion polypeptide contemplated herein.

In additional embodiments, an RNA encodes a first or a second polypeptide or a fusion polypeptide contemplated herein.

In various embodiments, a vector comprises a polynucleotide contemplated herein.

In particular embodiments, the vector is an expression vector.

In additional embodiments, the vector is a transposon.

In certain embodiments, the vector is a piggyBAC transposon or a Sleeping Beauty transposon.

In further embodiments, the vector is a viral vector.

In various embodiments, the vector is an adenoviral vector, an adeno-associated viral (AAV) vector, a herpes virus vector, a vaccinia virus vector, or a retroviral vector.

In some embodiments, the retroviral vector is a lentiviral vector.

In some embodiments, the lentiviral vector is selected from the group consisting of: human immunodeficiency virus 1 (HIV-1); human immunodeficiency virus 2 (HIV-2), visna-maedi virus (VMV) virus; caprine arthritis-encephalitis virus (CAEV); equine infectious anemia virus (EIAV); feline immunodeficiency virus (FIV); bovine immune deficiency virus (BIV); and simian immunodeficiency virus (SIV).

In particular embodiments, a composition comprises a non-natural cell, a fusion polypeptide, a polynucleotide, or a vector contemplated herein.

In various embodiments, a pharmaceutical composition comprises a pharmaceutically acceptable carrier and a non-natural cell, a fusion polypeptide, a polynucleotide, or a vector contemplated herein.

In additional embodiments, a method of treating a subject in need thereof comprises administering the subject an effective amount of a composition contemplated herein.

In further embodiments, a method of treating, preventing, or ameliorating at least one symptom of a cancer, infectious disease, autoimmune disease, inflammatory disease, and immunodeficiency, or condition associated therewith, comprising administering to the subject an effective amount of a composition contemplated herein.

In particular embodiments, a method of treating a solid cancer comprises administering to the subject an effective amount of a composition contemplated herein.

In various embodiments, the solid cancer comprises liver cancer, pancreatic cancer, lung cancer, breast cancer, ovarian cancer, prostate cancer, testicular cancer, bladder cancer, brain cancer, sarcoma, head and neck cancer, bone cancer, thyroid cancer, kidney cancer, or skin cancer.

In additional embodiments, the solid cancer is a pancreatic cancer, a lung cancer, or a breast cancer.

In certain embodiments, a method of treating a hematological malignancy comprises administering to the subject an effective amount of a composition contemplated herein.

In various embodiments, the hematological malignancy is a leukemia, lymphoma, or multiple myeloma.

In some embodiments, a polypeptide complex comprises a first polypeptide comprising: a first multimerization domain polypeptide or variant thereof; a first transmembrane domain; a first costimulatory domain; and/or a primary signaling domain; and a second polypeptide comprising: an extracellular binding domain; a second multimerization domain polypeptide or variant thereof; a second transmembrane domain;

and a second costimulatory domain; and a bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

In particular embodiments, the first and second multimerization domains are different.

In various embodiments, the first and second costimulatory domains are different.

In further embodiments, the first multimerization domain and the second multimerization domain associate with a bridging factor selected from the group consisting of: rapamycin or a rapalog thereof, coumermycin or a derivative thereof, gibberellin or a derivative thereof, abscisic acid (ABA) or a derivative thereof, methotrexate or a derivative thereof, cyclosporin A or a derivative thereof, FK506/cyclosporin A (FKCsA) or a derivative thereof, and trimethoprim (Tmp)-synthetic ligand for FK506 binding protein (FKBP) (SLF) or a derivative thereof.

In additional embodiments, the first multimerization domain and the second multimerization domain are a pair selected from the group consisting of: FKBP and FKBP-rapamycin binding (FRB), FKBP and calcineurin, FKBP and cyclophilin, FKBP and bacterial dihydrofolate reductase (DHFR), calcineurin and cyclophilin, and PYR1-like 1 (PYL1) and abscisic acid insensitive 1 (ABI1).

In various embodiments, the first multimerization domain comprises an FKBP polypeptide or variant thereof, and the second multimerization domain comprises an FRB polypeptide or variant thereof.

In particular embodiments, the first multimerization domain comprises an FRB polypeptide or variant thereof, and the second multimerization domain comprises an FKBP polypeptide or variant thereof.

In certain embodiments, the bridging factor is selected from the group consisting of: AP21967, sirolimus, everolimus, novolimus, pimecrolimus, ridaforolimus, tacrolimus, temsirolimus, umirolimus, and zotarolimus.

In some embodiments, the first and second multimerization domains are selected from FRB T2098L and FKBP12; and the bridging factor is sirolimus or AP21967.

In further embodiments, the first transmembrane domain and the second transmembrane domain are independently selected from a polypeptide selected from the group consisting of: alpha, beta, gamma, or delta chain of the T-cell receptor, CD3δ, CD3ε, CD3γ, CD3ζ, CD4, CD5, CD8α, CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD 134, CD137, CD152, CD154, CD278, amnionless (AMN), and programmed cell death 1 (PDCD1).

In various embodiments, the first transmembrane domain and the second transmembrane domain are independently selected from the group consisting of: a CD4 transmembrane domain, a CD8α transmembrane domain, and an AMN transmembrane domain.

In some embodiments, the first transmembrane domain and the second transmembrane domain are different.

In additional embodiments, the costimulatory domain and/or the primary signaling domain comprise an immunoreceptor tyrosine activation motif (ITAM).

In further embodiments, the first and second costimulatory domain are independently selected from a costimulatory molecule selected from the group consisting of: Toll-like receptor 1 (TLR1), TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, caspase recruitment domain family member 11 (CARD11), CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD94, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), DNAX-Activation Protein 10 (DAP10), Linker for activation of T-cells family member 1 (LAT), SH2 Domain-Containing Leukocyte Protein Of 76 kD (SLP76), T cell receptor associated transmembrane adaptor 1 (TRAT1), TNFR2, TNFRS14, TNFRS18, TNFRS25, and zeta chain of T cell receptor associated protein kinase 70 (ZAP70).

In particular embodiments, the first costimulatory domain is isolated from a costimulatory molecule selected from the group consisting of: CD28, CD134, and CD137 and the second costimulatory domain is isolated from CD28, CD278, TNFRS14, TNFRS18, TNFRS25, OX40 or TNFR2.

In additional embodiments, the first costimulatory domain is isolated from CD137 and the second costimulatory domain is isolated from OX40 or TNFR2.

In various embodiments, the first costimulatory domain is isolated from CD137 and the second costimulatory domain is isolated from OX40.

In certain embodiments, the first costimulatory domain is isolated from CD137 and the second costimulatory domain is isolated from TNFR2.

In particular embodiments, the primary signaling domain isolated from a polypeptide selected from the group consisting of: FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d.

In further embodiments, the primary signaling domain is isolated from a CD3ζ polypeptide.

In various embodiments, the extracellular binding domain comprises an antibody or antigen binding fragment thereof, a receptor ectodomain, or a ligand.

In some embodiments, the extracellular binding domain comprises an antibody or antigen binding fragment thereof selected from the group consisting of: a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a F(ab')2 fragment, a bispecific Fab dimer (Fab2), a trispecific Fab trimer (Fab3), an Fv, an single chain Fv protein ("scFv"), a bis-scFv, (scFv)2, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody).

In additional embodiments, the extracellular binding domain comprises a humanized antibody or antigen binding fragment thereof.

In certain embodiments, the extracellular binding domain comprises a human antibody or antigen binding fragment thereof.

In various embodiments, the extracellular binding domain comprises an scFv.

In various embodiments, the extracellular binding domain comprises one or more camelid VHH antibodies.

In additional embodiments, the extracellular binding domain binds an antigen selected from the group consisting of: tumor associated antigens (TAA), tumor specific antigens (TSA), NKG2D ligands, γδ T cell receptor (TCR) ligands, and αβ TCR ligands.

In particular embodiments, the extracellular binding domain binds an antigen selected from the group consisting of: FRα, $\alpha_v\beta_6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, CEA, CLDN6, CLDN18.2, CLL-1, CS-1, CSPG4, CTAGE1, DLL3, EGFR, EGFRvIII, EGP2, EGP40, EPCAM, EPHA2, ERBB4, FAP, FCRL5, AchR, GD2, GD3, GPC3, HER2, HER2 p95, IL-10Rα, IL-13Rα2, Kappa, LAGE-1A, Lambda, LeY, L1-CAM, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGEA10, MelanA or MART1, SLN), MUC1, MUC16, MICA, MICB, NCAM, NY-ESO-1, PLAC1, PRAME, PSCA, PSMA, ROR1, SSX2, Survivin, TAG72, TEM1/CD248, TEM7R, TPBG, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, VEGFR2, and WT-1.

In various embodiments, the extracellular binding domain binds an antigen selected from the group consisting of: BCMA, B7-H3, CLDN6, CLDN18.2, DLL3, ERBB4, HER2, HER2 p95, MUC16, MICA, MICB, TAG72, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and ULBP6.

In various embodiments, the extracellular binding domain binds an antigen selected from the group consisting of: BCMA, B7-H3, CD19, CD20, CD22, CD33, CD79A, CD79B, and/or EGFRvIII.

In various embodiments, a polypeptide complex comprises a first polypeptide comprising: a first multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain; a second polypeptide comprising: an extracellular binding domain; a second multimerization domain polypeptide or variant thereof; a second transmembrane domain; and an OX40 costimulatory domain; and a bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

In some embodiments, a polypeptide complex comprises a first polypeptide comprising: a first multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain; a second polypeptide comprising: an extracellular binding domain; a second multimerization

domain polypeptide or variant thereof; a second transmembrane domain; and a TNFR2 costimulatory domain; and a bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

In certain embodiments, the first and second multimerization domains are the same.

In further embodiments, the first and second multimerization domains are different.

In various embodiments, the first multimerization domain and the second multimerization domain associate with a bridging factor selected from the group consisting of: rapamycin or a rapalog thereof, coumermycin or a derivative thereof, gibberellin or a derivative thereof, ABA or a derivative thereof, methotrexate or a derivative thereof, cyclosporin A or a derivative thereof, FKCsA or a derivative thereof, and SLF or a derivative thereof.

In particular embodiments, the first multimerization domain and the second multimerization domain are a pair selected from the group consisting of: FKBP and FRB, FKBP and calcineurin, FKBP and cyclophilin, FKBP and DHFR, calcineurin and cyclophilin, and PYL1 and ABI1.

In some embodiments, the first multimerization domain comprises an FKBP polypeptide or variant thereof, and the second multimerization domain comprises an FRB polypeptide or variant thereof.

In particular embodiments, the first multimerization domain comprises an FRB polypeptide or variant thereof, and the second multimerization domain comprises an FKBP polypeptide or variant thereof.

In additional embodiments, the bridging factor is selected from the group consisting of: AP21967, sirolimus, everolimus, novolimus, pimecrolimus, ridaforolimus, tacrolimus, temsirolimus, umirolimus, and zotarolimus.

In various embodiments, the first and second multimerization domains are selected from FRB T2098L and FKBP12; and the bridging factor is sirolimus or AP21967.

In further embodiments, the first transmembrane domain and the second transmembrane domain are independently selected from a polypeptide selected from the group consisting of: alpha, beta, gamma, or delta chain of the T-cell receptor, CD3δ,

CD3ε, CD3γ, CD3ζ, CD4, CD5, CD8α, CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD 134, CD137, CD152, CD154, AMN, and PDCD1.

In some embodiments, the first transmembrane domain and the second transmembrane domain are independently selected from the group consisting of: a CD4 transmembrane domain and a CD8α transmembrane domain.

In additional embodiments, the first transmembrane domain and the second transmembrane domain are the same.

In particular embodiments, the first transmembrane domain and the second transmembrane domain are different.

In various embodiments, the costimulatory domain and/or the primary signaling domain comprise an immunoreceptor tyrosine activation motif (ITAM).

In further embodiments, the costimulatory domain is isolated from a costimulatory molecule selected from the group consisting of: TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, CARD11, CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD94, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), DAP10, LAT, SLP76, TRAT1, TNFR2, and ZAP70.

In certain embodiments, the costimulatory domain is isolated from a costimulatory molecule selected from the group consisting of: CD28, CD134, and CD137.

In additional embodiments, the costimulatory domain is isolated from a CD137 costimulatory molecule.

In various embodiments, the primary signaling domain isolated from a polypeptide selected from the group consisting of: FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d.

In further embodiments, the primary signaling domain is isolated from a CD3ζ polypeptide.

In particular embodiments, the extracellular binding domain comprises an antibody or antigen binding fragment thereof, a receptor ectodomain, or a ligand.

In some embodiments, the extracellular binding domain comprises an antibody or antigen binding fragment thereof selected from the group consisting of: a Camel Ig, a

Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a F(ab')2 fragment, a bispecific Fab dimer (Fab2), a trispecific Fab trimer (Fab3), an Fv, an single chain Fv protein ("scFv"), a bis-scFv, (scFv)2, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody).

In some embodiments, the extracellular binding domain comprises a humanized antibody or antigen binding fragment thereof.

In certain embodiments, the extracellular binding domain comprises a human antibody or antigen binding fragment thereof.

In various embodiments, the extracellular binding domain comprises an scFv.

In additional embodiments, the extracellular binding domain comprises one or more camelid VHH antibodies.

In various embodiments, the extracellular binding domain binds an antigen selected from the group consisting of: tumor associated antigens (TAA), tumor specific antigens (TSA), NKG2D ligands, γδ T cell receptor (TCR) ligands, and αβ TCR ligands.

In some embodiments, the extracellular binding domain binds an antigen selected from the group consisting of: FRα, $\alpha_v\beta_6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, CEA, CLDN6, CLDN18.2, CLL-1, CS-1, CSPG4, CTAGE1, DLL3, EGFR, EGFRvIII, EGP2, EGP40, EPCAM, EPHA2, ERBB4, FAP, FCRL5, AchR, GD2, GD3, GPC3, HER2, HER2 p95, IL-10Rα, IL-13Rα2, Kappa, LAGE-1A, Lambda, LeY, L1-CAM, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGEA10, MelanA or MART1, SLN), MUC1, MUC16, MICA, MICB, NCAM, NY-ESO-1, PLAC1, PRAME, PSCA, PSMA, ROR1, SSX2, Survivin, TAG72, TEM1/CD248, TEM7R, TPBG, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, VEGFR2, and WT-1.

In various embodiments, the extracellular binding domain binds an antigen selected from the group consisting of: BCMA, B7-H3, CLDN6, CLDN18.2, DLL3, ERBB4, HER2, HER2 p95, MUC16, MICA, MICB, TAG72, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and ULBP6.

In various embodiments, the extracellular binding domain binds an antigen selected from the group consisting of: BCMA, B7-H3, CD19, CD20, CD22, CD33, CD79A, CD79B, and/or EGFRvIII.

In particular embodiments, a polypeptide complex comprises a first polypeptide comprising: an FKBP multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain; a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FRB multimerization domain polypeptide or variant thereof; a second transmembrane domain; and an OX40 costimulatory domain; and a bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

In various embodiments, a polypeptide complex comprises a first polypeptide comprising: an FKBP multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain; a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FRB multimerization domain polypeptide or variant thereof; a second transmembrane domain; and a TNFR2 costimulatory domain; and a bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

In particular embodiments, a polypeptide complex comprises a first polypeptide comprising: an FRB multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain; a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP multimerization domain polypeptide or variant thereof; a second transmembrane domain; and an OX40 costimulatory domain; and a bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

In further embodiments, a polypeptide complex comprises a first polypeptide comprising: an FRB multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain; a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP multimerization domain polypeptide or variant thereof; a second transmembrane domain;

and a TNFR2 costimulatory domain; and a bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

In various embodiments, the bridging factor is selected from the group consisting of: AP21967, sirolimus, everolimus, novolimus, pimecrolimus, ridaforolimus, tacrolimus, temsirolimus, umirolimus, and zotarolimus.

In particular embodiments, the FRB multimerization domain is FRB T2098L; the FKBP multimerization domain is FKBP12; and the bridging factor is sirolimus or AP21967.

In certain embodiments, the first transmembrane domain and the second transmembrane domain are independently selected from a polypeptide selected from the group consisting of: alpha, beta, gamma, or delta chain of the T-cell receptor, CD3δ, CD3ε, CD3γ, CD3ζ, CD4, CD5, CD8α, CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD 134, CD137, CD152, CD154, AMN, and PDCD1.

In additional embodiments, the first transmembrane domain and the second transmembrane domain are independently selected from a polypeptide selected from the group consisting of: a CD4 transmembrane domain and a CD8α transmembrane domain.

In some embodiments, the costimulatory domain and/or the primary signaling domain comprise an immunoreceptor tyrosine activation motif (ITAM).

In further embodiments, the costimulatory signaling domaincostimulatory domain is isolated from a costimulatory molecule selected from the group consisting of: TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, CARD11, CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD94, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), DAP10, LAT, SLP76, TRAT1, TNFR2, and ZAP70.

In various embodiments, the costimulatory domain is isolated from a costimulatory molecule selected from the group consisting of: CD28, CD134, and CD137.

In certain embodiments, the costimulatory domain is isolated from a CD137 costimulatory molecule.

In particular embodiments, the primary signaling domain isolated from a polypeptide selected from the group consisting of: FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d.

In particular embodiments, the primary signaling domain is isolated from a CD3ζ polypeptide.

In some embodiments, the antibody or antigen binding fragment thereof selected is from the group consisting of: a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a F(ab')2 fragment, a bispecific Fab dimer (Fab2), a trispecific Fab trimer (Fab3), an Fv, an single chain Fv protein ("scFv"), a bis-scFv, (scFv)2, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody).

In various embodiments, the antibody or antigen binding fragment thereof is human or humanized.

In additional embodiments, the antibody or antigen binding fragment thereof comprises an scFv or one or more camelid VHH antibodies.

In further embodiments, the antibody or antigen binding fragment thereof binds an antigen selected from the group consisting of: tumor associated antigens (TAA), tumor specific antigens (TSA), NKG2D ligands, γδ T cell receptor (TCR) ligands, and αβ TCR ligands.

In various embodiments, the antibody or antigen binding fragment thereof binds an antigen selected from the group consisting of: FRα, $\alpha_v\beta_6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, CEA, CLDN6, CLDN18.2, CLL-1, CS-1, CSPG4, CTAGE1, DLL3, EGFR, EGFRvIII, EGP2, EGP40, EPCAM, EPHA2, ERBB4, FAP, FCRL5, AchR, GD2, GD3, GPC3, HER2, HER2 p95, IL-10Rα, IL-13Rα2, Kappa, LAGE-1A, Lambda, LeY, L1-CAM, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGEA10, MelanA or MART1, SLN), MUC1, MUC16, MICA, MICB, NCAM, NY-ESO-1, PLAC1, PRAME, PSCA, PSMA, ROR1, SSX2, Survivin,

TAG72, TEM1/CD248, TEM7R, TPBG, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, VEGFR2, and WT-1.

In additional embodiments, the antibody or antigen binding fragment thereof binds BCMA, B7-H3, CLDN6, CLDN18.2, DLL3, ERBB4, HER2, HER2 p95, MUC16, MICA, MICB, TAG72, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and ULBP6.

In some embodiments, the antibody or antigen binding fragment thereof binds BCMA, B7-H3, CD19, CD20, CD22, CD33, CD79A, CD79B, and/or EGFRvIII.

In particular embodiments, the antibody or antigen binding fragment thereof binds BCMA.

In additional embodiments, the antibody or antigen binding fragment thereof binds CD19.

In certain embodiments, the antibody or antigen binding fragment thereof binds CD20 or CD22.

In various embodiments, the antibody or antigen binding fragment thereof binds B7-H3.

In further embodiments, the antibody or antigen binding fragment thereof binds CD33.

In various embodiments, the antibody or antigen binding fragment thereof binds CD79A.

In particular embodiments, the antibody or antigen binding fragment thereof binds CD79B.

In various embodiments, the antibody or antigen binding fragment thereof binds EGFRvIII.

In some embodiments, a polypeptide complex comprises a first polypeptide comprising: an FKBP12 multimerization domain polypeptide or variant thereof; a CD8α transmembrane domain; a CD137 costimulatory domain; and a CD3ζ primary signaling domain; a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FRB T2098L multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and a TNFR2 costimulatory domain; and a bridging factor

associated with and disposed between the multimerization domains of the first and second polypeptides.

In certain embodiments, a polypeptide complex comprises a first polypeptide comprising: an FKBP12 multimerization domain polypeptide or variant thereof; a CD8α transmembrane domain; a CD137 costimulatory domain; and a CD3ζ primary signaling domain; a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FRB T2098L multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and an OX40 costimulatory domain; and a bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

In various embodiments, a polypeptide complex comprises a first polypeptide comprising: an FRB T2098L multimerization domain polypeptide or variant thereof; a CD8α transmembrane domain; a CD137 costimulatory domain; and a CD3ζ primary signaling domain; a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP12 multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and a TNFR2 costimulatory domain; and a bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

In particular embodiments, a polypeptide complex comprises a first polypeptide comprising: an FRB T2098L multimerization domain polypeptide or variant thereof; a CD8α transmembrane domain; a CD137 costimulatory domain; and a CD3ζ primary signaling domain; a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP12 multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and an OX40 costimulatory domain; and a bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

In some embodiments, the bridging factor is AP21967 or sirolimus.

In various embodiments, the antibody or antigen binding fragment thereof selected is from the group consisting of: a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab'

fragment, a F(ab')2 fragment, a bispecific Fab dimer (Fab2), a trispecific Fab trimer (Fab3), an Fv, an single chain Fv protein ("scFv"), a bis-scFv, (scFv)2, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody).

In additional embodiments, the antibody or antigen binding fragment thereof is human or humanized.

In further embodiments, the antibody or antigen binding fragment thereof comprises an scFv or one or more camelid VHH antibodies.

In various embodiments, the antibody or antigen binding fragment thereof binds an antigen selected from the group consisting of: tumor associated antigens (TAA), tumor specific antigens (TSA), NKG2D ligands, γδ T cell receptor (TCR) ligands, and αβ TCR ligands.

In certain embodiments, the antibody or antigen binding fragment thereof binds an antigen selected from the group consisting of: FRα, $\alpha_v\beta_6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, CEA, CLDN6, CLDN18.2, CLL-1, CS-1, CSPG4, CTAGE1, DLL3, EGFR, EGFRvIII, EGP2, EGP40, EPCAM, EPHA2, ERBB4, FAP, FCRL5, AchR, GD2, GD3, GPC3, HER2, HER2 p95, IL-10Rα, IL-13Rα2, Kappa, LAGE-1A, Lambda, LeY, L1-CAM, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGEA10, MelanA or MART1, SLN), MUC1, MUC16, MICA, MICB, NCAM, NY-ESO-1, PLAC1, PRAME, PSCA, PSMA, ROR1, SSX2, Survivin, TAG72, TEM1/CD248, TEM7R, TPBG, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, VEGFR2, and WT-1.

In additional embodiments, the antibody or antigen binding fragment thereof binds BCMA, B7-H3, CLDN6, CLDN18.2, DLL3, ERBB4, HER2, HER2 p95, MUC16, MICA, MICB, TAG72, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and ULBP6.

In particular embodiments, the antibody or antigen binding fragment thereof binds BCMA, B7-H3, CD19, CD20, CD22, CD33, CD79A, CD79B, and/or EGFRvIII.

In additional embodiments, the antibody or antigen binding fragment thereof binds BCMA.

In further embodiments, the antibody or antigen binding fragment thereof binds CD19.

In particular embodiments, the antibody or antigen binding fragment thereof binds CD20 or CD22.

In various embodiments, the antibody or antigen binding fragment thereof binds B7-H3.

In certain embodiments, the antibody or antigen binding fragment thereof binds CD33.

In some embodiments, the antibody or antigen binding fragment thereof binds CD79A.

In various embodiments, the antibody or antigen binding fragment thereof binds CD79B.

In particular embodiments, the antibody or antigen binding fragment thereof binds EGFRvIII, optionally wherein the antibody is EGFR806 or an antigen binding fragment thereof.

In various embodiments, the multimerization domains localize extracellularly when of the first polypeptide and the second polypeptide are expressed.

BRIEF DESCRIPTION OF SEVERAL VIEWS OF THE DRAWINGS

Figure 1 shows a cartoon of DARIC architectures comprising DARIC binding components with a costimulatory domain.

Figure 2A shows a cartoon of NKG2D DARIC architectures comprising DARIC binding components with various costimulatory domains.

Figure 2B shows NKG2D binding domain expression in the $CD4^+$ gate for untransduced T cells, NKG2D DARIC T cells, NKG2D.TNFR2 DARIC T cells, NKG2D.OX40 DARIC T cells, NKG2D.CD27 DARIC T cells, NKG2D.HVEM DARIC T cells, NKG2D.DR3 DARIC T cells, and NKG2D.GITR DARIC T cells .

Figure 2C shows the growth kinetics of untransduced T cells, NKG2D DARIC T cells, NKG2D.TNFR2 DARIC T cells, NKG2D.OX40 DARIC T cells, NKG2D.CD27 DARIC T cells, NKG2D.HVEM DARIC T cells, NKG2D.DR3 DARIC T cells, and NKG2D.GITR DARIC T cells.

Figure 2D shows IFNγ, TNFα, and GM-CSF production from culture supernatants of $EGFR^{+}NKG2DL^{+}$ HCT116 cells co-cultured for 24 hrs with untransduced control T cells, NKG2D DARIC T cells, NKG2D.TNFR2 DARIC T cells, NKG2D.OX40 DARIC T cells, NKG2D.CD27 DARIC T cells, NKG2D.HVEM DARIC T cells, NKG2D.DR3 DARIC T cells, or NKG2D.GITR DARIC T cells at a 1:1 E:T ratio in rapamycin.

Figure 3A shows IFNγ, TNFα, and GM-CSF production from culture supernatants of $EGFR^{+}NKG2DL^{+}$ A549 cells co-cultured for 24 hrs with NKG2D DARIC T cells, NKG2D.OX40 DARIC T cells or NKG2D.TNFR2 DARIC T cells at a 1:1 E:T ratio in vehicle, rapamycin or AP21967

Figure 3B shows IFNγ, TNFα, and GM-CSF production from culture supernatants of $EGFR^{+}NKG2DL^{+}$ A549 cells co-cultured for 24 hrs with NKG2D DARIC T cells, NKG2D.OX40 DARIC T cells or NKG2D.TNFR2 DARIC T cells at a 1:1 E:T ratio in vehicle, rapamycin or AP21967

Figure 3C shows the ratio of cytokine production when T cell co-cultures are treated with AP2167 vs. rapamycin. Anti-EGFR CAR T cells, NKG2D DARIC T cells, NKG2D.TNFR2 DARIC T cells, and NKG2D.OX40 DARIC T cells are co-cultured at a 1:1 E:T ratio in rapamycin or AP21967 with either A549 or HCT116 target cells. The ratio of cytokine production from AP2167 cultured divided by cytokine production from rapamycin cultures is shown. Arrows show rapamycin-mediated immunosuppression (>1) or rapamycin-mediated immunoboost (<1).

Figure 4A shows a cartoon of NKG2D DARIC architectures comprising DARIC binding components that have two costimulatory domains.

Figure 4B shows IFNγ and GM-CSF production from culture supernatants of $EGFR^{+}NKG2DL^{+}$ A549 cells co-cultured for 24 hrs with untransduced control T cells,

NKG2D DARIC T cells, NKG2D.DAP10 DARIC T cells, NKG2D.CD28 DARIC T cells, or NKG2D.CD28.DAP10 DARIC T cells at a 1:1 E:T ratio in vehicle or rapamycin.

Figure 4C shows IFNγ and GM-CSF production from culture supernatants of $EGFR^+NKG2DL^+$ A549 cells co-cultured for 24 hrs with untransduced control T cells, NKG2D DARIC T cells, NKG2D.DAP10 DARIC T cells, NKG2D.DAP10.OX40 DARIC T cells, or NKG2D.OX40.DAP10 DARIC T cells at a 1:1 E:T ratio in vehicle or rapamycin.

Figure 5A shows a cartoon of NKG2D DARIC architectures comprising DARIC binding components that have ICOS-based transmembrane and costimulatory domains.

Figure 5B shows IFNγ production from culture supernatants of $EGFR^+NKG2DL^+$ A549 cells co-cultured for 24 hrs with anti-EGFR CAR T cells, NKG2D DARIC T cells, or NKG2D DARIC T cells containing single or dual costimulatory and transmembrane domains derived from ICOS and DAP10 at a 1:1 E:T ratio in AP21967.

Figure 5C shows GM-CSF production from culture supernatants of $EGFR^+NKG2DL^+$ A549 cells co-cultured for 24 hrs with anti-EGFR CAR T cells, NKG2D DARIC T cells, or NKG2D DARIC T cells containing single or dual costimulatory and transmembrane domains derived from ICOS and DAP10 at a 1:1 E:T ratio in AP21967.

Figure 6A shows a cartoon of a dual targeting DARIC strategy: an NKG2D DARIC comprising a DARIC binding component with a costimulatory domain together with an anti-CD19 DARIC binding component.

Figure 6B shows NKG2D binding domain expression in the $CD4^+$ gate for untransduced T cells, NKG2D.TNFR2 DARIC T cells, and NKG2D.TNFR2 DARIC:CD19 DARIC T cells.

Figure 6C shows CD19-Fc binding efficiency for untransduced T cells, CD19 DARIC T cells, and NKG2D.TNFR2 DARIC:CD19 DARIC T cells.

Figure 6D shows GM-CSF production from culture supernatants of $NKG2DL^-CD19^-$ A20 cells (A20), $NKG2DL^-CD19^+$ A20 cells (A20-hCD19) and $NKG2DL^+CD19^-$ A549 cells (A549). Target cells were co-cultured for 24 hrs with untransduced control T

cells, CD19 DARIC T cells, NKG2D.TNFR2 DARIC T cells, or NKG2D.TNFR2 DARIC:CD19 DARIC T cells at a 1:1 E:T ratio in AP21967.

Figure 7A shows a cartoon of anti-CD19 DARIC architecture with costimulatory domains.

Figure 7B shows CD19-Fc binding efficiency for untransduced T cells, anti-CD19 CAR T cells, CD19 DARIC T cells, CD19.OX40 DARIC T cells and CD19.TNFR2 DARIC T cells.

Figure 7C shows IFNγ, GM-CSF and TNFα production from culture supernatants of $CD19^+$ Nalm6 cells co-cultured for 24 hrs with anti-CD19 CAR T cells, CD19 DARIC T cells, CD19.OX40 DARIC T cells, and CD19.TNFR2 DARIC T cells at a 1:1 E:T ratio in vehicle or Rapamycin.

Figure 7D shows IFNγ, GM-CSF and TNFα production from culture supernatants of $CD19^+$ Jeko-1 cells co-cultured for 24 hrs with anti-CD19 CAR T cells, CD19 DARIC T cells, CD19.OX40 DARIC T cells, and CD19.TNFR2 DARIC T cells at a 1:1 E:T ratio in vehicle or Rapamycin.

Figure 8A shows a cartoon of anti-CD33 DARIC architecture with costimulatory domains.

Figure 8B shows CD33-Fc binding efficiency for untransduced T cells, anti-CD33 CAR T cells, anti-CD33 DARIC T cells, CD33.OX40 DARIC T cells and CD33.TNFR2 DARIC T cells. Two different anti-CD33 scFvs are shown.

Figure 8C shows IFNγ production from culture supernatants of $CD33^+$ THP-1 or $CD33^+$ Molm-1 cells co-cultured for 24 hrs with ant-CD33 CAR T cells, CD33 DARIC T cells, CD33.OX40 DARIC T cells, and CD33.TNFR2 DARIC T cells at a 1:1 E:T ratio in Vehicle or Rapamycin. Two different anti-CD33 scFvs are shown.

BRIEF DESCRIPTION OF THE SEQUENCE IDENTIFIERS

SEQ ID NO: 1 sets forth the amino acid sequence for an FRB T2098L-CD8α TM-CD137-CD3ζ DARIC signaling component.

SEQ ID NO: 2 sets forth the amino acid sequence for an FRB T2098L-CD8α TM-CD134-CD3ζ DARIC signaling component.

SEQ ID NO: 3 sets forth the amino acid sequence for an FRB T2098L-CD8α TM-CD28-CD ζ DARIC signaling component.

SEQ ID NO: 4 sets forth the amino acid sequence for an anti-BCMA-FKBP12-CD4 TM DARIC.TNFR2 binding component.

SEQ ID NO: 5 sets forth the amino acid sequence for an anti-BCMA-FKBP12-AMN TM DARIC.TNFR2 binding component

SEQ ID NO: 6 sets forth the amino acid sequence for an anti-CD19-FKBP12-CD4 TM DARIC.TNFR2 binding component.

SEQ ID NO: 7 sets forth the amino acid sequence for an anti-CD19-FKBP12-CD4 TM DARIC.OX40 binding component.

SEQ ID NO: 8 sets forth the amino acid sequence for an anti-B7-H3-FKBP12-CD4 TM DARIC.OX40 binding component.

SEQ ID NO: 9 sets forth the amino acid sequence for an anti-B7-H3-FKBP12-CD4 TM DARIC.TNFR2 binding component.

SEQ ID NO: 10 sets forth the amino acid sequence for an anti-CD20-FKBP12-CD4 TM DARIC.OX40 binding component.

SEQ ID NO: 11 sets forth the amino acid sequence for an anti-CD20-FKBP12-CD4 TM DARIC.TNFR2 binding component.

SEQ ID NO: 12 sets forth the amino acid sequence for an anti-CD22-FKBP12-CD4 TM DARIC.OX40 binding component.

SEQ ID NO: 13 sets forth the amino acid sequence for an anti-CD22-FKBP12-CD4 TM DARIC.TNFR2 binding component.

SEQ ID NO: 14 sets forth the amino acid sequence for an anti-EGFRvIII-FKBP12-CD4 TM DARIC.OX40 binding component.

SEQ ID NO: 15 sets forth the amino acid sequence for an anti-EGFRvIII-FKBP12-CD4 TM DARIC.TNFR2 binding component.

SEQ ID NO: 16 sets forth the amino acid sequence for an anti-CD33-FKBP12-CD4 TM DARIC.OX40 binding component.

SEQ ID NO: 17 sets forth the amino acid sequence for an anti-CD33-FKBP12-CD4 TM DARIC.TNFR2 binding component.

SEQ ID NO: 18 sets forth the amino acid sequence for an anti-CD33-FKBP12-CD4 TM DARIC.OX40 binding component.

SEQ ID NO: 19 sets forth the amino acid sequence for an anti-CD33-FKBP12-CD4 TM DARIC.TNFR2 binding component.

SEQ ID NO: 20 sets forth the amino acid sequence for an NKG2D-FKBP12-CD4 TM DARIC.OX40 binding component.

SEQ ID NO: 21 sets forth the amino acid sequence for an NKG2D-FKBP12-CD4 TM DARIC.TNFR2 binding component.

SEQ ID NO: 22 sets forth the amino acid sequence for an NKG2D DARIC polyprotein.

SEQ ID NO: 23 sets forth the amino acid sequence for a CD19 DARIC polyprotein.

SEQ ID NO: 24 sets forth the amino acid sequence for a B7-H3 DARIC polyprotein.

SEQ ID NO: 25 sets forth the amino acid sequence for a CD20 DARIC polyprotein.

SEQ ID NO: 26 sets forth the amino acid sequence for a CD22 DARIC polyprotein.

SEQ ID NO: 27 sets forth the amino acid sequence for an EGFRvIII DARIC polyprotein.

SEQ ID NO: 28 sets forth the amino acid sequence for a CD33 DARIC-1 polyprotein.

SEQ ID NO: 29 sets forth the amino acid sequence for a CD33 DARIC-2 polyprotein.

SEQ ID NOs: 30-40 set forth the amino acid sequences of various linkers.

SEQ ID NOs: 41-65 set forth the amino acid sequences of protease cleavage sites and self-cleaving polypeptide cleavage sites.

DETAILED DESCRIPTION

A. OVERVIEW

Cancer is among the leading causes of death worldwide. Recently, oncologists introduced genetic approaches as a potential means to enhance immune recognition and elimination of cancer cells. One promising strategy is adoptive cellular immunotherapy using immune effector cells genetically engineered to express chimeric antigen receptors (CAR) that redirect cytotoxicity of these CAR T cells to cancer cells. A significant limitation of CAR T cell therapy is the lack of spatial and temporal control of the CAR T cell activity. Lack of control over CAR T cell activity can trigger a range of side effects, many of which begin subtly but can rapidly worsen. A particularly severe complication is cytokine release syndrome (CRS) or "cytokine storm" where CAR T cells induce massive and potentially fatal cytokine release. CRS can produce dangerously high fevers, extreme fatigue, difficulty breathing, and a sharp drop in blood pressure. CRS can also produce a second wave of side effects that involve the nervous system, including neurotoxicity, tremors, headaches, confusion, loss of balance, trouble speaking, seizures, and hallucinations. The compositions and methods contemplated herein offer solutions to these and other problems plaguing adoptive cell therapies.

The disclosure generally relates to improved compositions and methods for regulating the spatial and temporal control of adoptive cell therapies using costimulatory dimerizing agent regulated immunoreceptor complexes (DARIC). A DARIC comprises one or more DARIC binding components and/or one or more DARIC signaling components. Without wishing to be bound by any particular theory, DARIC compositions and methods contemplated herein provide numerous advantages over CAR T cell therapies existing in the art, including but not limited to, both spatial and temporal control over immune effector cell signal transduction binding and signaling activities. DARIC temporal

control primes the DARIC machinery for signaling through bridging factor mediated association of a DARIC binding component to a DARIC signaling component. DARIC spatial control engages the signaling machinery through target antigen recognition by the binding domain on the DARIC binding component. In this manner, DARIC immune effector cells become activated when both a target antigen and a bridging factor are present. In addition, DARICs comprising two or more binding components directed to different target antigens enable dual or multiplex targeting of target cells and may be advantageous in enhancing efficacy, tumor clearance, and safety; and in decreasing relapse, antigen escape, on-target off-tumor cell lysis.

In various embodiments, the disclosure contemplates improved DARIC components. Without wishing to be bound by any particular theory, the present inventors have unexpectedly discovered that DARIC binding components comprising an intracellular signaling domain increase the potency of DARIC immune effector cells by, for example, increasing inflammatory cytokine secretion and increasing antigen dependent cytotoxicity against target cells. Moreover, in particular embodiments, wherein DARIC multimerization relies on an immunosuppressive bridging factor molecule, *e.g.,* rapamycin, a DARIC binding component comprising an intracellular signaling domain, *e.g.,* a costimulatory domain, surprisingly reduces or eliminates the immunosuppressive activity associated with the bridging factor.

In particular embodiments, a DARIC includes a polypeptide (DARIC signaling component) that comprises a multimerization domain polypeptide or variant thereof, a transmembrane domain, and one or more intracellular signaling domains; and a polypeptide (DARIC binding component) that comprises a binding domain, a multimerization domain polypeptide or variant thereof, a transmembrane domain; and one or more intracellular signaling domains. In preferred embodiments, the one or more intracellular signaling domains in the DARIC signaling component are different than the one or more intracellular signaling domains in the DARIC binding component. In the presence of a bridging factor, the DARIC binding and signaling components associate with one another through the bridging factor to form a functionally active DARIC.

In particular embodiments, a DARIC includes a polypeptide (DARIC signaling component) that comprises a multimerization domain polypeptide or variant thereof, a transmembrane domain, a costimulatory domain; and/or a primary signaling domain; and a polypeptide (DARIC binding component) that comprises a binding domain, a multimerization domain polypeptide or variant thereof, a transmembrane domain; and a costimulatory domain. In preferred embodiments, the costimulatory domain in the DARIC signaling component is different than the costimulatory domain in the DARIC binding component. Without wishing to be bound by any particular theory, it is believed that the two different costimulatory domains synergistically enhance the cytokine secretion profile and cytotoxicity of DARIC T cells directed to target cells. In the presence of a bridging factor, the DARIC binding and signaling components associate with one another through the bridging factor to form a functionally active DARIC.

In various embodiments, the disclosure contemplates DARIC components that generate an anti-cancer response against cancers that express two or more target antigens expressed on one or more target cells.

In particular embodiments, a DARIC signaling component that comprises a multimerization domain polypeptide or variant thereof, a transmembrane domain, a costimulatory domain; and/or a primary signaling domain; a first DARIC binding component that comprises a binding domain that binds a first target antigen, a multimerization domain polypeptide or variant thereof, a transmembrane domain; and a costimulatory domain; and a second DARIC binding component that comprises a binding domain that binds a second target antigen, a multimerization domain polypeptide or variant thereof, a transmembrane domain, and optionally a costimulatory domain. In the presence of a bridging factor, the DARIC binding components each associate with the DARIC signaling component through the bridging factor to form functionally active DARICs.

In preferred embodiments, the multimerization domains of the DARIC binding and DARIC signaling components are positioned extracellularly. Extracellular position of the multimerization domains provides numerous advantages over intracellular positioning including, but not limited to, more efficient positioning of the binding domain, higher

temporal sensitivity to bridging factor regulation, and less toxicity due to ability to use non-immunosuppressive doses of particular bridging factors.

Polynucleotides encoding DARICs, DARIC binding components, and DARIC signaling components; DARIC binding components, DARIC signaling components, DARIC protein complexes, DARIC fusion proteins; cells comprising polynucleotides encoding DARICs, DARIC binding components, and DARIC signaling components and/or expressing the same; and methods of using the same to treat an immune disorder are also contemplated herein.

Techniques for recombinant (*i.e.*, engineered) DNA, peptide and oligonucleotide synthesis, immunoassays, tissue culture, transformation (*e.g.*, electroporation, lipofection), enzymatic reactions, purification and related techniques and procedures may be generally performed as described in various general and more specific references in microbiology, molecular biology, biochemistry, molecular genetics, cell biology, virology and immunology as cited and discussed throughout the present specification. *See*, *e.g.*, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 3d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; *Current Protocols in Molecular Biology* (John Wiley and Sons, updated July 2008); *Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*, Greene Pub. Associates and Wiley-Interscience; Glover, *DNA Cloning: A Practical Approach*, vol. I & II (IRL Press, Oxford Univ. Press USA, 1985); *Current Protocols in Immunology* (Edited by: John E. Coligan, Ada M. Kruisbeek, David H. Margulies, Ethan M. Shevach, Warren Strober 2001 John Wiley & Sons, NY, NY); *Real-Time PCR: Current Technology and Applications*, Edited by Julie Logan, Kirstin Edwards and Nick Saunders, 2009, Caister Academic Press, Norfolk, UK; Anand, *Techniques for the Analysis of Complex Genomes*, (Academic Press, New York, 1992); Guthrie and Fink, *Guide to Yeast Genetics and Molecular Biology* (Academic Press, New York, 1991); *Oligonucleotide Synthesis* (N. Gait, Ed., 1984); *Nucleic Acid* The *Hybridization* (B. Hames & S. Higgins, Eds., 1985); *Transcription and Translation* (B. Hames & S. Higgins, Eds., 1984); *Animal Cell Culture* (R. Freshney, Ed., 1986); Perbal, *A Practical Guide to Molecular Cloning* (1984); *Next-*

Generation Genome Sequencing (Janitz, 2008 Wiley-VCH); *PCR Protocols (Methods in Molecular Biology)* (Park, Ed., 3rd Edition, 2010 Humana Press); *Immobilized Cells And Enzymes* (IRL Press, 1986); the treatise, *Methods In Enzymology* (Academic Press, Inc., N.Y.); *Gene Transfer Vectors For Mammalian Cells* (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); Harlow and Lane, *Antibodies*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1998); *Immunochemical Methods In Cell And Molecular Biology* (Mayer and Walker, eds., Academic Press, London, 1987); *Handbook Of Experimental Immunology*, Volumes I-IV (D. M. Weir andCC Blackwell, eds., 1986); Roitt, *Essential Immunology,* 6th Edition, (Blackwell Scientific Publications, Oxford, 1988); *Current Protocols in Immunology* (Q. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach and W. Strober, eds., 1991); *Annual Review of Immunology*; as well as monographs in journals such as *Advances in Immunology*.

B. DEFINITIONS

Prior to setting forth this disclosure in more detail, it may be helpful to an understanding thereof to provide definitions of certain terms to be used herein.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of particular embodiments, preferred embodiments of compositions, methods and materials are described herein. For the purposes of the present disclosure, the following terms are defined below.

The articles "a," "an," and "the" are used herein to refer to one or to more than one (*i.e.,* to at least one, or to one or more) of the grammatical object of the article. By way of example, "an element" means one element or one or more elements.

The use of the alternative (*e.g.,* "or") should be understood to mean either one, both, or any combination thereof of the alternatives.

The term "and/or" should be understood to mean either one, or both of the alternatives.

As used herein, the term "about" or "approximately" refers to a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that varies by as much as 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length. In one embodiment, the term "about" or "approximately" refers a range of quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length ± 15%, ± 10%, ± 9%, ± 8%, ± 7%, ± 6%, ± 5%, ± 4%, ± 3%, ± 2%, or ± 1% about a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

In one embodiment, a range, *e.g.,* 1 to 5, about 1 to 5, or about 1 to about 5, refers to each numerical value encompassed by the range. For example, in one non-limiting and merely illustrative embodiment, the range "1 to 5" is equivalent to the expression 1, 2, 3, 4, 5; or 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, or 5.0; or 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, or 5.0.

As used herein, the term "substantially" refers to a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that is 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher compared to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length. In one embodiment, "substantially the same" refers to a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that produces an effect, e.g., a physiological effect, that is approximately the same as a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

Throughout this specification, unless the context requires otherwise, the words "comprise," "comprises," and "comprising" will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements. By "consisting of" is meant including, and limited to, whatever follows the phrase "consisting of." Thus, the phrase "consisting of" indicates that the listed elements are required or mandatory, and that no other elements may

be present. By "consisting essentially of" is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase "consisting essentially of" indicates that the listed elements are required or mandatory, but that no other elements are present that materially affect the activity or action of the listed elements.

Reference throughout this specification to "one embodiment," "an embodiment," "a particular embodiment," "a related embodiment," "a certain embodiment," "an additional embodiment," or "a further embodiment" or combinations thereof means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment. Thus, the appearances of the foregoing phrases in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more embodiments. It is also understood that the positive recitation of a feature in one embodiment, serves as a basis for excluding the feature in a particular embodiment.

An "antigen (Ag)" refers to a compound, composition, or substance that can stimulate the production of antibodies or a T cell response in an animal, including compositions (such as one that includes a cancer-specific protein) that are injected or absorbed into an animal. Exemplary antigens include but are not limited to lipids, carbohydrates, polysaccharides, glycoproteins, peptides, or nucleic acids. An antigen reacts with the products of specific humoral or cellular immunity, including those induced by heterologous antigens, such as the disclosed antigens.

A "target antigen" or "target antigen of interest" is an antigen that a binding domain contemplated herein, is designed to bind. In particular embodiments, one or more target antigens are selected from the group consisting of: alpha folate receptor (FRα), $\alpha_v\beta_6$ integrin, B cell maturation antigen (BCMA), B7-H3 (CD276), B7-H6, carbonic anhydrase IX (CAIX), CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, carcinoembryonic antigen (CEA), claudin 6, (CLDN6), claudin 18 isoform 2 (CLDN18.2), C-type lectin-like

molecule-1 (CLL-1), CD2 subset 1 (CS-1), chondroitin sulfate proteoglycan 4 (CSPG4), cutaneous T cell lymphoma-associated antigen 1 (CTAGE1), delta like canonical Notch ligand 3 (DLL3), epidermal growth factor receptor (EGFR), epidermal growth factor receptor variant III (EGFRvIII), epithelial glycoprotein 2 (EGP2), epithelial glycoprotein 40 (EGP40), epithelial cell adhesion molecule (EPCAM), ephrin type-A receptor 2 (EPHA2), erb-b2 receptor tyrosine kinase 4 (ERBB4), fibroblast activation protein (FAP), Fc Receptor Like 5 (FCRL5), fetal acetylcholinesterase receptor (AchR), ganglioside G2 (GD2), ganglioside G3 (GD3), Glypican-3 (GPC3), EGFR family including ErbB2 (HER2), HER2 p95, IL-10Rα, IL-13Rα2, Kappa, cancer/testis antigen 2 (LAGE-1A), Lambda, Lewis-Y (LeY), L1 cell adhesion molecule (L1-CAM), melanoma antigen gene (MAGE)-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGEA10, melanoma antigen recognized by T cells 1 (MelanA or MART1), Mesothelin (MSLN), MUC1, MUC16, MHC class I chain related proteins A (MICA), MHC class I chain related proteins B (MICB), neural cell adhesion molecule (NCAM), cancer/testis antigen 1 (NY-ESO-1), polysialic acid; placenta-specific 1 (PLAC1), preferentially expressed antigen in melanoma (PRAME), prostate stem cell antigen (PSCA), prostate-specific membrane antigen (PSMA), receptor tyrosine kinase-like orphan receptor 1 (ROR1), synovial sarcoma, X breakpoint 2 (SSX2), Survivin, tumor associated glycoprotein 72 (TAG72), tumor endothelial marker 1 (TEM1/CD248), tumor endothelial marker 7-related (TEM7R), trophoblast glycoprotein (TPBG), UL16-binding protein (ULBP) 1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, vascular endothelial growth factor receptor 2 (VEGFR2), and Wilms tumor 1 (WT-1).

In one embodiment, the antigen is an MHC-peptide complex, such as a class I MHC-peptide complex or a class II MHC-peptide complex.

An "NKG2D ligand" refers to a polypeptide that is recognized and/or bound by a natural-killer group 2, member D (NKG2D) receptor. Two families of NKG2D ligands have been identified in humans: MHC class I chain related proteins A (MICA) and B (MICB) and HCMV UL16-binding proteins, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and ULBP6. MICA and MICB each have an α1, α2, α3, and transmembrane domain;

ULBP1, ULBP2, ULBP3, and ULBP6 each have an α1 and α2 domain and are glycosylphosphatidylinositol (GPI)-linked to the cell membrane; and ULBP4 and ULBP 5 each have an α1 and α2 domain and a transmembrane domain. NKG2D ligands are expressed, in various combinations, on many human cancer cells and immunosuppressive cells (T-regs and myeloid derived suppressor cells (MDSCs) within tumor microenvironments). Cancers expressing one or more NKG2D ligands include, but are not limited to, carcinomas (ovarian, bladder, breast, lung, liver, colon, kidney, prostate, melanoma, Ewing's sarcoma, glioma, and neuroblastoma), leukemias (AML, CML, CLL), lymphomas, and multiple myeloma. NKG2D ligands can also be induced at sites of chronic inflammation, transiently after some infections, following local irradiation, and after treatment with particular drugs, *e.g.,* HDAC inhibitors and bortezomib.

An "NKG2D receptor binding domain or NKG2D ligand binding portion thereof" refers to the NKG2D receptor or a portion thereof necessary or sufficient to bind one or more NKG2D ligands. Natural-killer group 2, member D (NKG2D), also known as Klrk1, is a C-type lectin-like receptor, that was first identified in natural killer (NK) cells as an activating immune receptor. In human, NKG2D is expressed on NK cells, $CD8^+$ T cells, subsets of $CD4^+$ T cells, and subsets of γδ T cells as a costimulatory receptor. NKG2D receptor binding domain or NKG2D ligand binding portion thereof binds one or more NKG2D ligands including, but not limited to MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and ULBP6.

As used herein, the terms, "binding domain," "extracellular domain," "antigen binding domain," "extracellular binding domain," "extracellular antigen binding domain," "antigen-specific binding domain," and "extracellular antigen specific binding domain," are used interchangeably and refer to a polypeptide with the ability to specifically and/or selectively bind to the target antigen of interest. The binding domain may be derived either from a natural, synthetic, semi-synthetic, or recombinant source.

The terms "specific binding affinity" or "specifically binds" or "specifically bound" or "specific binding" or "specifically targets" as used herein, describe binding of binding domain to a target antigen at greater binding affinity than background binding. A binding

domain "specifically binds" to a target antigen, if it binds to or associates with the antigen with an affinity or K_a (*i.e.*, an equilibrium association constant of a particular binding interaction with units of 1/M) of, for example, greater than or equal to about 10^5 M^{-1}. In certain embodiments, a binding domain (or a fusion protein comprising the same) binds to a target with a K_a greater than or equal to about 10^6 M^{-1}, 10^7 M^{-1}, 10^8 M^{-1}, 10^9 M^{-1}, 10^{10} M^{-1}, 10^{11} M^{-1}, 10^{12} M^{-1}, or 10^{13} M^{-1}. "High affinity" binding domains (or single chain fusion proteins thereof) refer to those binding domains with a K_a of at least 10^7 M^{-1}, at least 10^8 M^{-1}, at least 10^9 M^{-1}, at least 10^{10} M^{-1}, at least 10^{11} M^{-1}, at least 10^{12} M^{-1}, at least 10^{13} M^{-1}, or greater.

The terms "selectively binds" or "selectively bound" or "selectively binding" or "selectively targets" and describe preferential binding of one molecule to a target molecule (on-target binding) in the presence of a plurality of off-target molecules.

An "antibody" refers to a binding agent that is a polypeptide comprising at least a light chain or heavy chain immunoglobulin variable region which specifically recognizes and binds an epitope of an antigen, such as a lipid, carbohydrate, polysaccharide, glycoprotein, peptide, or nucleic acid containing an antigenic determinant, such as those recognized by an immune cell.

An "epitope" or "antigenic determinant" refers to the region of an antigen to which a binding agent binds. In particular embodiments, the polypeptide is intracellular and the epitope is a short oligopeptide (about 2 to about 20 amino acids) displayed in complex with an MHC.

Antibodies include antigen binding fragments thereof, such as a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a $F(ab')_2$ fragment, a bispecific Fab dimer (Fab2), a trispecific Fab trimer (Fab3), an Fv, an single chain Fv protein ("scFv"), a bis-scFv, $(scFv)_2$, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody) and portions of full length antibodies responsible for antigen binding. Antibodies also include: polyclonal and monoclonal antibodies and antigen binding fragments thereof; murine antibodies, camelid antibodies, and human antibodies, and antigen binding fragments

thereof; and chimeric antibodies, heteroconjugate antibodies, and humanized antibodies, and antigen binding fragments thereof. *See also*, Pierce Catalog and Handbook, 1994-1995 (Pierce Chemical Co., Rockford, IL); Kuby, J., Immunology, 3rd Ed., W. H. Freeman & Co., New York, 1997.

A "linker" refers to a plurality of amino acid residues between the various polypeptide domains added for appropriate spacing and conformation of the molecule. In particular embodiments, the linker is a variable region linking sequence. A "variable region linking sequence," is an amino acid sequence that connects the V_H and V_L domains and provides a spacer function compatible with interaction of the two sub-binding domains so that the resulting polypeptide retains a specific binding affinity to the same target molecule as an antibody that comprises the same light and heavy chain variable regions. In particular embodiments, a linker separates one or more heavy or light chain variable domains, hinge domains, multimerization domains, transmembrane domains, costimulatory domains, and/or primary signaling domains.

Illustrated examples of linkers suitable for use in particular embodiments contemplated herein include, but are not limited to the following amino acid sequences: GGG; DGGGS (SEQ ID NO: 30); TGEKP (SEQ ID NO: 31) (see, *e.g.,* Liu *et al.*, PNAS 5525-5530 (1997)); GGRR (SEQ ID NO: 32) (Pomerantz *et al.* 1995, supra); $(GGGGS)_n$ wherein n = 1, 2, 3, 4 or 5 (SEQ ID NO: 33) (Kim *et al.*, PNAS 93, 1156-1160 (1996.); EGKSSGSGSESKVD (SEQ ID NO: 34) (Chaudhary *et al.*, 1990, Proc. Natl. Acad. Sci. U.S.A. 87:1066-1070); KESGSVSSEQLAQFRSLD (SEQ ID NO: 35) (Bird *et al.*, 1988, Science 242:423-426), GGRRGGGS (SEQ ID NO: 36); LRQRDGERP (SEQ ID NO: 37); LRQKDGGGSERP (SEQ ID NO: 38); LRQKD$(GGGS)_2$ ERP (SEQ ID NO: 39). Alternatively, flexible linkers can be rationally designed using a computer program capable of modeling both DNA-binding sites and the peptides themselves (Desjarlais & Berg, *PNAS* 90:2256-2260 (1993), *PNAS* 91:11099-11103 (1994) or by phage display methods. In one embodiment, the linker comprises the following amino acid sequence: GSTSGSGKPGSGEGSTKG (SEQ ID NO: 40) (Cooper *et al., Blood*, 101(4): 1637-1644 (2003)).

A "spacer domain," refers to a polypeptide that separates two domains. In one embodiment, a spacer domain moves an antigen binding domain away from the effector cell surface to enable proper cell/cell contact, antigen binding and activation (Patel *et al.*, *Gene Therapy*, 1999; 6: 412-419). In particular embodiments, a spacer domain separates one or more binding domains, multimerization domains, transmembrane domains, costimulatory domains, and/or primary signaling domains. The spacer domain may be derived either from a natural, synthetic, semi-synthetic, or recombinant source. In certain embodiments, a spacer domain is a portion of an immunoglobulin, including, but not limited to, one or more heavy chain constant regions, *e.g.,* CH2 and CH3. The spacer domain can include the amino acid sequence of a naturally occurring immunoglobulin hinge region or an altered immunoglobulin hinge region.

A "hinge domain," refers to a polypeptide that plays a role in positioning the antigen binding domain away from the effector cell surface to enable proper cell/cell contact, antigen binding and activation. In particular embodiments, polypeptides may comprise one or more hinge domains between the binding domain and the multimerization domain, between the binding domain and the transmembrane domain (TM), or between the multimerization domain and the transmembrane domain. The hinge domain may be derived either from a natural, synthetic, semi-synthetic, or recombinant source. The hinge domain can include the amino acid sequence of a naturally occurring immunoglobulin hinge region or an altered immunoglobulin hinge region.

A "multimerization domain," as used herein, refers to a polypeptide that preferentially interacts or associates with another different polypeptide directly or via a bridging molecule, *e.g.,* a chemically inducible dimerizer, wherein the interaction of different multimerization domains substantially contributes to or efficiently promotes multimerization (*i.e.*, the formation of a dimer, trimer, or multipartite complex, which may be a homodimer, heterodimer, homotrimer, heterotrimer, homomultimer, heteromultimer). A multimerization domain may be derived either from a natural, synthetic, semi-synthetic, or recombinant source.

Illustrative examples of multimerization domains suitable for use in particular embodiments contemplated herein include an FK506 binding protein (FKBP) polypeptide or variants thereof, an FKBP-rapamycin binding (FRB) polypeptide or variants thereof, a calcineurin polypeptide or variants thereof, a cyclophilin polypeptide or variants thereof, a bacterial dihydrofolate reductase (DHFR) polypeptide or variants thereof, a PYR1-like 1 (PYL1) polypeptide or variants thereof, an abscisic acid insensitive 1 (ABI1) polypeptide or variants thereof, a GIB1 polypeptide or variants thereof, or a GAI polypeptide or variants thereof.

As used herein, the term "FKBP-rapamycin binding polypeptide" refers to an FRB polypeptide. In particular embodiments, the FRB polypeptide is an FKBP12-rapamycin binding polypeptide. FRB polypeptides suitable for use in particular embodiments contemplated herein generally contain at least about 85 to about 100 amino acid residues. In certain embodiments, the FRB polypeptide comprises a 93 amino acid sequence Ile-2021 through Lys-2113 and a mutation of T2098L, with reference to GenBank Accession No. L34075.1. An FRB polypeptide contemplated herein binds to an FKBP polypeptide through a bridging factor, thereby forming a ternary complex.

As used herein, the term "FK506 binding protein" refers to an FKBP polypeptide. In particular embodiments, the FKBP polypeptide is an FKBP12 polypeptide or an FKBP12 polypeptide comprising an F36V mutation. In certain embodiments, an FKBP domain may also be referred to as a "rapamycin binding domain". Information concerning the nucleotide sequences, cloning, and other aspects of various FKBP species is known in the art (*see*, *e.g.,* Staendart *et al*., *Nature 346*:671, 1990 (human FKBP12); Kay, *Biochem. J. 314*:361, 1996). An FKBP polypeptide contemplated herein binds to an FRB polypeptide through a bridging factor, thereby forming a ternary complex.

A "bridging factor" refers to a molecule that associates with and that is disposed between two or more multimerization domains. In particular embodiments, multimerization domains substantially contribute to or efficiently promote formation of a polypeptide complex only in the presence of a bridging factor. In particular embodiments, multimerization domains do not contribute to or do not efficiently promote formation of a

polypeptide complex in the absence of a bridging factor. Illustrative examples of bridging factors suitable for use in particular embodiments contemplated herein include, but are not limited to AP21967, rapamycin (sirolimus) or a rapalog thereof, coumermycin or a derivative thereof, gibberellin or a derivative thereof, abscisic acid (ABA) or a derivative thereof, methotrexate or a derivative thereof, cyclosporin A or a derivative thereof, FKCsA or a derivative thereof, trimethoprim (Tmp)-synthetic ligand for FKBP (SLF) or a derivative thereof, or any combination thereof.

Rapamycin analogs (rapalogs) include but are not limited to those disclosed in U.S. Pat. No. 6,649,595, which rapalog structures are incorporated herein by reference in their entirety. In certain embodiments, a bridging factor is a rapalog with substantially reduced immunosuppressive effect as compared to rapamycin. In a preferred embodiment, the rapalog is AP21967 (also known as C-16-(S)-7-methylindolerapamycin, IC_{50} = 10nM, a chemically modified non-immunosuppressive rapamycin analogue). Other illustrative rapalogs suitable for use in particular embodiments contemplated herein include, but are not limited to, everolimus, novolimus, pimecrolimus, ridaforolimus, tacrolimus, temsirolimus, umirolimus, and zotarolimus.

A "substantially reduced immunosuppressive effect" refers to at least less than 0.1 to 0.005 times the immunosuppressive effect observed or expected for the same dose measured either clinically or in an appropriate *in vitro* (*e.g.*, inhibition of T cell proliferation) or *in vivo* surrogate of human immunosuppressive activity.

A "transmembrane domain" or "TM domain" is a domain that anchors a polypeptide to the plasma membrane of a cell. The TM domain may be derived either from a natural, synthetic, semi-synthetic, or recombinant source.

The term "effector function" or "effector cell function" refers to a specialized function of an immune effector cell. Effector function includes, but is not limited to, activation, cytokine production, proliferation and cytotoxic activity, including the release of cytotoxic factors, or other cellular responses elicited with antigen binding to the receptor expressed on the immune effector cell.

An "intracellular signaling domain" or "endodomain" refers to the portion of a protein which transduces the effector function signal and that directs the cell to perform a specialized function. While usually the entire intracellular signaling domain can be employed, in many cases it is not necessary to use the entire domain. To the extent that a truncated portion of an intracellular signaling domain is used, such truncated portion may be used in place of the entire domain as long as it transduces an effector function signal. The term intracellular signaling domain is meant to include any truncated portion of an intracellular signaling domain necessary or sufficient to transduce an effector function signal.

It is known that signals generated through the TCR alone are insufficient for full activation of the T cell and that a secondary or costimulatory signal is also required. Thus, T cell activation can be said to be mediated by two distinct classes of intracellular signaling domains: primary signaling domains that initiate antigen-dependent primary activation through the TCR (*e.g.*, a TCR/CD3 complex) and costimulatory domains that act in an antigen-independent manner to provide a secondary or costimulatory signal.

A "primary signaling domain" refers to an intracellular signaling domain that regulates the primary activation of the TCR complex either in a stimulatory way, or in an inhibitory way. Primary signaling domains that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs. Illustrative examples of ITAM containing primary signaling domains that are suitable for use in particular embodiments include, but are not limited to those derived from FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d.

As used herein, the term, "costimulatory domain," or "costimulatory domain" refers to an intracellular signaling domain of a costimulatory molecule. Costimulatory molecules are cell surface molecules other than antigen receptors or Fc receptors that provide a second signal required for efficient activation and function of T lymphocytes upon binding to antigen. Illustrative examples of such costimulatory molecules from which costimulatory domains may be isolated include, but are not limited to: Toll-like receptor 1 (TLR1), TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, caspase

recruitment domain family member 11 (CARD11), CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD94, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), DNAX-Activation Protein 10 (DAP10), Linker for activation of T-cells family member 1 (LAT), SH2 Domain-Containing Leukocyte Protein Of 76 kD (SLP76), T cell receptor associated transmembrane adaptor 1 (TRAT1), TNFR2, TNF receptor superfamily member 14 (TNFRS14; HVEM), TNF receptor superfamily member 18 (TNFRS18; GITR), TNF receptor superfamily member 25 (TNFRS25; DR3), and zeta chain of T cell receptor associated protein kinase 70 (ZAP70).

An "immune disorder" refers to a disease that evokes a response from the immune system. In particular embodiments, the term "immune disorder" refers to a cancer, an autoimmune disease, or an immunodeficiency. In one embodiment, immune disorders encompass infectious disease.

As used herein, the term "cancer" relates generally to a class of diseases or conditions in which abnormal cells divide without control and can invade nearby tissues.

As used herein, the term "malignant" refers to a cancer in which a group of tumor cells display one or more of uncontrolled growth (*i.e.,* division beyond normal limits), invasion (*i.e.,* intrusion on and destruction of adjacent tissues), and metastasis (*i.e.,* spread to other locations in the body via lymph or blood). As used herein, the term "metastasize" refers to the spread of cancer from one part of the body to another. A tumor formed by cells that have spread is called a "metastatic tumor" or a "metastasis." The metastatic tumor contains cells that are like those in the original (primary) tumor.

As used herein, the term "benign" or "non-malignant" refers to tumors that may grow larger but do not spread to other parts of the body. Benign tumors are self-limited and typically do not invade or metastasize.

A "cancer cell" refers to an individual cell of a cancerous growth or tissue. Cancer cells include both solid cancers and liquid cancers. A "tumor" or "tumor cell" refers generally to a swelling or lesion formed by an abnormal growth of cells, which may be benign, pre-malignant, or malignant. Most cancers form tumors, but liquid cancers, *e.g.,* leukemia, do not necessarily form tumors. For those cancers that form tumors, the terms

cancer (cell) and tumor (cell) are used interchangeably. The amount of a tumor in an individual is the "tumor burden" which can be measured as the number, volume, or weight of the tumor.

The term "relapse" refers to the diagnosis of return, or signs and symptoms of return, of a cancer after a period of improvement or remission.

"Remission," is also referred to as "clinical remission," and includes both partial and complete remission. In partial remission, some, but not all, signs and symptoms of cancer have disappeared. In complete remission, all signs and symptoms of cancer have disappeared, although cancer still may be in the body.

"Refractory" refers to a cancer that is resistant to, or non-responsive to, therapy with a particular therapeutic agent. A cancer can be refractory from the onset of treatment (*i.e.*, non-responsive to initial exposure to the therapeutic agent), or as a result of developing resistance to the therapeutic agent, either over the course of a first treatment period or during a subsequent treatment period.

"Antigen negative" refers to a cell that does not express antigen or expresses a negligible amount of antigen that is undetectable. In one embodiment, antigen negative cells do not bind receptors directed to the antigen. In one embodiment, antigen negative cells do not substantially bind receptors directed to the antigen.

An "autoimmune disease" refers to a disease in which the body produces an immunogenic (*i.e.*, immune system) response to some constituent of its own tissue. In other words, the immune system loses its ability to recognize some tissue or system within the body as "self" and targets and attacks it as if it were foreign. Autoimmune diseases can be classified into those in which predominantly one organ is affected (*e.g.*, hemolytic anemia and anti-immune thyroiditis), and those in which the autoimmune disease process is diffused through many tissues (*e.g.*, systemic lupus erythematosus). For example, multiple sclerosis is thought to be caused by T cells attacking the sheaths that surround the nerve fibers of the brain and spinal cord. This results in loss of coordination, weakness, and blurred vision. Autoimmune diseases are known in the art and include, for instance, Hashimoto's thyroiditis, Grave's disease, lupus, multiple sclerosis, rheumatic arthritis,

hemolytic anemia, anti-immune thyroiditis, systemic lupus erythematosus, celiac disease, Crohn's disease, colitis, diabetes, scleroderma, psoriasis, and the like.

An "immunodeficiency" means the state of a patient whose immune system has been compromised by disease or by administration of chemicals. This condition makes the system deficient in the number and type of blood cells needed to defend against a foreign substance. Immunodeficiency conditions or diseases are known in the art and include, for example, AIDS (acquired immunodeficiency syndrome), SCID (severe combined immunodeficiency disease), selective IgA deficiency, common variable immunodeficiency, X-linked agammaglobulinemia, chronic granulomatous disease, hyper-IgM syndrome, and diabetes.

An "infectious disease" refers to a disease that can be transmitted from person to person or from organism to organism and is caused by a microbial or viral agent (*e.g.,* common cold). Infectious diseases are known in the art and include, for example, hepatitis, sexually transmitted diseases (*e.g.,* Chlamydia, gonorrhea), tuberculosis, HIV/AIDS, diphtheria, hepatitis B, hepatitis C, cholera, and influenza.

As used herein, the terms "individual" and "subject" are often used interchangeably and refer to any animal that exhibits a symptom of cancer or other immune disorder that can be treated with the compositions and methods contemplated elsewhere herein. Suitable subjects (*e.g.,* patients) include laboratory animals (such as mouse, rat, rabbit, or guinea pig), farm animals, and domestic animals or pets (such as a cat or dog). Non-human primates and, preferably, human patients, are included. Typical subjects include human patients that have, have been diagnosed with, or are at risk or having, cancer or another immune disorder.

As used herein, the term "patient" refers to a subject that has been diagnosed with cancer or another immune disorder that can be treated with the compositions and methods disclosed elsewhere herein.

As used herein "treatment" or "treating," includes any beneficial or desirable effect on the symptoms or pathology of a disease or pathological condition and may include even minimal reductions in one or more measurable markers of the disease or condition being

treated. Treatment can involve optionally either the reduction of the disease or condition, or the delaying of the progression of the disease or condition, *e.g.*, delaying tumor outgrowth. "Treatment" does not necessarily indicate complete eradication or cure of the disease or condition, or associated symptoms thereof.

As used herein, "prevent," and similar words such as "prevented," "preventing" *etc.*, indicate an approach for preventing, inhibiting, or reducing the likelihood of the occurrence or recurrence of, a disease or condition. It also refers to delaying the onset or recurrence of a disease or condition or delaying the occurrence or recurrence of the symptoms of a disease or condition. As used herein, "prevention" and similar words also includes reducing the intensity, effect, symptoms and/or burden of a disease or condition prior to onset or recurrence of the disease or condition.

As used herein, the phrase "ameliorating at least one symptom of" refers to decreasing one or more symptoms of the disease or condition for which the subject is being treated. In particular embodiments, the disease or condition being treated is a cancer, wherein the one or more symptoms ameliorated include, but are not limited to, weakness, fatigue, shortness of breath, easy bruising and bleeding, frequent infections, enlarged lymph nodes, distended or painful abdomen (due to enlarged abdominal organs), bone or joint pain, fractures, unplanned weight loss, poor appetite, night sweats, persistent mild fever, and decreased urination (due to impaired kidney function).

By "enhance" or "promote," or "increase" or "expand" refers generally to the ability of a composition contemplated herein to produce, elicit, or cause a greater physiological response (*i.e.*, downstream effects) compared to the response caused by either vehicle or a control molecule/composition. A measurable physiological response may include an increase in T cell expansion, activation, persistence, cytokine secretion, and/or an increase in cancer cell killing ability, among others apparent from the understanding in the art and the description herein. An "increased" or "enhanced" amount is typically a "statistically significant" amount, and may include an increase that is 1.1, 1.2, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 or more times (*e.g.*, 500, 1000 times) (including all

integers and decimal points in between and above 1, *e.g.,* 1.5, 1.6, 1.7. 1.8, *etc.*) the response produced by vehicle or a control composition.

By "decrease" or "lower," or "lessen," or "reduce," or "abate" refers generally to the ability of composition contemplated herein to produce, elicit, or cause a lesser physiological response (*i.e.*, downstream effects) compared to the response caused by either vehicle or a control molecule/composition. A "decrease" or "reduced" amount is typically a "statistically significant" amount, and may include a decrease that is 1.1, 1.2, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 or more times (*e.g.,* 500, 1000 times) (including all integers and decimal points in between and above 1, *e.g.,* 1.5, 1.6, 1.7. 1.8, *etc.*) the response (reference response) produced by vehicle, a control composition, or the response in a particular cell lineage.

By "maintain," or "preserve," or "maintenance," or "no change," or "no substantial change," or "no substantial decrease" refers generally to the ability of a composition contemplated herein to produce, elicit, or cause a substantially similar or comparable physiological response (*i.e.*, downstream effects) in a cell, as compared to the response caused by either vehicle, a control molecule/composition, or the response in a particular cell lineage. A comparable response is one that is not significantly different or measurable different from the reference response.

Additional definitions are set forth throughout this disclosure.

C. DARICs

In particular embodiments, one or more costimulatory DARICs redirect cytotoxicity of an immune effector cell to a cancer cell that expresses at least one or more target antigens. As used herein, the term "DARIC" refers to a dimerizing agent regulated immunoreceptor complex. A DARIC comprises one or more non-naturally occurring polypeptides that transduces an immunostimulatory signal in an immune effector cell upon exposure to a multimerizing agent or bridging factor, *e.g.,* stimulating immune effector cell activity and function, increasing production and/or secretion of proinflammatory cytokines.

In particular embodiments, the DARICs contemplated herein reduce the immunosuppressive effects of particular bridging factors and improve the spatial and temporal control of an immunostimulatory signal in an immune effector. In preferred embodiments, a DARIC is a multi-chain chimeric receptor comprising one or more DARIC signaling components that each comprise a multimerization domain polypeptide or variant thereof, a transmembrane domain, and one or more intracellular signaling domains and one or more DARIC binding components that each comprise a binding domain, a multimerization domain polypeptide or variant thereof, a transmembrane domain, and one or more intracellular signaling domains. In preferred embodiments, the one or more intracellular signaling domains in the DARIC signaling component are different than the one or more intracellular signaling domains in the DARIC binding component.

In one embodiment, a DARIC signaling component and a DARIC binding component are expressed from one or more polynucleotides in the same cell. In another embodiment, a DARIC signaling component and a DARIC binding component are expressed from a polycistronic polynucleotide in the same cell.

1. DARIC SIGNALING COMPONENT

A "DARIC signaling component" or "DARIC signaling polypeptide" refers to a polypeptide comprising one or more multimerization domains, a transmembrane domain, and one or more intracellular signaling domains. In particular embodiments, the DARIC signaling component comprises a multimerization domain, a transmembrane domain, a costimulatory domain and/or a primary signaling domain. In particular embodiments, the DARIC signaling component comprises a first multimerization domain, a first transmembrane domain, a first costimulatory domain and/or a primary signaling domain.

In particular embodiments, a DARIC signaling component comprises one or more multimerization domains.

Illustrative examples of multimerization domains suitable for use in particular DARIC signaling components contemplated herein include, but are not limited to, an FK506 binding protein (FKBP) polypeptide or variants thereof, an FKBP-rapamycin

binding (FRB) polypeptide or variants thereof, a calcineurin polypeptide or variants thereof, a cyclophilin polypeptide or variants thereof, a bacterial dihydrofolate reductase (DHFR) polypeptide or variants thereof, a PYR1-like 1 (PYL1) polypeptide or variants thereof and an abscisic acid insensitive 1 (ABI1) polypeptide or variants thereof.

In particular embodiments, a DARIC signaling component comprises an FRB polypeptide. In a preferred embodiment, a DARIC signaling component comprises an FRB polypeptide comprising a T2098L mutation, or variant thereof.

In particular embodiments, a DARIC signaling component comprises an FKBP polypeptide or variant thereof. In a preferred embodiment, a DARIC signaling component comprises an FK506-binding protein 12 (FKBP12) polypeptide, or variant thereof.

In particular embodiments, a DARIC signaling component comprises a transmembrane domain.

Illustrative examples of transmembrane domains suitable for use in particular DARIC signaling components contemplated herein include, but are not limited to, the transmembrane region(s) of the alpha, beta, gamma, or delta chain of a T-cell receptor, CD3ε, CD3ζ, CD4, CD5, CD8α, CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD71, CD80, CD86, CD 134, CD137, CD152, CD 154, amnionless (AMN), programmed cell death 1 (PDCD1), NKG2A, NKG2B, NKG2C, and NKG2D. In a preferred embodiment, a DARIC signaling component comprises a CD4 transmembrane domain. In a preferred embodiment, a DARIC signaling component comprises a CD8α transmembrane domain.

In particular embodiments, a DARIC signaling component comprises a linker that links the C-terminus of the transmembrane domain to the N-terminus of an intracellular signaling domain. In various preferred embodiments, a short oligo- or poly-peptide linker, preferably between 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids in length links the transmembrane domain and an intracellular signaling domain. A glycine-serine based linker provides a particularly suitable linker.

DARIC signaling components contemplated in particular embodiments herein comprise one or more intracellular signaling domains. In one embodiment, a DARIC

signaling component comprises one or more costimulatory domains and/or a primary signaling domain. In one embodiment, the intracellular signaling domain comprises an immunoreceptor tyrosine activation motif (ITAM).

Illustrative examples of ITAM containing primary signaling domains that are suitable for use in particular DARIC signaling components contemplated herein include, but are not limited to those derived from FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d. In preferred embodiments, a DARIC signaling component comprises a CD3ζ primary signaling domain and one or more costimulatory domains. The primary signaling and costimulatory domains may be linked in any order in tandem to the carboxyl terminus of the transmembrane domain.

Illustrative examples of costimulatory domains suitable for use in particular DARIC signaling components contemplated herein include, but are not limited to those domains isolated from the following costimulatory molecules: Toll-like receptor 1 (TLR1), TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, caspase recruitment domain family member 11 (CARD11), CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD94, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), DNAX-Activation Protein 10 (DAP10), Linker for activation of T-cells family member 1 (LAT), SH2 Domain-Containing Leukocyte Protein Of 76 kD (SLP76), T cell receptor associated transmembrane adaptor 1 (TRAT1), TNFR2, TNFRS14, TNFRS18, TNFRS25, and zeta chain of T cell receptor associated protein kinase 70 (ZAP70).

In particular embodiments, a DARIC signaling component contemplated herein comprises a signal peptide, *e.g.,* secretion signal peptide, and do not comprise a transmembrane domain. Illustrative examples of signal peptides suitable for use in particular DARIC signaling components include but are not limited to an IgG1 heavy chain signal polypeptide, an Igκ light chain signal polypeptide, a CD8α signal polypeptide, or a human GM-CSF receptor alpha signal polypeptide. In various preferred embodiments, a DARIC signaling component comprises a CD8α signal polypeptide.

In particular embodiments, a DARIC signaling component comprises one or more costimulatory domains selected from the group consisting of CD28, CD137, and CD134.

In particular embodiments, a DARIC signaling component comprises one or more costimulatory domains selected from the group consisting of CD28, CD137, and CD134, and a CD3ζ primary signaling domain. In a particular embodiment, a DARIC signaling component comprises a CD137 costimulatory domain and a CD3ζ primary signaling domain.

In a preferred embodiment, a DARIC signaling component comprises an FRB T2098L multimerization domain, a CD8α transmembrane domain, a CD137 costimulatory domain and a CD3ζ primary signaling domain.

2. DARIC BINDING COMPONENT

A "DARIC binding component" or "DARIC binding polypeptide" refers to a polypeptide comprising a binding domain that binds a target antigen, one or more multimerization domains, a transmembrane domain, and one or more intracellular signaling domains. In particular embodiments, the DARIC binding component comprises a binding domain that binds a target antigen, a multimerization domain, a transmembrane domain, and a costimulatory domain. In particular embodiments, the DARIC binding component comprises a binding domain that binds a target antigen, a second multimerization domain, a second transmembrane domain, and a second costimulatory domain.

In particular embodiments, a DARIC comprises two or more DARIC binding components that each comprise a binding domain that binds to a different antigen, a multimerization domain, a transmembrane domain, and a costimulatory domain. In particular embodiments, a DARIC comprises two or more DARIC binding components that each comprise a binding domain that binds to a different antigen, a second or third multimerization domain, a second or third transmembrane domain, and a second or third costimulatory domain. In some embodiments, two or more DARIC binding components comprise different binding domains but comprise the same multimerization, transmembrane, and/or intracellular signaling domains.

Illustrative examples of binding domains suitable for use in particular DARIC binding components include, but are not limited to, antibodies or antigen binding fragments thereof.

In particular embodiments, antibodies and antigen binding fragments thereof suitable for use in particular DARIC binding components include, but are not limited to, murine antibodies, camelid antibodies, chimeric antibodies, humanized antibodies, or human antibodies. In preferred embodiments, the antibody or antigen binding fragment thereof is derived from a monoclonal antibody.

Illustrative examples of antibodies and antigen binding fragments thereof suitable for use in particular DARIC binding components include, but are not limited to, a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a $F(ab')_2$ fragment, a bispecific Fab dimer (Fab2), a trispecific Fab trimer (Fab3), an Fv, an single chain Fv protein ("scFv"), a bis-scFv, $(scFv)_2$, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody).

In a preferred embodiment, the binding domain comprises an scFv.

In a preferred embodiment, the binding domain comprises one or more camelid VHH antibodies.

In particular embodiments, a DARIC binding component comprises a binding domain that binds a tumor associated antigen (TAA), a tumor specific antigen (TSA), an NKG2D ligand, a γδ T cell receptor (γδTCR) ligand, or an αβTCR ligand.

In particular embodiments, a DARIC binding component comprises a binding domain that binds a target antigen selected from the group consisting of: FRα, $\alpha_v\beta_6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, CEA, CLDN6, CLDN18.2, CLL-1, CS-1, CSPG4, CTAGE1, DLL3, EGFR, EGFRvIII, EGP2, EGP40, EPCAM, EPHA2, ERBB4, FAP, FCRL5, AchR, GD2, GD3, GPC3, HER2, HER2 p95, IL-10Rα, IL-13Rα2, Kappa, LAGE-1A, Lambda, LeY, L1-CAM, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGEA10, MelanA or MART1, SLN), MUC1, MUC16, MICA, MICB, NCAM, NY-ESO-1, PLAC1, PRAME, PSCA,

PSMA, ROR1, SSX2, Survivin, TAG72, TEM1/CD248, TEM7R, TPBG, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, VEGFR2, and WT-1.

In a particular embodiment, a DARIC binding component comprises a binding domain that binds BCMA, B7-H3, CD19, CD20, CD22, CD33, CD79A, CD79B, CD123, CLL-1, or EGFRvIII, a multimerization domain, a transmembrane domain, and one or more intracellular signaling domains.

In particular embodiments, a DARIC binding component comprises one or more multimerization domains.

Illustrative examples of multimerization domains suitable for use in particular DARIC binding components contemplated herein include, but are not limited to, an FKBP polypeptide or variants thereof, an FRB polypeptide or variants thereof, a calcineurin polypeptide or variants thereof, a cyclophilin polypeptide or variants thereof, a DHFR polypeptide or variants thereof, a PYL1 polypeptide or variants thereof and an ABI1 polypeptide or variants thereof.

In particular embodiments, a DARIC binding component comprises an FRB polypeptide or variant thereof and a DARIC signaling component comprises an FKBP polypeptide or variant thereof. In a preferred embodiment, a DARIC binding component comprises an FRB polypeptide comprising a T2098L mutation, or variant thereof and a DARIC signaling component comprises an FKBP12 polypeptide or variant thereof.

In particular embodiments, a DARIC binding component comprises an FKBP polypeptide or variant thereof and a DARIC signaling component comprises an FRB polypeptide, or variant thereof. In a preferred embodiment, a DARIC binding component comprises an FKBP12 polypeptide, or variant thereof and a DARIC signaling component comprises an FRB polypeptide comprising a T2098L mutation, or variant thereof.

In particular embodiments, a DARIC binding component comprises a binding domain, an FKBP polypeptide or variant thereof, a transmembrane domain, and one or more intracellular signaling domains; and a DARIC signaling component comprises an FRB polypeptide or variant thereof, a transmembrane domain, and one or more intracellular signaling domains. In a preferred embodiment, a DARIC binding component

comprises a binding domain, an FKBP12 polypeptide or variant thereof, a transmembrane domain, and a costimulatory domain; and a DARIC signaling component comprises an FRB polypeptide comprising a T2098L mutation or variant thereof, a transmembrane domain, a costimulatory domain, and a primary signaling domain.

In particular embodiments, a DARIC binding component comprises a transmembrane domain. In one embodiment, the transmembrane domain may be the same as the transmembrane domain used in the DARIC signaling component. In one embodiment, the transmembrane domain may be different from the transmembrane domain used in the DARIC signaling component.

Illustrative examples of transmembrane domains suitable for use in particular DARIC signaling components contemplated herein include, but are not limited to, the transmembrane region(s) of the alpha, beta, gamma, or delta chain of a T-cell receptor, CD3ε, CD3ζ, CD4, CD5, CD8α, CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD71, CD80, CD86, CD 134, CD137, CD152, CD 154, amnionless (AMN), programmed cell death 1 (PDCD1), NKG2A, NKG2B, NKG2C, and NKG2D. In a particular embodiment, a DARIC binding component comprises a CD8α transmembrane domain. In a preferred embodiment, a DARIC binding component comprises a CD4 transmembrane domain.

In various preferred embodiments, a short oligo- or poly-peptide linker, preferably between 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids in length links the transmembrane domain and the intracellular signaling domain. A glycine-serine based linker provides a particularly suitable linker.

DARIC binding components contemplated in particular embodiments herein comprise one or more intracellular signaling domains. In one embodiment, a DARIC binding component comprises a costimulatory domain.

Illustrative examples of costimulatory domains suitable for use in particular DARIC signaling components contemplated herein include, but are not limited to those domains isolated from the following costimulatory molecules: Toll-like receptor 1 (TLR1), TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, caspase recruitment domain

family member 11 (CARD11), CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD94, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), DNAX-Activation Protein 10 (DAP10), Linker for activation of T-cells family member 1 (LAT), SH2 Domain-Containing Leukocyte Protein Of 76 kD (SLP76), T cell receptor associated transmembrane adaptor 1 (TRAT1), TNFR2, TNFRS14, TNFRS18, TNFRS25, and zeta chain of T cell receptor associated protein kinase 70 (ZAP70).

In particular embodiments, a DARIC binding component comprises a CD27 or CD28 costimulatory domain.

In particular embodiments, a DARIC binding component comprises a TNFRS14, TNFRS18, or TNFRS25 costimulatory domain.

In particular embodiments, a DARIC binding component comprises an OX40 costimulatory domain.

In some preferred embodiments, a DARIC binding component comprises a TNFR2 costimulatory domain.

In particular embodiments, a DARIC binding component contemplated herein comprises a signal peptide, *e.g.,* secretion signal peptide, and do not comprise a transmembrane domain. Illustrative examples of signal peptides suitable for use in particular DARIC binding components include but are not limited to an IgG1 heavy chain signal polypeptide, an Igκ light chain signal polypeptide, a CD8α signal polypeptide, or a human GM-CSF receptor alpha signal polypeptide. In various preferred embodiments, a DARIC binding component comprises a CD8α signal polypeptide.

In particular embodiments, a DARIC binding component comprises a binding domain that binds BCMA, CD19, CD20, CD22, CD33, B7H3, CD33, CD79A, CD79B, CD123, CLL-1, or EGFRvIII, an FKBP12 multimerization domain polypeptide, a CD4 transmembrane domain or AMN transmembrane domain, and a CD27, CD28, TNFRS14, TNFRS18, TNFRS25, OX40 or TNFR2 costimulatory domain.

In particular embodiments, a DARIC binding component comprises a binding domain that binds BCMA, CD19, CD20, CD22, CD33, B7H3, CD33, CD79A, CD79B, CD123, CLL-1, or EGFRvIII, an FKBP12 multimerization domain polypeptide, a CD4

transmembrane domain or AMN transmembrane domain, and a CD27 or CD28 costimulatory domain.

In particular embodiments, a DARIC binding component comprises a binding domain that binds BCMA, CD19, CD20, CD22, CD33, B7H3, CD33, CD79A, CD79B, CD123, CLL-1, or EGFRvIII, an FKBP12 multimerization domain polypeptide, a CD4 transmembrane domain or AMN transmembrane domain, and a TNFRS14, TNFRS18, or TNFRS25 costimulatory domain.

In a certain embodiment, a DARIC binding component comprises a binding domain that binds BCMA, CD19, CD20, CD22, CD33, B7H3, CD33, CD79A, CD79B, CD123, CLL-1, or EGFRvIII, an FKBP12 multimerization domain polypeptide, a CD4 transmembrane domain or AMN transmembrane domain, and an OX40 costimulatory domain.

In another embodiment, a DARIC binding component comprises a binding domain that binds BCMA, CD19, CD20, CD22, CD33, B7H3, CD33, CD79A, CD79B, CD123, CLL-1, or EGFRvIII, an FKBP12 multimerization domain polypeptide, a CD4 transmembrane domain or AMN transmembrane domain, and an TNFR2 costimulatory domain.

In particular embodiments, a DARIC signaling component comprises an FRB T2098L multimerization domain, a CD8α transmembrane domain, a CD137 costimulatory domain and a CD3ζ primary signaling domain and a DARIC binding component comprises a binding domain that binds BCMA, CD19, CD20, CD22, CD33, B7H3, CD33, CD79A, CD79B, CD123, CLL-1, or EGFRvIII, an FKBP12 multimerization domain polypeptide, a CD4 transmembrane domain or AMN transmembrane domain, and a CD27, CD28, TNFRS14, TNFRS18, TNFRS25, OX40 or TNFR2 costimulatory domain.

In particular embodiments, a DARIC signaling component comprises an FRB T2098L multimerization domain, a CD8α transmembrane domain, a CD137 costimulatory domain and a CD3ζ primary signaling domain and a DARIC binding component comprises a binding domain that binds BCMA, CD19, CD20, CD22, CD33, B7H3, CD33, CD79A, CD79B, CD123, CLL-1, or EGFRvIII, an FKBP12 multimerization domain polypeptide, a

CD4 transmembrane domain or AMN transmembrane domain, and a CD27 or CD28 costimulatory domain.

In particular embodiments, a DARIC signaling component comprises an FRB T2098L multimerization domain, a CD8α transmembrane domain, a CD137 costimulatory domain and a CD3ζ primary signaling domain and a DARIC binding component comprises a binding domain that binds BCMA, CD19, CD20, CD22, CD33, B7H3, CD33, CD79A, CD79B, CD123, CLL-1, or EGFRvIII, an FKBP12 multimerization domain polypeptide, a CD4 transmembrane domain or AMN transmembrane domain, and a TNFRS14, TNFRS18, or TNFRS25 costimulatory domain.

In particular embodiments, a DARIC signaling component comprises an FRB T2098L multimerization domain, a CD8α transmembrane domain, a CD137 costimulatory domain and a CD3ζ primary signaling domain and a DARIC binding component comprises a binding domain that binds BCMA, CD19, CD20, CD22, CD33, B7H3, CD33, CD79A, CD79B, CD123, CLL-1, or EGFRvIII, an FKBP12 multimerization domain polypeptide, a CD4 transmembrane domain or AMN transmembrane domain, and an OX40 costimulatory domain.

In particular embodiments, a DARIC signaling component comprises an FRB T2098L multimerization domain, a CD8α transmembrane domain, a CD137 costimulatory domain and a CD3ζ primary signaling domain and a DARIC binding component comprises a binding domain that binds BCMA, CD19, CD20, CD22, CD33, B7H3, CD33, CD79A, CD79B, CD123, CLL-1, or EGFRvIII, an FKBP12 multimerization domain polypeptide, a CD4 transmembrane domain or AMN transmembrane domain, and a TNFR2 costimulatory domain.

3. BRIDGING FACTOR

Bridging factors contemplated in particular embodiments herein, mediate or promote the association of one or more DARIC signaling components with one or more DARIC binding components through multimerization domains in the respective components. A bridging factor associates with and is disposed between the

multimerization domains to promote association of a DARIC signaling component and a DARIC binding component. In the presence of a bridging factor, the DARIC binding component and the DARIC signaling component associate and initiate immune effector cell activity against a target cell when the DARIC binding polypeptide is bound to a target antigen on the target cell. In the absence of a bridging factor, the DARIC binding component does not associate with the DARIC signaling component and the DARIC is inactive.

In particular embodiments, a DARIC signaling component and a DARIC binding component comprise a cognate pair of multimerization domains selected from the group consisting of: FKBP and FKBP-rapamycin binding (FRB), FKBP and calcineurin, FKBP and cyclophilin, FKBP and bacterial dihydrofolate reductase (DHFR), calcineurin and cyclophilin, and PYR1-like 1 (PYL1) and abscisic acid insensitive 1 (ABI1).

In certain embodiments, the multimerization domains of DARIC signaling and binding components associate with a bridging factor selected from the group consisting of: rapamycin or a rapalog thereof, coumermycin or a derivative thereof, gibberellin or a derivative thereof, abscisic acid (ABA) or a derivative thereof, methotrexate or a derivative thereof, cyclosporin A or a derivative thereof, FK506/cyclosporin A (FKCsA) or a derivative thereof, and trimethoprim (Tmp)-synthetic ligand for FK506 binding protein (FKBP) (SLF) or a derivative thereof.

In particular embodiments, a DARIC signaling component and a DARIC binding component comprise one or more FRB and/or FKBP multimerization domains or variants thereof. In certain embodiments, a DARIC signaling component comprises an FRB multimerization domain or variant thereof and a DARIC binding component comprises an FKBP multimerization domain or variant thereof. In particular preferred embodiments, a DARIC signaling component comprises an FRB T2098L multimerization domain or variant thereof and a DARIC binding component comprises an FKBP12 or FKBP12 F36V multimerization domains or variant thereof.

Illustrative examples of bridging factors suitable for use in particular embodiments contemplated herein include, but are not limited to, AP1903, AP20187, AP21967 (also

known as C-16-(S)-7-methylindolerapamycin), everolimus, novolimus, pimecrolimus, ridaforolimus, tacrolimus, temsirolimus, umirolimus, and zotarolimus. In particular preferred embodiments, the bridging factor is AP21967. In certain preferred embodiments, the bridging factor is a non-immunosuppressive dose of sirolimus (rapamycin).

D. ENGINEERED ANTIGEN RECEPTORS

In particular embodiments, a cell is engineered or modified to express one or more DARIC binding and/or signaling components and an engineered antigen receptor. In particular embodiments, a nucleic acid or vector encodes a fusion polypeptide comprising an engineered receptor and a DARIC binding component and/or DARIC signaling component, and one or more polypeptide cleavage signals interspersed between the receptor and the components. In other particular embodiments, a polynucleotide or vector encoding a DARIC is introduced into an immune effector cell that comprises an engineered antigen receptor. Without wishing to be bound by any particular theory, it is contemplated in particular embodiments, that any suitable mechanism known in the art may be used to introduce and co-express an engineered antigen receptor and a DARIC in the same immune effector cell or population of cells to the efficiency, potency, and durability of the immune effector cell response. In preferred embodiments, the intracellular signaling domains, *e.g.*, costimulatory domains, of the engineered antigen receptor and the DARIC binding and/or DARIC signaling domains will be different from each other.

In particular embodiments, immune effector cells contemplated herein comprise an engineered antigen receptor and one or more components of a DARIC. In particular embodiments, the engineered antigen receptor is an engineered T cell receptor (TCR), a chimeric antigen receptor (CAR), or a zetakine.

1. ENGINEERED TCRS

In particular embodiments, immune effector cells contemplated herein comprise an engineered TCR and one or more components of a DARIC. In one embodiment, T cells are engineered by introducing a polynucleotide or vector encoding an engineered TCR and

one or more components of a DARIC separated by one or more polypeptide cleavage signals. In one embodiment, T cells are engineered by introducing a polynucleotide or vector encoding an engineered TCR and a polynucleotide or vector encoding one or more components of a DARIC. In one embodiment, T cells engineered to express an engineered TCR are further engineered by introducing a polynucleotide or vector encoding one or more components of a DARIC.

Naturally occurring T cell receptors comprise two subunits, an alpha chain and a beta chain subunit (αβTCR), or a gamma chain and a delta chain subunit (γδTCR), each of which is a unique protein produced by recombination event in each T cell's genome. Libraries of TCRs may be screened for their selectivity to particular target antigens. In this manner, natural TCRs, which have a high-avidity and reactivity toward target antigens may be selected, cloned, and subsequently introduced into a population of T cells used for adoptive immunotherapy. In one embodiment, the TCR is an αβTCR. In one embodiment, the TCR is a γδTCR.

In one embodiment, T cells are modified by introducing a TCR subunit that has the ability to form TCRs that confer specificity to T cells for tumor cells expressing a target antigen. In particular embodiments, the subunits have one or more amino acid substitutions, deletions, insertions, or modifications compared to the naturally occurring subunit, so long as the subunits retain the ability to form TCRs and confer upon transfected T cells the ability to home to target cells and participate in immunologically-relevant cytokine signaling. The engineered TCRs preferably also bind target cells displaying the relevant tumor-associated peptide with high avidity, and optionally mediate efficient killing of target cells presenting the relevant peptide in vivo.

The nucleic acids encoding engineered TCRs are preferably isolated from their natural context in a (naturally-occurring) chromosome of a T cell and can be incorporated into suitable vectors as described elsewhere herein. Both the nucleic acids and the vectors comprising them can be transferred into a cell, preferably a T cell in particular embodiments. The modified T cells are then able to express one or more chains of a TCR encoded by the transduced nucleic acid or nucleic acids. In preferred embodiments, the

engineered TCR is an exogenous TCR because it is introduced into T cells that do not normally express the particular TCR. The essential aspect of the engineered TCRs is that it has high avidity for a tumor antigen presented by a major histocompatibility complex (MHC) or similar immunological component. In contrast to engineered TCRs, CARs are engineered to bind target antigens in an MHC independent manner.

The TCR can be expressed with additional polypeptides attached to the amino-terminal or carboxyl-terminal portion of the alpha chain or beta chain of a TCR, or of the gamma chain or delta chain of a TCR so long as the attached additional polypeptide does not interfere with the ability of the alpha chain or beta chain to form a functional T cell receptor and the MHC dependent antigen recognition.

Antigens that are recognized by the engineered TCRs contemplated in particular embodiments include, but are not limited to cancer antigens, including antigens on both hematological cancers and solid tumors. Illustrative antigens include, but are not limited to FRα, $\alpha_v\beta_6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, CEA, CLDN6, CLDN18.2, CLL-1, CS-1, CSPG4, CTAGE1, DLL3, EGFR, EGFRvIII, EGP2, EGP40, EPCAM, EPHA2, ERBB4, FAP, FCRL5, AchR, GD2, GD3, GPC3, HER2, HER2 p95, IL-10Rα, IL-13Rα2, Kappa, LAGE-1A, Lambda, LeY, L1-CAM, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGEA10, MelanA or MART1, SLN), MUC1, MUC16, MICA, MICB, NCAM, NY-ESO-1, PLAC1, PRAME, PSCA, PSMA, ROR1, SSX2, Survivin, TAG72, TEM1/CD248, TEM7R, TPBG, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, VEGFR2, and WT-1.

In preferred embodiments, the target antigen is expressed on one or more cells of a cancer and is selected from the group consisting of: BCMA, B7-H3, CD19, CD20, CD22, CD33, CD79A, CD79B, CD123, CLL-1, or EGFRvIII.

2. CHIMERIC ANTIGEN RECEPTORS

In particular embodiments, immune effector cells contemplated herein comprise a CAR and one or more components of a DARIC. Chimeric antigen receptors (CARs) are molecules

that combine antibody-based specificity for a target antigen (*e.g.,* tumor antigen) with a T cell receptor-activating intracellular domain to generate a chimeric protein that exhibits a specific anti-tumor cellular immune activity. As used herein, the term, "chimeric," describes being composed of parts of different proteins or DNAs from different origins.

In one embodiment, T cells are engineered by introducing a polynucleotide or vector encoding a CAR and one or more DARIC components separated by one or more polypeptide cleavage signals. In one embodiment, T cells are engineered by introducing a polynucleotide or vector encoding a CAR and a polynucleotide or vector encoding one or more DARIC components. In one embodiment, T cells that are engineered to express a CAR are further engineered by introducing a polynucleotide or vector encoding one or more DARIC components.

In various embodiments, a CAR comprises an extracellular domain that binds to a specific target antigen (also referred to as a binding domain or antigen-specific binding domain), a transmembrane domain and one or more intracellular signaling domains. The main characteristic of CARs is their ability to redirect immune effector cell specificity, thereby triggering proliferation, cytokine production, phagocytosis or production of molecules that can mediate cell death of the target antigen expressing cell in a major histocompatibility (MHC) independent manner, exploiting the cell specific targeting abilities of monoclonal antibodies, soluble ligands or cell specific coreceptors.

In particular embodiments, CARs comprise an extracellular binding domain that specifically binds to a target polypeptide. In preferred embodiments, a CAR binds a target polypeptide that is different than the target polypeptide(s) bound by a DARIC binding component. A binding domain includes any naturally occurring, synthetic, semi-synthetic, or recombinantly produced binding partner for a biological molecule of interest.

In particular embodiments, the extracellular binding domain comprises an antibody or antigen binding fragment thereof.

In one preferred embodiment, the binding domain comprises an scFv.

In another preferred embodiment, the binding domain comprises one or more camelid antibodies.

In particular embodiments, a CAR comprises an extracellular domain that binds an antigen selected from the group consisting of: FRα, $\alpha_v\beta_6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, CEA, CLDN6, CLDN18.2, CLL-1, CS-1, CSPG4, CTAGE1, DLL3, EGFR, EGFRvIII, EGP2, EGP40, EPCAM, EPHA2, ERBB4, FAP, FCRL5, AchR, GD2, GD3, GPC3, HER2, HER2 p95, IL-10Rα, IL-13Rα2, Kappa, LAGE-1A, Lambda, LeY, L1-CAM, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGEA10, MelanA or MART1, SLN), MUC1, MUC16, MICA, MICB, NCAM, NY-ESO-1, PLAC1, PRAME, PSCA, PSMA, ROR1, SSX2, Survivin, TAG72, TEM1/CD248, TEM7R, TPBG, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, VEGFR2, and WT-1.

In preferred embodiments, a CAR comprises an extracellular domain that binds an antigen selected from the group consisting of: BCMA, B7-H3, CD19, CD20, CD22, CD33, CD79A, CD79B, CD123, CLL-1, or EGFRvIII.

In particular embodiments, the CARs comprise an extracellular binding domain, *e.g.,* antibody or antigen binding fragment thereof that binds an antigen, wherein the antigen is an MHC-peptide complex, such as a class I MHC-peptide complex or a class II MHC-peptide complex.

In one embodiment, the spacer domain comprises the CH2 and CH3 of IgG1, IgG4, or IgD.

Illustrative hinge domains suitable for use in the CARs described herein include the hinge region derived from the extracellular regions of type 1 membrane proteins such as CD8α, and CD4, which may be wild-type hinge regions from these molecules or may be altered. In another embodiment, the hinge domain comprises a CD8α hinge region.

In one embodiment, the hinge is a PD-1 hinge or CD152 hinge.

The transmembrane (TM) domain of the CAR fuses the extracellular binding portion and intracellular signaling domain and anchors the CAR to the plasma membrane of the immune effector cell. The TM domain may be derived either from a natural, synthetic, semi-synthetic, or recombinant source.

Illustrative TM domains may be derived from (i.e., comprise at least the transmembrane region(s) of the alpha, beta, gamma, or delta chain of a T-cell receptor, CD3ε,

CD3ζ, CD4, CD5, CD8α, CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD71, CD80, CD86, CD 134, CD137, CD152, CD 154, AMN, PDCD1, NKG2A, NKG2B, NKG2C, and NKG2D.

In one embodiment, a CAR comprises a TM domain derived from CD8α. In another embodiment, a CAR contemplated herein comprises a TM domain derived from CD8α and a short oligo- or polypeptide linker, preferably between 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids in length that links the TM domain and the intracellular signaling domain of the CAR. A glycine-serine linker provides a particularly suitable linker.

In preferred embodiments, a CAR comprises an intracellular signaling domain that comprises one or more costimulatory domains and a primary signaling domain.

Primary signaling domains that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs.

Illustrative examples of ITAM containing primary signaling domains suitable for use in CARs contemplated in particular embodiments include those derived from FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d. In particular preferred embodiments, a CAR comprises a CD3ζ primary signaling domain and one or more costimulatory domains. The intracellular primary signaling and costimulatory domains may be linked in any order in tandem to the carboxyl terminus of the transmembrane domain.

In particular embodiments, a CAR comprises one or more costimulatory domains to enhance the efficacy and expansion of T cells expressing CAR receptors.

Illustrative examples of such costimulatory molecules suitable for use in CARs contemplated in particular embodiments include, but are not limited to, TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, CARD11, CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD94, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), DAP10, LAT, SLP76, TRAT1, TNFR2, TNFRS14, TNFRS18, TNFRS25, and ZAP70. In one embodiment, a CAR comprises one or more costimulatory domains selected from the group consisting of CD28, CD137, and CD134, and a CD3ζ primary signaling domain.

In various embodiments, the CAR comprises: an extracellular domain that binds an antigen selected from the group consisting of: BCMA, B7-H3, CD19, CD20, CD22, CD33, CD79A, CD79B, CD123, CLL-1, or EGFRvIII; a CD4, CD8α or CD28 transmembrane

domain; one or more intracellular costimulatory domains isolated from a polypeptide selected from the group consisting of: CD28, CD134, and CD137; and a CD3ζ primary signaling domain.

3. ZETAKINES

In various embodiments, immune effector cells contemplated herein comprise one or more chains of a zetakine receptor and one or more DARIC components. Zetakines are chimeric transmembrane immunoreceptors that comprise an extracellular domain comprising a soluble receptor ligand linked to a support region capable of tethering the extracellular domain to a cell surface, a transmembrane region and an intracellular signaling domain. Zetakines, when expressed on the surface of T lymphocytes, direct T cell activity to those cells expressing a receptor for which the soluble receptor ligand is specific. Zetakine chimeric immunoreceptors redirect the antigen specificity of T cells, with application to treatment of a variety of cancers, particularly via the autocrine/paracrine cytokine systems utilized by human malignancy.

In one embodiment, T cells are engineered by introducing a polynucleotide or vector encoding one or more chains of a zetakine receptor and one or more DARIC components separated by one or more polypeptide cleavage signals. In one embodiment, T cells are engineered by introducing a polynucleotide or vector encoding one or more chains of a zetakine receptor and a polynucleotide or vector encoding one or more DARIC components. In one embodiment, T cells are engineered to express one or more chains of a zetakine receptor are further engineered by introducing a polynucleotide or vector encoding one or more DARIC components.

In particular embodiments, the zetakine comprises an immunosuppressive cytokine or cytokine receptor binding variant thereof, a linker, a transmembrane domain, and an intracellular signaling domain.

In particular embodiments, the cytokine or cytokine receptor binding variant thereof is selected from the group consisting of: interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), and interleukin-13 (IL-13).

In certain embodiments, the linker comprises a CH2CH3 domain, hinge domain, or the like. In one embodiment, a linker comprises the CH2 and CH3 domains of IgG1, IgG4, or IgD. In one embodiment, a linker comprises a CD8α or CD4 hinge domain.

In particular embodiments, the transmembrane domain is selected from the group consisting of: the alpha, beta, gamma, or delta chain of the T-cell receptor, CD3δ, CD3ε, CD3γ, CD3ζ, CD4, CD5, CD8α, CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD 134, CD137, CD152, CD154, AMN, and PD-1.

In particular embodiments, the intracellular signaling domain is selected from the group consisting of: an ITAM containing primary signaling domain and/or a costimulatory domain.

In particular embodiments, the intracellular signaling domain is selected from the group consisting of: FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d.

In particular embodiments, the intracellular signaling domain is selected from the group consisting of: TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, CARD11, CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD94, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), DAP10, LAT, SLP76, TRAT1, TNFR2, and ZAP70.

In one embodiment, a chimeric cytokine receptor comprises one or more costimulatory domains selected from the group consisting of CD28, CD137, and CD134, and a CD3ζ primary signaling domain.

E. POLYPEPTIDES

Various polypeptides are contemplated herein, including, but not limited to, DARIC binding components, DARIC signaling components, engineered TCRs, CARs, zetakines, fusion proteins comprising the foregoing polypeptides and fragments thereof. In preferred embodiments, a polypeptide comprises an amino acid sequence set forth in any one of SEQ ID NOs: 1-29. "Polypeptide," "peptide" and "protein" are used interchangeably, unless specified to the contrary, and according to conventional meaning, *i.e.*, as a sequence of amino acids. In one embodiment, a "polypeptide" includes fusion polypeptides and other variants. Polypeptides can be prepared using any of a variety of well-known recombinant and/or synthetic techniques. Polypeptides are not limited to a specific length, *e.g.*, they

may comprise a full-length protein sequence, a fragment of a full-length protein, or a fusion protein, and may include post-translational modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like, as well as other modifications known in the art, both naturally occurring and non-naturally occurring. In particular preferred embodiments, fusion polypeptides, polypeptides, fragments and other variants thereof are prepared, obtained, or isolated from one or more human polypeptides.

An "isolated peptide" or an "isolated polypeptide" and the like, as used herein, refer to *in vitro* isolation and/or purification of a peptide or polypeptide molecule from a cellular environment, and from association with other components of the cell, *i.e.*, it is not significantly associated with *in vivo* substances. In particular embodiments, an isolated polypeptide is a synthetic polypeptide, a semi-synthetic polypeptide, or a polypeptide obtained or derived from a recombinant source.

Polypeptides include "polypeptide variants." Polypeptide variants may differ from a naturally occurring polypeptide in one or more substitutions, deletions, additions and/or insertions. Such variants may be naturally occurring or may be synthetically generated, for example, by modifying one or more of the above polypeptide sequences. For example, in particular embodiments, it may be desirable to improve the binding affinity and/or other biological properties of a polypeptide by introducing one or more substitutions, deletions, additions and/or insertions the polypeptide. In particular embodiments, polypeptides include polypeptides having at least about 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 86%, 97%, 98%, or 99% amino acid identity to any of the reference sequences contemplated herein, typically where the variant maintains at least one biological activity of the reference sequence. In particular embodiments, the biological activity is binding affinity. In particular embodiments, the biological activity is enzymatic activity.

In certain embodiments, a DARIC comprises a polypeptide complex comprising (i) a first polypeptide, *e.g.,* first fusion polypeptide, having a first multimerization domain and (ii) second polypeptide, *e.g.,* second fusion polypeptide, having a second multimerization

domain. In particular embodiments, the multimerization domains are the same; in certain embodiments, the first multimerization domain is different than the second multimerization domain. The first and second multimerization domains substantially contribute to or efficiently promote formation of the polypeptide complex in the presence of a bridging factor. The interaction(s) between the first and second multimerization domains substantially contributes to or efficiently promotes the multimerization of the first and second fusion polypeptides if there is a statistically significant reduction in the association between the first and second fusion polypeptides in the absence of the first multimerization domain, the second multimerization domain, or the bridging factor. In certain embodiments, when the first and second fusion polypeptides are co-expressed, at least about 60%, for instance, at least about 60% to about 70%, at least about 70% to about 80%, at least about 80% to about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, and at least about 90% to about 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the first and second single chain polypeptides form multimers with each other in the presence of a bridging factor.

Polypeptides variants include biologically active "polypeptide fragments." Illustrative examples of biologically active polypeptide fragments include binding domains, intracellular signaling domains, and the like. As used herein, the term "biologically active fragment" or "minimal biologically active fragment" refers to a polypeptide fragment that retains at least 100%, at least 90%, at least 80%, at least 70%, at least 60%, at least 50%, at least 40%, at least 30%, at least 20%, at least 10%, or at least 5% of the naturally occurring polypeptide activity. In certain embodiments, a polypeptide fragment can comprise an amino acid chain at least 5 to about 1700 amino acids long. It will be appreciated that in certain embodiments, fragments are at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700 or more amino acids long.

In particular embodiments, the polypeptides set forth herein may comprise one or more amino acids denoted as "X." "X" if present in an amino acid SEQ ID NO, refers to any one or more amino acids. In particular embodiments, SEQ ID NOs denoting a fusion protein comprise a sequence of continuous X residues that cumulatively represent any amino acid sequence.

As noted above, polypeptides may be altered in various ways including amino acid substitutions, deletions, truncations, and insertions. Methods for such manipulations are generally known in the art. For example, amino acid sequence variants of a reference polypeptide can be prepared by mutations in the DNA. Methods for mutagenesis and nucleotide sequence alterations are well known in the art. *See*, for example, Kunkel (1985, *Proc. Natl. Acad. Sci. USA*. 82: 488-492), Kunkel *et al.*, (1987, *Methods in Enzymol*, 154: 367-382), U.S. Pat. No. 4,873,192, Watson, J. D. *et al.*, (*Molecular Biology of the Gene*, Fourth Edition, Benjamin/Cummings, Menlo Park, Calif., 1987) and the references cited therein. Guidance as to appropriate amino acid substitutions that do not affect biological activity of the protein of interest may be found in the model of Dayhoff *et al.*, (1978) *Atlas of Protein Sequence and Structure* (*Natl. Biomed. Res. Found.*, Washington, D.C.).

In certain embodiments, a polypeptide variant comprises one or more conservative substitutions. A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. Modifications may be made in the structure of the polynucleotides and polypeptides contemplated in particular embodiments and still obtain a functional molecule that encodes a variant or derivative polypeptide with desirable characteristics. When it is desired to alter the amino acid sequence of a polypeptide to create an equivalent, or even an improved, variant polypeptide, one skilled in the art, for example, can change one or more of the codons of the encoding DNA sequence, *e.g.*, according to Table 1.

TABLE 1- Amino Acid Codons

Amino Acids	One letter code	Three letter code	Codons					
Alanine	A	Ala	GCA	GCC	GCG	GCU		
Cysteine	C	Cys	UGC	UGU				
Aspartic acid	D	Asp	GAC	GAU				
Glutamic acid	E	Glu	GAA	GAG				
Phenylalanine	F	Phe	UUC	UUU				
Glycine	G	Gly	GGA	GGC	GGG	GGU		
Histidine	H	His	CAC	CAU				
Isoleucine	I	Iso	AUA	AUC	AUU			
Lysine	K	Lys	AAA	AAG				
Leucine	L	Leu	UUA	UUG	CUA	CUC	CUG	CUU
Methionine	M	Met	AUG					
Asparagine	N	Asn	AAC	AAU				
Proline	P	Pro	CCA	CCC	CCG	CCU		
Glutamine	Q	Gln	CAA	CAG				
Arginine	R	Arg	AGA	AGG	CGA	CGC	CGG	CGU
Serine	S	Ser	AGC	AGU	UCA	UCC	UCG	UCU
Threonine	T	Thr	ACA	ACC	ACG	ACU		
Valine	V	Val	GUA	GUC	GUG	GUU		
Tryptophan	W	Trp	UGG					
Tyrosine	Y	Tyr	UAC	UAU				

Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological activity can be found using computer programs well known in the art, such as DNASTAR, DNA Strider, Geneious, Mac Vector, or Vector NTI software. Preferably, amino acid changes in the protein variants disclosed herein are conservative amino acid changes, *i.e.,* substitutions of similarly charged or uncharged amino acids. A conservative amino acid change involves substitution of one of a family of amino acids which are related in their side chains. Naturally occurring amino acids are generally divided into four families: acidic (aspartate, glutamate), basic (lysine, arginine,

histidine), non-polar (alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), and uncharged polar (glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine) amino acids. Phenylalanine, tryptophan, and tyrosine are sometimes classified jointly as aromatic amino acids. In a peptide or protein, suitable conservative substitutions of amino acids are known to those of skill in this art and generally can be made without altering a biological activity of a resulting molecule. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, *e.g.,* Watson *et al. Molecular Biology of the Gene*, 4th Edition, 1987, The Benjamin/Cummings Pub. Co., p.224).

In one embodiment, where expression of two or more polypeptides is desired, the polynucleotide sequences encoding them can be separated by an IRES sequence as disclosed elsewhere herein.

Polypeptides contemplated in particular embodiments include fusion polypeptides. In particular embodiments, fusion polypeptides and polynucleotides encoding fusion polypeptides are provided. Fusion polypeptides and fusion proteins refer to a polypeptide having at least two, three, four, five, six, seven, eight, nine, or ten polypeptide segments. In preferred embodiments, a fusion polypeptide comprises one or more DARIC components. In other preferred embodiments, the fusion polypeptide comprises one or more DARICs.

In another embodiment, two or more polypeptides can be expressed as a fusion protein that comprises one or more self-cleaving peptide sequences between the polypeptides as disclosed elsewhere herein.

Fusion polypeptides can comprise one or more polypeptide domains or segments including, but are not limited to signal peptides, cell permeable peptide domains (CPP), binding domains, signaling domains, *etc.*, epitope tags (*e.g.,* maltose binding protein ("MBP"), glutathione S transferase (GST), HIS6, MYC, FLAG, V5, VSV-G, and HA), polypeptide linkers, and polypeptide cleavage signals. Fusion polypeptides are typically linked C-terminus to N-terminus, although they can also be linked C-terminus to C-terminus, N-terminus to N-terminus, or N-terminus to C-terminus. In particular

embodiments, the polypeptides of the fusion protein can be in any order. Fusion polypeptides or fusion proteins can also include conservatively modified variants, polymorphic variants, alleles, mutants, subsequences, and interspecies homologs, so long as the desired activity of the fusion polypeptide is preserved. Fusion polypeptides may be produced by chemical synthetic methods or by chemical linkage between the two moieties or may generally be prepared using other standard techniques. Ligated DNA sequences comprising the fusion polypeptide are operably linked to suitable transcriptional or translational control elements as disclosed elsewhere herein.

Fusion polypeptides may optionally comprise one or more linkers that can be used to link the one or more polypeptides or domains within a polypeptide. A peptide linker sequence may be employed to separate any two or more polypeptide components by a distance sufficient to ensure that each polypeptide folds into its appropriate secondary and tertiary structures so as to allow the polypeptide domains to exert their desired functions. Such a peptide linker sequence is incorporated into the fusion polypeptide using standard techniques in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. In particular embodiments, preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea *et al.*, *Gene* 40:39-46, 1985; Murphy *et al.*, *Proc. Natl. Acad. Sci. USA* 83:8258-8262, 1986; U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180. Linker sequences are not required when a particular fusion polypeptide segment contains non-essential N-terminal amino acid regions that can be used to separate the functional domains and prevent steric interference. In particular embodiments, preferred linkers are typically flexible amino acid subsequences which are synthesized as part of a recombinant fusion protein. Linker polypeptides can be

between 1 and 200 amino acids in length, between 1 and 100 amino acids in length, or between 1 and 50 amino acids in length, including all integer values in between.

Exemplary polypeptide cleavage signals include polypeptide cleavage recognition sites such as protease cleavage sites, nuclease cleavage sites (*e.g.,* rare restriction enzyme recognition sites, self-cleaving ribozyme recognition sites), and self-cleaving viral oligopeptides (*see* deFelipe and Ryan, 2004. *Traffic*, 5(8); 616-26).

Suitable protease cleavages sites and self-cleaving peptides are known to the skilled person (*see, e.g.,* in Ryan *et al.*, 1997. *J. Gener. Virol.* 78, 699-722; Scymczak *et al.* (2004) Nature Biotech. 5, 589-594). Exemplary protease cleavage sites include, but are not limited to the cleavage sites of potyvirus NIa proteases (*e.g.,* tobacco etch virus protease), potyvirus HC proteases, potyvirus P1 (P35) proteases, byovirus NIa proteases, byovirus RNA-2-encoded proteases, aphthovirus L proteases, enterovirus 2A proteases, rhinovirus 2A proteases, picorna 3C proteases, comovirus 24K proteases, nepovirus 24K proteases, RTSV (rice tungro spherical virus) 3C-like protease, PYVF (parsnip yellow fleck virus) 3C-like protease, heparin, thrombin, factor Xa and enterokinase. Due to its high cleavage stringency, TEV (tobacco etch virus) protease cleavage sites are preferred in one embodiment, *e.g.,* EXXYXQ(G/S) (SEQ ID NO: 41), for example, ENLYFQG (SEQ ID NO: 42) and ENLYFQS (SEQ ID NO: 43), wherein X represents any amino acid (cleavage by TEV occurs between Q and G or Q and S).

In particular embodiments, the polypeptide cleavage signal is a viral self-cleaving peptide or ribosomal skipping sequence.

Illustrative examples of ribosomal skipping sequences include but are not limited to: a 2A or 2A-like site, sequence or domain (Donnelly et al., 2001. J. Gen. Virol. 82:1027-1041). In a particular embodiment, the viral 2A peptide is an aphthovirus 2A peptide, a potyvirus 2A peptide, or a cardiovirus 2A peptide.

In one embodiment, the viral 2A peptide is selected from the group consisting of: a foot-and-mouth disease virus (FMDV) 2A peptide, an equine rhinitis A virus (ERAV) 2A peptide, a Thosea asigna virus (TaV) 2A peptide, a porcine teschovirus-1 (PTV-1) 2A peptide, a Theilovirus 2A peptide, and an encephalomyocarditis virus 2A peptide.

Illustrative examples of 2A sites are provided in Table 2.

TABLE 2:

SEQ ID NO: 44	GSGATNFSLLKQAGDVEENPGP
SEQ ID NO: 45	ATNFSLLKQAGDVEENPGP
SEQ ID NO: 46	LLKQAGDVEENPGP
SEQ ID NO: 47	GSGEGRGSLLTCGDVEENPGP
SEQ ID NO: 48	EGRGSLLTCGDVEENPGP
SEQ ID NO: 49	LLTCGDVEENPGP
SEQ ID NO: 50	GSGQCTNYALLKLAGDVESNPGP
SEQ ID NO: 51	QCTNYALLKLAGDVESNPGP
SEQ ID NO: 52	LLKLAGDVESNPGP
SEQ ID NO: 53	GSGVKQTLNFDLLKLAGDVESNPGP
SEQ ID NO: 54	VKQTLNFDLLKLAGDVESNPGP
SEQ ID NO: 55	LLKLAGDVESNPGP
SEQ ID NO: 56	LLNFDLLKLAGDVESNPGP
SEQ ID NO: 57	TLNFDLLKLAGDVESNPGP
SEQ ID NO: 58	LLKLAGDVESNPGP
SEQ ID NO: 59	NFDLLKLAGDVESNPGP
SEQ ID NO: 50	QLLNFDLLKLAGDVESNPGP
SEQ ID NO: 61	APVKQTLNFDLLKLAGDVESNPGP
SEQ ID NO: 62	VTELLYRMKRAETYCPRPLLAIHPTEARHKQKIVAPVKQT
SEQ ID NO: 63	LNFDLLKLAGDVESNPGP
SEQ ID NO: 64	LLAIHPTEARHKQKIVAPVKQTLNFDLLKLAGDVESNPGP
SEQ ID NO: 65	EARHKQKIVAPVKQTLNFDLLKLAGDVESNPGP

In preferred embodiments, a polypeptide or fusion polypeptide comprises one or more DARIC components or DARICs.

In particular embodiments, a fusion polypeptide comprises a DARIC signaling component comprising an FRB T2098L multimerization domain, a CD8α transmembrane domain, a CD137 costimulatory domain and a CD3ζ primary signaling domain; a viral self-

cleaving 2A polypeptide; and a DARIC binding component comprising a binding domain that binds BCMA, CD19, CD20, CD22, CD33, B7H3, CD33, CD79A, CD79B, CD123, CLL-1, or EGFRvIII, an FKBP12 multimerization domain polypeptide, a CD4 transmembrane domain or AMN transmembrane domain, and a CD27, CD28, TNFRS14, TNFRS18, TNFRS25, OX40 or TNFR2 costimulatory domain.

In particular embodiments, a fusion polypeptide comprises a DARIC signaling component comprising an FRB T2098L multimerization domain, a CD8α transmembrane domain, a CD137 costimulatory domain and a CD3ζ primary signaling domain; a viral self-cleaving 2A polypeptide; and a DARIC binding component comprising a binding domain that binds BCMA, CD19, CD20, CD22, CD33, B7H3, CD33, CD79A, CD79B, CD123, CLL-1, or EGFRvIII, an FKBP12 multimerization domain polypeptide, a CD4 transmembrane domain or AMN transmembrane domain, and a CD27 or CD28 costimulatory domain.

In particular embodiments, a fusion polypeptide comprises a DARIC signaling component comprising an FRB T2098L multimerization domain, a CD8α transmembrane domain, a CD137 costimulatory domain and a CD3ζ primary signaling domain; a viral self-cleaving 2A polypeptide; and a DARIC binding component comprising a binding domain that binds BCMA, CD19, CD20, CD22, CD33, B7H3, CD33, CD79A, CD79B, CD123, CLL-1, or EGFRvIII, an FKBP12 multimerization domain polypeptide, a CD4 transmembrane domain or AMN transmembrane domain, and a TNFRS14, TNFRS18, or TNFRS25 costimulatory domain.

In particular embodiments, a fusion polypeptide comprises a DARIC signaling component comprising an FRB T2098L multimerization domain, a CD8α transmembrane domain, a CD137 costimulatory domain and a CD3ζ primary signaling domain; a viral self-cleaving 2A polypeptide; and a DARIC binding component comprising a binding domain that binds BCMA, CD19, CD20, CD22, CD33, B7H3, CD33, CD79A, CD79B, CD123, CLL-1, or EGFRvIII, an FKBP12 multimerization domain polypeptide, a CD4 transmembrane domain or AMN transmembrane domain, and an OX40 costimulatory domain.

In particular embodiments, a fusion polypeptide comprises a DARIC signaling component comprising an FRB T2098L multimerization domain, a CD8α transmembrane domain, a CD137 costimulatory domain and a CD3ζ primary signaling domain; a viral self-cleaving 2A polypeptide; and a DARIC binding component comprising a binding domain that binds BCMA, CD19, CD20, CD22, CD33, B7H3, CD33, CD79A, CD79B, CD123, CLL-1, or EGFRvIII, an FKBP12 multimerization domain polypeptide, a CD4 transmembrane domain or AMN transmembrane domain, and a TNFR2 costimulatory domain.

F. POLYNUCLEOTIDES

In particular embodiments, polynucleotides encoding one or more DARIC components, engineered TCRs, CARs, zetakines, fusion proteins comprising the foregoing polypeptides and fragments thereof are provided. As used herein, the terms "polynucleotide" or "nucleic acid" refer to deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and DNA/RNA hybrids. Polynucleotides may be single-stranded or double-stranded and either recombinant, synthetic, or isolated. Polynucleotides include, but are not limited to: pre-messenger RNA (pre-mRNA), messenger RNA (mRNA), RNA, short interfering RNA (siRNA), short hairpin RNA (shRNA), microRNA (miRNA), ribozymes, genomic RNA (gRNA), plus strand RNA (RNA(+)), minus strand RNA (RNA(-)), tracrRNA, crRNA, single guide RNA (sgRNA), synthetic RNA, synthetic mRNA, genomic DNA (gDNA), PCR amplified DNA, complementary DNA (cDNA), synthetic DNA, or recombinant DNA. Polynucleotides refer to a polymeric form of nucleotides of at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, at least 100, at least 200, at least 300, at least 400, at least 500, at least 1000, at least 5000, at least 10000, or at least 15000 or more nucleotides in length, either ribonucleotides or deoxyribonucleotides or a modified form of either type of nucleotide, as well as all intermediate lengths. It will be readily understood that "intermediate lengths," in this context, means any length between the quoted values, such as 6, 7, 8, 9, *etc.*, 101, 102, 103, *etc.*; 151, 152, 153, *etc.*; 201, 202, 203, *etc.* In particular embodiments, polynucleotides or

variants have at least or about 50%, 55%, 60%, 65%, 70%, 71%, 72%, 73%, 74%, 75%,76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%,85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a reference sequence.

As used herein, "isolated polynucleotide" refers to a polynucleotide that has been purified from the sequences which flank it in a naturally-occurring state, *e.g.,* a DNA fragment that has been removed from the sequences that are normally adjacent to the fragment. An "isolated polynucleotide" also refers to a complementary DNA (cDNA), a recombinant DNA, or other polynucleotide that does not exist in nature and that has been made by the hand of man. In particular embodiments, an isolated polynucleotide is a synthetic polynucleotide, a semi-synthetic polynucleotide, or a polynucleotide obtained or derived from a recombinant source.

In various embodiments, a polynucleotide comprises an mRNA encoding a polypeptide contemplated herein. In certain embodiments, the mRNA comprises a cap, one or more nucleotides, and a poly(A) tail.

In particular embodiments, polynucleotides encoding one or more DARIC components may be codon-optimized. As used herein, the term "codon-optimized" refers to substituting codons in a polynucleotide encoding a polypeptide in order to increase the expression, stability and/or activity of the polypeptide. Factors that influence codon optimization include, but are not limited to one or more of: (i) variation of codon biases between two or more organisms or genes or synthetically constructed bias tables, (ii) variation in the degree of codon bias within an organism, gene, or set of genes, (iii) systematic variation of codons including context, (iv) variation of codons according to their decoding tRNAs, (v) variation of codons according to GC %, either overall or in one position of the triplet, (vi) variation in degree of similarity to a reference sequence for example a naturally occurring sequence, (vii) variation in the codon frequency cutoff, (viii) structural properties of mRNAs transcribed from the DNA sequence, (ix) prior knowledge about the function of the DNA sequences upon which design of the codon substitution set is to be based, (x) systematic variation of codon sets for each amino acid, and/or (xi)

isolated removal of spurious translation initiation sites. Moreover, it will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide, or fragment of variant thereof, as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated in particular embodiments, for example polynucleotides that are optimized for human and/or primate codon selection. In particular embodiments, the polynucleotides are codon optimized for expression and/or stability.

As used herein the term "nucleotide" refers to a heterocyclic nitrogenous base in N-glycosidic linkage with a phosphorylated sugar. Nucleotides are understood to include natural bases, and a wide variety of art-recognized modified bases. Such bases are generally located at the 1′ position of a nucleotide sugar moiety. Nucleotides generally comprise a base, sugar and a phosphate group. In ribonucleic acid (RNA), the sugar is a ribose, and in deoxyribonucleic acid (DNA) the sugar is a deoxyribose, *i.e.*, a sugar lacking a hydroxyl group that is present in ribose. Exemplary natural nitrogenous bases include the purines, adenosine (A) and guanidine (G), and the pyrimidines, cytidine (C) and thymidine (T) (or in the context of RNA, uracil (U)). The C-1 atom of deoxyribose is bonded to N-1 of a pyrimidine or N-9 of a purine. Nucleotides are usually mono, di- or triphosphates. The nucleotides can be unmodified or modified at the sugar, phosphate and/or base moiety, (also referred to interchangeably as nucleotide analogs, nucleotide derivatives, modified nucleotides, non-natural nucleotides, and non-standard nucleotides; see for example, WO 92/07065 and WO 93/15187). Examples of modified nucleic acid bases are summarized by Limbach *et al.*, (1994, *Nucleic Acids Res.* 22, 2183-2196).

A nucleotide may also be regarded as a phosphate ester of a nucleoside, with esterification occurring on the hydroxyl group attached to C-5 of the sugar. As used herein, the term "nucleoside" refers to a heterocyclic nitrogenous base in N-glycosidic linkage with a sugar. Nucleosides are recognized in the art to include natural bases, and also to include well known modified bases. Such bases are generally located at the 1′ position of a nucleoside sugar moiety. Nucleosides generally comprise a base and sugar

group. The nucleosides can be unmodified or modified at the sugar, and/or base moiety, (also referred to interchangeably as nucleoside analogs, nucleoside derivatives, modified nucleosides, non-natural nucleosides, or non-standard nucleosides). As also noted above, examples of modified nucleic acid bases are summarized by Limbach *et al.*, (1994, *Nucleic Acids Res.* 22, 2183-2196).

Illustrative examples of polynucleotides include, but are not limited to, polynucleotides encoding polypeptides set forth in SEQ ID NOs: 1-29.

In various illustrative embodiments, polynucleotides contemplated herein include, but are not limited to polynucleotides encoding one or more DARIC components, DARICs, engineered antigen receptors, fusion polypeptides, and expression vectors, viral vectors, and transfer plasmids comprising polynucleotides contemplated herein.

As used herein, the terms "polynucleotide variant" and "variant" and the like refer to polynucleotides displaying substantial sequence identity with a reference polynucleotide sequence or polynucleotides that hybridize with a reference sequence under stringent conditions that are defined hereinafter. These terms also encompass polynucleotides that are distinguished from a reference polynucleotide by the addition, deletion, substitution, or modification of at least one nucleotide. Accordingly, the terms "polynucleotide variant" and "variant" include polynucleotides in which one or more nucleotides have been added or deleted, or modified, or replaced with different nucleotides. In this regard, it is well understood in the art that certain alterations inclusive of mutations, additions, deletions and substitutions can be made to a reference polynucleotide whereby the altered polynucleotide retains the biological function or activity of the reference polynucleotide.

The recitations "sequence identity" or, for example, comprising a "sequence 50% identical to," as used herein, refer to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a "percentage of sequence identity" may be calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.,* A, T, C, G, I) or the identical amino acid residue (*e.g.,* Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn,

Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. Included are polynucleotides and polypeptides having at least about 50%, 55%, 60%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 86%, 97%, 98%, or 99% sequence identity to any of the reference sequences described herein.

The term "nucleic acid cassette" or "expression cassette" as used herein refers to genetic sequences within the vector which can express an RNA, and subsequently a polypeptide. In one embodiment, the nucleic acid cassette contains a gene(s)-of-interest, *e.g.*, a polynucleotide(s)-of-interest. In another embodiment, the nucleic acid cassette contains one or more expression control sequences, *e.g.*, a promoter, enhancer, poly(A) sequence, and a gene(s)-of-interest, *e.g.,* a polynucleotide(s)-of-interest. Vectors may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more nucleic acid cassettes. The nucleic acid cassette is positionally and sequentially oriented within the vector such that the nucleic acid in the cassette can be transcribed into RNA, and when necessary, translated into a protein or a polypeptide, undergo appropriate post-translational modifications required for activity in the transformed cell, and be translocated to the appropriate compartment for biological activity by targeting to appropriate intracellular compartments or secretion into extracellular compartments. Preferably, the cassette has its 3′ and 5′ ends adapted for ready insertion into a vector, *e.g.*, it has restriction endonuclease sites at each end. The cassette can be removed and inserted into a plasmid or viral vector as a single unit.

Polynucleotides include polynucleotide(s)-of-interest. As used herein, the term "polynucleotide-of-interest" refers to a polynucleotide encoding a polypeptide or fusion polypeptide or a polynucleotide that serves as a template for the transcription of an inhibitory polynucleotide, as contemplated herein.

The polynucleotides contemplated herein, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, *e.g.,* expression control

sequences such as promoters and/or enhancers, untranslated regions (UTRs), signal sequences, Kozak sequences, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, internal ribosomal entry sites (IRES), recombinase recognition sites (*e.g.*, LoxP, FRT, and Att sites), termination codons, transcriptional termination signals, and polynucleotides encoding self-cleaving polypeptides, epitope tags, as disclosed elsewhere herein or as known in the art, such that their overall length may vary considerably. It is therefore contemplated that a polynucleotide fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol.

Polynucleotides can be prepared, manipulated, expressed and/or delivered using any of a variety of well-established techniques known and available in the art. In order to express a desired polypeptide, a nucleotide sequence encoding the polypeptide, can be inserted into appropriate vector.

Illustrative examples of vectors include, but are not limited to plasmid, autonomously replicating sequences, and transposable elements, *e.g.*, Sleeping Beauty, PiggyBac.

Additional Illustrative examples of vectors include, without limitation, plasmids, phagemids, cosmids, artificial chromosomes such as yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), or P1-derived artificial chromosome (PAC), bacteriophages such as lambda phage or M13 phage, and animal viruses.

Illustrative examples of viruses useful as vectors include, without limitation, retrovirus (including lentivirus), adenovirus, adeno-associated virus, herpesvirus (*e.g.*, herpes simplex virus), poxvirus, baculovirus, papillomavirus, and papovavirus (*e.g.*, SV40).

Illustrative examples of expression vectors include, but are not limited to, pClneo vectors (Promega) for expression in mammalian cells; pLenti4/V5-DEST™, pLenti6/V5-DEST™, and pLenti6.2/V5-GW/lacZ (Invitrogen) for lentivirus-mediated gene transfer and expression in mammalian cells. In particular embodiments, coding sequences of

polypeptides disclosed herein can be ligated into such expression vectors for the expression of the polypeptides in mammalian cells.

In particular embodiments, the vector is an episomal vector or a vector that is maintained extrachromosomally. As used herein, the term "episomal" refers to a vector that is able to replicate without integration into host's chromosomal DNA and without gradual loss from a dividing host cell also meaning that said vector replicates extrachromosomally or episomally.

"Expression control sequences," "control elements," or "regulatory sequences" present in an expression vector are those non-translated regions of the vector including an origin of replication, selection cassettes, promoters, enhancers, translation initiation signals (Shine Dalgarno sequence or Kozak sequence) introns, a polyadenylation sequence, 5' and 3' untranslated regions, all of which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including ubiquitous promoters and inducible promoters may be used.

In particular embodiments, a polynucleotide comprises a vector, including but not limited to expression vectors and viral vectors. A vector may comprise one or more exogenous, endogenous, or heterologous control sequences such as promoters and/or enhancers. An "endogenous control sequence" is one which is naturally linked with a given gene in the genome. An "exogenous control sequence" is one which is placed in juxtaposition to a gene by means of genetic manipulation (*i.e.*, molecular biological techniques) such that transcription of that gene is directed by the linked enhancer/promoter. A "heterologous control sequence" is an exogenous sequence that is from a different species than the cell being genetically manipulated. A "synthetic" control sequence may comprise elements of one more endogenous and/or exogenous sequences, and/or sequences determined *in vitro* or *in silico* that provide optimal promoter and/or enhancer activity for the particular therapy.

The term "promoter" as used herein refers to a recognition site of a polynucleotide (DNA or RNA) to which an RNA polymerase binds. An RNA polymerase initiates and

transcribes polynucleotides operably linked to the promoter. In particular embodiments, promoters operative in mammalian cells comprise an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated and/or another sequence found 70 to 80 bases upstream from the start of transcription, a CNCAAT region where N may be any nucleotide.

The term "enhancer" refers to a segment of DNA which contains sequences capable of providing enhanced transcription and in some instances can function independent of their orientation relative to another control sequence. An enhancer can function cooperatively or additively with promoters and/or other enhancer elements. The term "promoter/enhancer" refers to a segment of DNA which contains sequences capable of providing both promoter and enhancer functions.

The term "operably linked", refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. In one embodiment, the term refers to a functional linkage between a nucleic acid expression control sequence (such as a promoter, and/or enhancer) and a second polynucleotide sequence, e.g., a polynucleotide-of-interest, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence.

As used herein, the term "constitutive expression control sequence" refers to a promoter, enhancer, or promoter/enhancer that continually or continuously allows for transcription of an operably linked sequence. A constitutive expression control sequence may be a "ubiquitous" promoter, enhancer, or promoter/enhancer that allows expression in a wide variety of cell and tissue types or a "cell specific," "cell type specific," "cell lineage specific," or "tissue specific" promoter, enhancer, or promoter/enhancer that allows expression in a restricted variety of cell and tissue types, respectively.

Illustrative ubiquitous expression control sequences suitable for use in particular embodiments include, but are not limited to, a cytomegalovirus (CMV) immediate early promoter, a viral simian virus 40 (SV40) (*e.g.,* early or late), a Moloney murine leukemia virus (MoMLV) LTR promoter, a Rous sarcoma virus (RSV) LTR, a herpes simplex virus (HSV) (thymidine kinase) promoter, H5, P7.5, and P11 promoters from vaccinia virus, an

elongation factor 1-alpha (EF1a) promoter, early growth response 1 (EGR1), ferritin H (FerH), ferritin L (FerL), Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), eukaryotic translation initiation factor 4A1 (EIF4A1), heat shock 70kDa protein 5 (HSPA5), heat shock protein 90kDa beta, member 1 (HSP90B1), heat shock protein 70kDa (HSP70), β-kinesin (β-KIN), the human ROSA 26 locus (Irions *et al.*, *Nature Biotechnology* 25, 1477 - 1482 (2007)), a Ubiquitin C promoter (UBC), a phosphoglycerate kinase-1 (PGK) promoter, a cytomegalovirus enhancer/chicken β-actin (CAG) promoter, a β-actin promoter and a myeloproliferative sarcoma virus enhancer, negative control region deleted, dl587rev primer-binding site substituted (MND) U3 promoter (Haas *et al. Journal of Virology*. 2003;77(17): 9439-9450).

In one embodiment, a vector comprises an MNDU3 promoter.

In one embodiment, a vector comprises an EF1a promoter comprising the first intron of the human EF1a gene.

In one embodiment, a vector comprises an EF1a promoter that lacks the first intron of the human EF1a gene.

In a particular embodiment, it may be desirable to use a cell, cell type, cell lineage or tissue specific expression control sequence to achieve cell type specific, lineage specific, or tissue specific expression of a desired polynucleotide sequence (e.g., to express a particular nucleic acid encoding a polypeptide in only a subset of cell types, cell lineages, or tissues or during specific stages of development).

In a particular embodiment, it may be desirable to express a polynucleotide a T cell specific promoter.

As used herein, "conditional expression" may refer to any type of conditional expression including, but not limited to, inducible expression; repressible expression; expression in cells or tissues having a particular physiological, biological, or disease state, *etc.* This definition is not intended to exclude cell type or tissue specific expression. Certain embodiments provide conditional expression of a polynucleotide-of-interest, *e.g.*, expression is controlled by subjecting a cell, tissue, organism, *etc.*, to a treatment or

condition that causes the polynucleotide to be expressed or that causes an increase or decrease in expression of the polynucleotide encoded by the polynucleotide-of-interest.

Illustrative examples of inducible promoters/systems include, but are not limited to, steroid-inducible promoters such as promoters for genes encoding glucocorticoid or estrogen receptors (inducible by treatment with the corresponding hormone), metallothionine promoter (inducible by treatment with various heavy metals), MX-1 promoter (inducible by interferon), the "GeneSwitch" mifepristone-regulatable system (Sirin *et al.*, 2003, *Gene*, 323:67), the cumate inducible gene switch (WO 2002/088346), tetracycline-dependent regulatory systems, *etc.* Inducer agents include, but are not limited to glucocorticoids, estrogens, mifepristone (RU486), metals, interferons, small molecules, cumate, tetracycline, doxycycline, and variants thereof.

As used herein, an "internal ribosome entry site" or "IRES" refers to an element that promotes direct internal ribosome entry to the initiation codon, such as ATG, of a cistron (a protein encoding region), thereby leading to the cap-independent translation of the gene. *See, e.g.,* Jackson *et al.*, 1990. *Trends Biochem Sci* 15(12):477-83) and Jackson and Kaminski. 1995. *RNA* 1(10):985-1000. Examples of IRES generally employed by those of skill in the art include those described in U.S. Pat. No. 6,692,736. Further examples of "IRES" known in the art include but are not limited to IRES obtainable from picornavirus (Jackson *et al.*, 1990) and IRES obtainable from viral or cellular mRNA sources, such as for example, immunoglobulin heavy-chain binding protein (BiP), the vascular endothelial growth factor (VEGF) (Huez *et al.* 1998. *Mol. Cell. Biol.* 18(11):6178-6190), the fibroblast growth factor 2 (FGF-2), and insulin-like growth factor (IGFII), the translational initiation factor eIF4G and yeast transcription factors TFIID and HAP4, the encephelomycarditis virus (EMCV) which is commercially available from Novagen (Duke *et al.*, 1992. J. Virol 66(3):1602-9) and the VEGF IRES (Huez *et al.*, 1998. Mol Cell Biol 18(11):6178-90). IRES have also been reported in viral genomes of Picornaviridae, Dicistroviridae and Flaviviridae species and in HCV, Friend murine leukemia virus (FrMLV) and Moloney murine leukemia virus (MoMLV).

In one embodiment, the IRES used in polynucleotides contemplated herein is an EMCV IRES.

In particular embodiments, the polynucleotides comprise a consensus Kozak sequence. As used herein, the term "Kozak sequence" refers to a short nucleotide sequence that greatly facilitates the initial binding of mRNA to the small subunit of the ribosome and increases translation. The consensus Kozak sequence is (GCC)RCCATGG (SEQ ID NO: 66), where R is a purine (A or G) (Kozak, 1986. *Cell.* 44(2):283-92, and Kozak, 1987. *Nucleic Acids Res.* 15(20):8125-48).

Elements directing the efficient termination and polyadenylation of the heterologous nucleic acid transcripts increases heterologous gene expression. Transcription termination signals are generally found downstream of the polyadenylation signal. In particular embodiments, vectors comprise a polyadenylation sequence 3′ of a polynucleotide encoding a polypeptide to be expressed. The term "polyA site" or "polyA sequence" as used herein denotes a DNA sequence which directs both the termination and polyadenylation of the nascent RNA transcript by RNA polymerase II. Polyadenylation sequences can promote mRNA stability by addition of a polyA tail to the 3′ end of the coding sequence and thus, contribute to increased translational efficiency. Cleavage and polyadenylation are directed by a poly(A) sequence in the RNA. The core poly(A) sequence for mammalian pre-mRNAs has two recognition elements flanking a cleavage-polyadenylation site. Typically, an almost invariant AAUAAA hexamer lies 20-50 nucleotides upstream of a more variable element rich in U or GU residues. Cleavage of the nascent transcript occurs between these two elements and is coupled to the addition of up to 250 adenosines to the 5' cleavage product. In particular embodiments, the core poly(A) sequence is an ideal polyA sequence (*e.g.*, AATAAA, ATTAAA, AGTAAA). In particular embodiments, the poly(A) sequence is an SV40 polyA sequence, a bovine growth hormone polyA sequence (BGHpA), a rabbit β-globin polyA sequence (rβgpA), variants thereof, or another suitable heterologous or endogenous polyA sequence known in the art. In particular embodiments, the poly(A) sequence is synthetic.

In some embodiments, a polynucleotide or cell harboring the polynucleotide utilizes a suicide gene, including an inducible suicide gene to reduce the risk of direct toxicity and/or uncontrolled proliferation. In specific embodiments, the suicide gene is not immunogenic to the host harboring the polynucleotide or cell. A certain example of a suicide gene that may be used is caspase-9 or caspase-8 or cytosine deaminase. Caspase-9 can be activated using a specific chemical inducer of dimerization (CID).

In particular embodiments, polynucleotides encoding one or more polypeptides, or fusion polypeptides may be introduced into immune effector cells, *e.g.,* T cells, by both non-viral and viral methods. In particular embodiments, delivery of one or more polynucleotides may be provided by the same method or by different methods, and/or by the same vector or by different vectors.

The term "vector" is used herein to refer to a nucleic acid molecule capable transferring or transporting another nucleic acid molecule. The transferred nucleic acid is generally linked to, *e.g.,* inserted into, the vector nucleic acid molecule. A vector may include sequences that direct autonomous replication in a cell or may include sequences sufficient to allow integration into host cell DNA. In particular embodiments, non-viral vectors are used to deliver one or more polynucleotides contemplated herein to a T cell.

Illustrative examples of non-viral vectors include, but are not limited to plasmids (*e.g.,* DNA plasmids or RNA plasmids), transposons, cosmids, and bacterial artificial chromosomes.

Illustrative methods of non-viral delivery of polynucleotides contemplated in particular embodiments include, but are not limited to: electroporation, sonoporation, lipofection, microinjection, biolistics, virosomes, liposomes, immunoliposomes, nanoparticles, polycation or lipid:nucleic acid conjugates, naked DNA, artificial virions, DEAE-dextran-mediated transfer, gene gun, and heat-shock.

Illustrative examples of polynucleotide delivery systems suitable for use in particular embodiments contemplated in particular embodiments include, but are not limited to those provided by Amaxa Biosystems, Maxcyte, Inc., BTX Molecular Delivery Systems, and Copernicus Therapeutics Inc. Lipofection reagents are sold commercially (*e.g.,*

Transfectam™ and Lipofectin™). Cationic and neutral lipids that are suitable for efficient receptor-recognition lipofection of polynucleotides have been described in the literature. See *e.g.,* Liu *et al.* (2003) *Gene Therapy.* 10:180–187; and Balazs *et al.* (2011) *Journal of Drug Delivery.* 2011:1-12. Antibody-targeted, bacterially derived, non-living nanocell-based delivery is also contemplated in particular embodiments.

Viral vectors comprising polynucleotides contemplated in particular embodiments can be delivered *in vivo* by administration to an individual patient, typically by systemic administration (*e.g.,* intravenous, intraperitoneal, intramuscular, subdermal, or intracranial infusion) or topical application, as described below. Alternatively, vectors can be delivered to cells *ex vivo,* such as cells explanted from an individual patient (*e.g.,* mobilized peripheral blood, lymphocytes, bone marrow aspirates, tissue biopsy, *etc.*) or universal donor hematopoietic stem cells, followed by reimplantation of the cells into a patient.

In one embodiment, viral vectors comprising polynucleotides contemplated herein are administered directly to an organism for transduction of cells *in vivo.* Alternatively, naked DNA can be administered. Administration is by any of the routes normally used for introducing a molecule into ultimate contact with blood or tissue cells including, but not limited to, injection, infusion, topical application and electroporation. Suitable methods of administering such nucleic acids are available and well known to those of skill in the art, and, although more than one route can be used to administer a particular composition, a particular route can often provide a more immediate and more effective reaction than another route.

Illustrative examples of viral vector systems suitable for use in particular embodiments contemplated in particular embodiments include, but are not limited to, adeno-associated virus (AAV), retrovirus, herpes simplex virus, adenovirus, and vaccinia virus vectors.

In various embodiments, one or more polynucleotides encoding one or more DARIC components and/or other polypeptides contemplated herein are introduced into an immune effector cell, *e.g.,* T cell, by transducing the cell with a recombinant adeno-associated virus (rAAV), comprising the one or more polynucleotides.

AAV is a small (~26 nm) replication-defective, primarily episomal, non-enveloped virus. AAV can infect both dividing and non-dividing cells and may incorporate its genome into that of the host cell. Recombinant AAV (rAAV) are typically composed of, at a minimum, a transgene and its regulatory sequences, and 5' and 3' AAV inverted terminal repeats (ITRs). The ITR sequences are about 145 bp in length. In particular embodiments, the rAAV comprises ITRs and capsid sequences isolated from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, or AAV10.

In some embodiments, a chimeric rAAV is used the ITR sequences are isolated from one AAV serotype and the capsid sequences are isolated from a different AAV serotype. For example, a rAAV with ITR sequences derived from AAV2 and capsid sequences derived from AAV6 is referred to as AAV2/AAV6. In particular embodiments, the rAAV vector may comprise ITRs from AAV2, and capsid proteins from any one of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, or AAV10. In a preferred embodiment, the rAAV comprises ITR sequences derived from AAV2 and capsid sequences derived from AAV6. In a preferred embodiment, the rAAV comprises ITR sequences derived from AAV2 and capsid sequences derived from AAV2.

In some embodiments, engineering and selection methods can be applied to AAV capsids to make them more likely to transduce cells of interest.

Construction of rAAV vectors, production, and purification thereof have been disclosed, *e.g.,* in U.S. Patent Nos. 9,169,494; 9,169,492; 9,012,224; 8,889,641; 8,809,058; and 8,784,799, each of which is incorporated by reference herein, in its entirety.

In various embodiments, one or more polynucleotides encoding one or more DARIC components and/or other polypeptides contemplated herein are introduced into an immune effector cell, *e.g.,* T cell, by transducing the cell with a retrovirus, *e.g.,* lentivirus, comprising the one or more polynucleotides.

As used herein, the term "retrovirus" refers to an RNA virus that reverse transcribes its genomic RNA into a linear double-stranded DNA copy and subsequently covalently integrates its genomic DNA into a host genome. Illustrative retroviruses suitable for use in particular embodiments, include, but are not limited to: Moloney murine leukemia virus (M-

MuLV), Moloney murine sarcoma virus (MoMSV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), gibbon ape leukemia virus (GaLV), feline leukemia virus (FLV), spumavirus, Friend murine leukemia virus, Murine Stem Cell Virus (MSCV) and Rous Sarcoma Virus (RSV)) and lentivirus.

As used herein, the term "lentivirus" refers to a group (or genus) of complex retroviruses. Illustrative lentiviruses include, but are not limited to, HIV (human immunodeficiency virus; including HIV type 1, and HIV type 2); visna-maedi virus (VMV) virus; the caprine arthritis-encephalitis virus (CAEV); equine infectious anemia virus (EIAV); feline immunodeficiency virus (FIV); bovine immune deficiency virus (BIV); and simian immunodeficiency virus (SIV). In one embodiment, HIV based vector backbones (*i.e.,* HIV cis-acting sequence elements) are preferred.

In various embodiments, a lentiviral vector contemplated herein comprises one or more LTRs, and one or more, or all, of the following accessory elements: a cPPT/FLAP, a Psi (Ψ) packaging signal, an export element, poly (A) sequences, and may optionally comprise a WPRE or HPRE, an insulator element, a selectable marker, and a cell suicide gene, as discussed elsewhere herein.

In particular embodiments, lentiviral vectors contemplated herein may be integrative or non-integrating or integration defective lentivirus. As used herein, the term "integration defective lentivirus" or "IDLV" refers to a lentivirus having an integrase that lacks the capacity to integrate the viral genome into the genome of the host cells. Integration-incompetent viral vectors have been described in patent application WO 2006/010834, which is herein incorporated by reference in its entirety.

Illustrative mutations in the HIV-1 pol gene suitable to reduce integrase activity include, but are not limited to: H12N, H12C, H16C, H16V, S81 R, D41A, K42A, H51A, Q53C, D55V, D64E, D64V, E69A, K71A, E85A, E87A, D116N, D1161, D116A, N120G, N1201, N120E, E152G, E152A, D35E, K156E, K156A, E157A, K159E, K159A, K160A, R166A, D167A, E170A, H171A, K173A, K186Q, K186T, K188T, E198A, R199c, R199T, R199A, D202A, K211A, Q214L, Q216L, Q221 L, W235F, W235E, K236S, K236A, K246A, G247W, D253A, R262A, R263A and K264H.

The term "long terminal repeat (LTR)" refers to domains of base pairs located at the ends of retroviral DNAs which, in their natural sequence context, are direct repeats and contain U3, R and U5 regions.

As used herein, the term "FLAP element" or "cPPT/FLAP" refers to a nucleic acid whose sequence includes the central polypurine tract and central termination sequences (cPPT and CTS) of a retrovirus, *e.g.,* HIV-1 or HIV-2. Suitable FLAP elements are described in U.S. Pat. No. 6,682,907 and in Zennou, *et al.,* 2000, *Cell*, 101:173.

As used herein, the term "packaging signal" or "packaging sequence" refers to psi [Ψ] sequences located within the retroviral genome which are required for insertion of the viral RNA into the viral capsid or particle, *see e.g.,* Clever *et al.,* 1995. *J. of Virology*, Vol. 69, No. 4; pp. 2101–2109.

The term "export element" refers to a cis-acting post-transcriptional regulatory element which regulates the transport of an RNA transcript from the nucleus to the cytoplasm of a cell. Examples of RNA export elements include, but are not limited to, the human immunodeficiency virus (HIV) rev response element (RRE) (*see e.g.,* Cullen *et al.*, 1991. *J. Virol.* 65: 1053; and Cullen *et al.,* 1991. *Cell* 58: 423), and the hepatitis B virus post-transcriptional regulatory element (HPRE).

In particular embodiments, expression of heterologous sequences in viral vectors is increased by incorporating posttranscriptional regulatory elements, efficient polyadenylation sites, and optionally, transcription termination signals into the vectors. A variety of posttranscriptional regulatory elements can increase expression of a heterologous nucleic acid at the protein, *e.g.,* woodchuck hepatitis virus posttranscriptional regulatory element (WPRE; Zufferey *et al.*, 1999, *J. Virol.*, 73:2886); the posttranscriptional regulatory element present in hepatitis B virus (HPRE) (Huang *et al., Mol. Cell. Biol.*, 5:3864); and the like (Liu *et al.,* 1995, *Genes Dev.*, 9:1766).

Lentiviral vectors preferably contain several safety enhancements as a result of modifying the LTRs. "Self-inactivating" (SIN) vectors refers to replication-defective vectors, *e.g.,* retroviral or lentiviral vectors, in which the right (3') LTR enhancer-promoter region, known as the U3 region, has been modified (*e.g.,* by deletion or substitution) to

prevent viral transcription beyond the first round of viral replication. Self-inactivation is preferably achieved through in the introduction of a deletion in the U3 region of the 3' LTR of the vector DNA, *i.e.*, the DNA used to produce the vector RNA. Thus, during reverse transcription, this deletion is transferred to the 5' LTR of the proviral DNA. In particular embodiments, it is desirable to eliminate enough of the U3 sequence to greatly diminish or abolish altogether the transcriptional activity of the LTR, thereby greatly diminishing or abolishing the production of full-length vector RNA in transduced cells. In the case of HIV based lentivectors, it has been discovered that such vectors tolerate significant U3 deletions, including the removal of the LTR TATA box (*e.g.*, deletions from -418 to -18), without significant reductions in vector titers.

An additional safety enhancement is provided by replacing the U3 region of the 5' LTR with a heterologous promoter to drive transcription of the viral genome during production of viral particles. Examples of heterologous promoters which can be used include, for example, viral simian virus 40 (SV40) (*e.g.*, early or late), cytomegalovirus (CMV) (*e.g.*, immediate early), Moloney murine leukemia virus (MoMLV), Rous sarcoma virus (RSV), and herpes simplex virus (HSV) (thymidine kinase) promoters.

The terms "pseudotype" or "pseudotyping" as used herein, refer to a virus whose viral envelope proteins have been substituted with those of another virus possessing preferable characteristics. For example, HIV can be pseudotyped with vesicular stomatitis virus G-protein (VSV-G) envelope proteins, which allows HIV to infect a wider range of cells because HIV envelope proteins (encoded by the env gene) normally target the virus to $CD4^+$ presenting cells.

In certain embodiments, lentiviral vectors are produced according to known methods. *See e.g.*, Kutner *et al., BMC Biotechnol.* 2009;9:10. doi: 10.1186/1472-6750-9-10; Kutner *et al. Nat. Protoc.* 2009;4(4):495–505. doi: 10.1038/nprot.2009.22.

According to certain specific embodiments contemplated herein, most or all of the viral vector backbone sequences are derived from a lentivirus, *e.g.*, HIV-1. However, it is to be understood that many different sources of retroviral and/or lentiviral sequences can be used or combined and numerous substitutions and alterations in certain of the

lentiviral sequences may be accommodated without impairing the ability of a transfer vector to perform the functions described herein. Moreover, a variety of lentiviral vectors are known in the art, *see* Naldini *et al.,* (1996a, 1996b, and 1998); Zufferey *et al.*, (1997); Dull *et al.*, 1998, U.S. Pat. Nos. 6,013,516; and 5,994,136, many of which may be adapted to produce a viral vector or transfer plasmid contemplated herein.

In various embodiments, one or more polynucleotides encoding one or more DARIC components and/or other polypeptides contemplated herein are introduced into an immune effector cell, by transducing the cell with an adenovirus comprising the one or more polynucleotides.

Adenoviral based vectors are capable of very high transduction efficiency in many cell types and do not require cell division. With such vectors, high titer and high levels of expression have been obtained. This vector can be produced in large quantities in a relatively simple system. Most adenovirus vectors are engineered such that a transgene replaces the Ad E1a, E1b, and/or E3 genes; subsequently the replication defective vector is propagated in human 293 cells that supply deleted gene function in trans. Ad vectors can transduce multiple types of tissues *in vivo*, including non-dividing, differentiated cells such as those found in liver, kidney and muscle. Conventional Ad vectors have a large carrying capacity.

Generation and propagation of the current adenovirus vectors, which are replication deficient, may utilize a unique helper cell line, designated 293, which was transformed from human embryonic kidney cells by Ad5 DNA fragments and constitutively expresses E1 proteins (Graham *et al.*, 1977). Since the E3 region is dispensable from the adenovirus genome (Jones & Shenk, 1978), the current adenovirus vectors, with the help of 293 cells, carry foreign DNA in either the E1, the D3 or both regions (Graham & Prevec, 1991). Adenovirus vectors have been used in eukaryotic gene expression (Levrero *et al.*, 1991; Gomez-Foix *et al.*, 1992) and vaccine development (Grunhaus & Horwitz, 1992; Graham & Prevec, 1992). Studies in administering recombinant adenovirus to different tissues include trachea instillation (Rosenfeld *et al.*, 1991; Rosenfeld *et al.,* 1992), muscle injection (Ragot *et al.*, 1993), peripheral intravenous injections (Herz & Gerard, 1993) and stereotactic inoculation into the brain (Le Gal La Salle *et al.*, 1993). An example of the use of an Ad

vector in a clinical trial involved polynucleotide therapy for antitumor immunization with intramuscular injection (Sterman *et al., Hum. Gene Ther.* 7:1083-9 (1998)).

In various embodiments, one or more polynucleotides encoding one or more DARIC components and/or other polypeptides contemplated herein are introduced into an immune effector cell by transducing the cell with a herpes simplex virus, *e.g.,* HSV-1, HSV-2, comprising the one or more polynucleotides.

The mature HSV virion consists of an enveloped icosahedral capsid with a viral genome consisting of a linear double-stranded DNA molecule that is 152 kb. In one embodiment, the HSV based viral vector is deficient in one or more essential or non-essential HSV genes. In one embodiment, the HSV based viral vector is replication deficient. Most replication deficient HSV vectors contain a deletion to remove one or more intermediate-early, early, or late HSV genes to prevent replication. For example, the HSV vector may be deficient in an immediate early gene selected from the group consisting of: ICP4, ICP22, ICP27, ICP47, and a combination thereof. Advantages of the HSV vector are its ability to enter a latent stage that can result in long-term DNA expression and its large viral DNA genome that can accommodate exogenous DNA inserts of up to 25 kb. HSV-based vectors are described in, for example, U.S. Pat. Nos. 5,837,532, 5,846,782, and 5,804,413, and International Patent Applications WO 91/02788, WO 96/04394, WO 98/15637, and WO 99/06583, each of which are incorporated by reference herein in its entirety.

G. GENETICALLY MODIFIED CELLS

In various embodiments, cells are modified to express one or more DARIC components, DARICs, engineered TCRs, CARs, zetakines, and/or fusion proteins contemplated herein, for use in the treatment of cancer. Cells may be non-genetically modified to express one or more of the polypeptides contemplated herein, or in particular preferred embodiments, cells may be genetically modified to express one or more of the polypeptides contemplated herein. As used herein, the term "genetically engineered" or "genetically modified" refers to the addition of extra genetic material in the form of DNA

or RNA into the total genetic material in a cell. The terms, "genetically modified cells," "modified cells," and "redirected cells," are used interchangeably in particular embodiments.

In particular embodiments, one or more DARIC components contemplated herein are introduced and expressed in immune effector cells to improve the efficacy of the immune effector cells. In particular embodiments, a dual targeting immune effector cell is contemplated where the target cell expresses an antigen recognized by a first DARIC binding component and another antigen recognized by a second DARIC binding component. In particular embodiments, one or more DARIC components are introduced and expressed in immune effector cells that have been redirected to a target cell by virtue of co-expressing an engineered antigen receptor, *e.g.,* a CAR, in the cell. In particular embodiments, a dual targeting immune effector cell is contemplated where the target cell expresses an antigen recognized by the engineered antigen receptor and a different antigen recognized by a DARIC.

An "immune effector cell," is any cell of the immune system that has one or more effector functions (*e.g.*, cytotoxic cell killing activity, secretion of cytokines, induction of ADCC and/or CDC). The illustrative immune effector cells contemplated herein are T lymphocytes, including but not limited to cytotoxic T cells (CTLs; $CD8^+$ T cells), TILs, and helper T cells (HTLs; $CD4^+$ T cells). In a particular embodiment, the cells comprise αβ T cells. In a particular embodiment, the cells comprise γδ T cells. In one embodiment, immune effector cells include natural killer (NK) cells. In one embodiment, immune effector cells include natural killer T (NKT) cells. Immune effector cells can be autologous/autogeneic ("self") or non-autologous ("non-self," *e.g.,* allogeneic, syngeneic or xenogeneic).

"Autologous," as used herein, refers to cells from the same subject. "Allogeneic," as used herein, refers to cells of the same species that differ genetically to the cell in comparison. "Syngeneic," as used herein, refers to cells of a different subject that are genetically identical to the cell in comparison. "Xenogeneic," as used herein, refers to cells

of a different species to the cell in comparison. In preferred embodiments, the cells are human autologous immune effector cells.

Illustrative immune effector cells suitable for introducing one or more DARIC components or a DARIC contemplated herein include T lymphocytes. The terms "T cell" or "T lymphocyte" are art-recognized and are intended to include thymocytes, immature T lymphocytes, mature T lymphocytes, resting T lymphocytes, or activated T lymphocytes. A T cell can be a T helper (Th) cell, for example a T helper 1 (Th1) or a T helper 2 (Th2) cell. The T cell can be a helper T cell (HTL; $CD4^+$ T cell) $CD4^+$ T cell, a cytotoxic T cell (CTL; $CD8^+$ T cell), $CD4^+CD8^+$ T cell, $CD4^-CD8^-$ T cell, or any other subset of T cells. Other illustrative populations of T cells suitable for use in particular embodiments include naïve T cells and memory T cells.

As would be understood by the skilled person, other cells may also be used as immune effector cells comprising one or more DARIC components or DARICs contemplated herein. In particular embodiments, immune effector cells also include NK cells, NKT cells, neutrophils, and macrophages. Immune effector cells also include progenitors of effector cells wherein such progenitor cells can be induced to differentiate into immune effector cells *in vivo* or *in vitro*. Thus, in particular embodiments, immune effector cells include progenitors of immune effectors cells such as hematopoietic stem cells (HSCs) contained within the $CD34^+$ population of cells derived from cord blood, bone marrow or mobilized peripheral blood which upon administration in a subject differentiate into mature immune effector cells, or which can be induced *in vitro* to differentiate into mature immune effector cells.

The term, "$CD34^+$ cell," as used herein refers to a cell expressing the CD34 protein on its cell surface. "CD34," as used herein refers to a cell surface glycoprotein (*e.g.*, sialomucin protein) that often acts as a cell-cell adhesion factor and is involved in T cell entrance into lymph nodes. The $CD34^+$ cell population contains hematopoietic stem cells (HSC), which upon administration to a patient differentiate and contribute to all hematopoietic lineages, including T cells, NK cells, NKT cells, neutrophils and cells of the monocyte/macrophage lineage.

Methods for making the immune effector cells which express one or more DARIC components contemplated herein are provided in particular embodiments. In one embodiment, the method comprises transfecting or transducing immune effector cells isolated from an individual such that the immune effector cells with one or more nucleic acids and/or vectors or combination thereof comprising one or more DARIC components contemplated herein. In one embodiment, the method comprises transfecting or transducing immune effector cells isolated from an individual such that the immune effector cells express one or more DARIC components and engineered antigen receptors contemplated herein. In certain embodiments, the immune effector cells are isolated from an individual and genetically modified without further manipulation *in vitro*. Such cells can then be directly re-administered into the individual. In further embodiments, the immune effector cells are first activated and stimulated to proliferate *in vitro* prior to being genetically modified. In this regard, the immune effector cells may be cultured before and/or after being genetically modified.

In particular embodiments, prior to *in vitro* manipulation or genetic modification of the immune effector cells described herein, the source of cells is obtained from a subject. In particular embodiments, the modified immune effector cells comprise T cells.

T cells can be obtained from a number of sources including, but not limited to, peripheral blood mononuclear cells, bone marrow, lymph nodes tissue, cord blood, thymus issue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In certain embodiments, T cells can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled person, such as sedimentation, *e.g.*, FICOLL™ separation.

In other embodiments, an isolated or purified population of T cells is used. In some embodiments, after isolation of PBMC, both cytotoxic and helper T lymphocytes can be sorted into naïve, memory, and effector T cell subpopulations either before or after activation, expansion, and/or genetic modification.

In one embodiment, an isolated or purified population of T cells expresses one or more of the markers including, but not limited to a $CD3^+$, $CD4^+$, $CD8^+$, or a combination thereof

In certain embodiments, the T cells are isolated from an individual and first activated and stimulated to proliferate *in vitro* prior to being modified to express one or more DARIC components.

In order to achieve sufficient therapeutic doses of T cell compositions, T cells are often subjected to one or more rounds of stimulation, activation and/or expansion. In particular embodiments, T cells can be activated and expanded generally using methods as described, for example, in U.S. Patents 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; and 6,867,041, each of which is incorporated herein by reference in its entirety. In particular embodiments, T cells are activated and expanded for about 6 hours, about 12 hours, about 18 hours or about 24 hours prior to introduction of vectors or polynucleotides encoding one or more DARIC components, optionally in combination with an engineered antigen receptor contemplated herein.

In one embodiment, T cells are activated at the same time that they are modified.

In various embodiments, a method of generating an immune effector cell comprises activating a population of cells comprising T cells and expanding the population of T cells. T cell activation can be accomplished by providing a primary stimulation signal through the T cell TCR/CD3 complex and by providing a secondary costimulation signal through an accessory molecule, *e.g.,* CD28.

The TCR/CD3 complex may be stimulated by contacting the T cell with a suitable CD3 binding agent, *e.g.,* a CD3 ligand or an anti-CD3 monoclonal antibody. Illustrative examples of CD3 antibodies include, but are not limited to, OKT3, G19-4, BC3, and 64.1.

In addition to the primary stimulation signal provided through the TCR/CD3 complex, induction of T cell responses requires a second, costimulatory signal. In particular embodiments, a CD28 binding agent can be used to provide a costimulatory signal. Illustrative examples of CD28 binding agents include but are not limited to: natural

CD 28 ligands, *e.g.,* a natural ligand for CD28 (*e.g.,* a member of the B7 family of proteins, such as B7-1(CD80) and B7-2 (CD86); and anti-CD28 monoclonal antibody or fragment thereof capable of crosslinking the CD28 molecule, *e.g.,* monoclonal antibodies 9.3, B-T3, XR-CD28, KOLT-2, 15E8, 248.23.2, and EX5.3D10.

In one embodiment, the molecule providing the primary stimulation signal, for example a molecule which provides stimulation through the TCR/CD3 complex and the costimulatory molecule are coupled to the same surface.

In certain embodiments, binding agents that provide stimulatory and costimulatory signals are localized on the surface of a cell. This can be accomplished by transfecting or transducing a cell with a nucleic acid encoding the binding agent in a form suitable for its expression on the cell surface or alternatively by coupling a binding agent to the cell surface.

In another embodiment, the molecule providing the primary stimulation signal, for example a molecule which provides stimulation through the TCR/CD3 complex and the costimulatory molecule are displayed on antigen presenting cells.

In one embodiment, the molecule providing the primary stimulation signal, for example a molecule which provides stimulation through the TCR/CD3 complex and the costimulatory molecule are provided on separate surfaces.

In a certain embodiment, one of the binding agents that provides stimulatory and costimulatory signals is soluble (provided in solution) and the other agent(s) is provided on one or more surfaces.

In a particular embodiment, the binding agents that provide stimulatory and costimulatory signals are both provided in a soluble form (provided in solution).

In various embodiments, the methods for making T cells contemplated herein comprise activating T cells with anti-CD3 and anti-CD28 antibodies.

In one embodiment, expanding T cells activated by the methods contemplated herein further comprises culturing a population of cells comprising T cells for several hours (about 3 hours) to about 7 days to about 28 days or any hourly integer value in between. In another embodiment, the T cell composition may be cultured for 14 days. In a particular

embodiment, T cells are cultured for about 21 days. In another embodiment, the T cell compositions are cultured for about 2-3 days. Several cycles of stimulation/activation/expansion may also be desired such that culture time of T cells can be 60 days or more.

In particular embodiments, conditions appropriate for T cell culture include an appropriate media (*e.g.,* Minimal Essential Media or RPMI Media 1640 or, X-vivo 15, (Lonza)) and one or more factors necessary for proliferation and viability including, but not limited to serum (*e.g.,* fetal bovine or human serum), interleukin-2 (IL-2), insulin, IFN-γ, IL-4, IL-7, IL-21, GM-CSF, IL-10, IL-12, IL-15, TGFβ, and TNF-α or any other additives suitable for the growth of cells known to the skilled artisan.

Further illustrative examples of cell culture media include, but are not limited to RPMI 1640, Clicks, AIM-V, DMEM, MEM, a-MEM, F-12, X-Vivo 15, and X-Vivo 20, Optimizer, with added amino acids, sodium pyruvate, and vitamins, either serum-free or supplemented with an appropriate amount of serum (or plasma) or a defined set of hormones, and/or an amount of cytokine(s) sufficient for the growth and expansion of T cells.

Antibiotics, *e.g.,* penicillin and streptomycin, are included only in experimental cultures, not in cultures of cells that are to be infused into a subject. The target cells are maintained under conditions necessary to support growth, for example, an appropriate temperature (*e.g.,* 37° C) and atmosphere (*e.g.,* air plus 5% C02).

In particular embodiments, PBMCs or isolated T cells are contacted with a stimulatory agent and costimulatory agent, such as anti-CD3 and anti-CD28 antibodies, generally attached to a bead or other surface, in a culture medium with appropriate cytokines, such as IL-2, IL-7, and/or IL-15.

In other embodiments, artificial APC (aAPC) made by engineering K562, U937, 721.221, T2, and C1R cells to direct the stable expression and secretion, of a variety of costimulatory molecules and cytokines. In a particular embodiment K32 or U32 aAPCs are used to direct the display of one or more antibody-based stimulatory molecules on the AAPC cell surface. Populations of T cells can be expanded by aAPCs expressing a variety

of costimulatory molecules including, but not limited to, CD137L (4-1BBL), CD134L (OX40L), and/or CD80 or CD86. Finally, the aAPCs provide an efficient platform to expand genetically modified T cells and to maintain CD28 expression on CD8 T cells. aAPCs provided in WO 03/057171 and US2003/0147869 are hereby incorporated by reference in their entirety.

In a particular embodiment, a polynucleotide encoding one or more DARIC components is introduced into the population of T cells. In a particular embodiment, a polynucleotide encoding one or more DARIC components is introduced into a population of T cells that express an engineered antigen receptor. The polynucleotides may be introduced into the T cells by microinjection, transfection, lipofection, heat-shock, electroporation, transduction, gene gun, microinjection, DEAE-dextran-mediated transfer, and the like.

In a preferred embodiment, polynucleotides are introduced into a T cell by viral transduction.

Illustrative examples of viral vector systems suitable for introducing a polynucleotide into an immune effector cell or $CD34^+$ cell include but are not limited to adeno-associated virus (AAV), retrovirus, herpes simplex virus, adenovirus, vaccinia virus vectors for gene transfer.

In one embodiment, polynucleotides are introduced into a T cell by AAV transduction.

In one embodiment, polynucleotides are introduced into a T cell by retroviral transduction.

In one embodiment, polynucleotides are introduced into a T cell by lentiviral transduction.

In one embodiment, polynucleotides are introduced into a T cell by adenovirus transduction.

In one embodiment, polynucleotides are introduced into a T cell by herpes simplex virus transduction.

In one embodiment, polynucleotides are introduced into a T cell by vaccinia virus transduction.

H. COMPOSITIONS AND FORMULATIONS

The compositions contemplated herein may comprise one or more DARIC polypeptides, polynucleotides encoding DARIC polypeptides, vectors comprising same, genetically modified immune effector cells, bridging factors, *etc.* Compositions include, but are not limited to, pharmaceutical compositions. A "pharmaceutical composition" refers to a composition formulated in pharmaceutically-acceptable or physiologically-acceptable solutions for administration to a cell or an animal, either alone, or in combination with one or more other modalities of therapy. It will also be understood that, if desired, the compositions may be administered in combination with other agents as well, such as, *e.g.,* cytokines, growth factors, hormones, small molecules, chemotherapeutics, pro-drugs, drugs, antibodies, or other various pharmaceutically-active agents. There is virtually no limit to other components that may also be included in the compositions, provided that the additional agents do not adversely affect the ability of the composition to deliver the intended therapy.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The term "pharmaceutically acceptable carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the bridging factors, polypeptides, polynucleotides, vectors comprising same, or genetically modified immune effector cells are administered. Illustrative examples of pharmaceutical carriers can be sterile liquids, such as cell culture media, water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients in particular embodiments, include starch, glucose, lactose, sucrose,

gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

In one embodiment, a composition comprising a pharmaceutically acceptable carrier is suitable for administration to a subject. In particular embodiments, a composition comprising a carrier is suitable for parenteral administration, *e.g.*, intravascular (intravenous or intraarterial), intraperitoneal or intramuscular administration. In particular embodiments, a composition comprising a pharmaceutically acceptable carrier is suitable for intraventricular, intraspinal, or intrathecal administration. Pharmaceutically acceptable carriers include sterile aqueous solutions, cell culture media, or dispersions. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the bridging factors, polypeptides, polynucleotides, vectors comprising same, or genetically modified immune effector cells, use thereof in the pharmaceutical compositions is contemplated.

In particular embodiments, compositions contemplated herein comprise T cells genetically modified to express one or more DARIC components and/or engineered antigen receptors and a pharmaceutically acceptable carrier. A composition comprising a cell-based composition contemplated herein can be administered separately by enteral or parenteral administration methods or in combination with other suitable compounds to effect the desired treatment goals.

In particular embodiments, compositions contemplated herein comprise a bridging factor and a pharmaceutically acceptable carrier.

The pharmaceutically acceptable carrier must be of sufficiently high purity and of sufficiently low toxicity to render it suitable for administration to the human subject being treated. It further should maintain or increase the stability of the composition. The pharmaceutically acceptable carrier can be liquid or solid and is selected, with the planned manner of administration in mind, to provide for the desired bulk, consistency, *etc.*, when

combined with other components of the composition. For example, the pharmaceutically acceptable carrier can be, without limitation, a binding agent (*e.g.*, pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, *etc.*), a filler (*e.g.*, lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates, calcium hydrogen phosphate, *etc.*), a lubricant (*e.g.,* magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, *etc.*), a disintegrant (*e.g.*, starch, sodium starch glycolate, *etc.*), or a wetting agent (*e.g.*, sodium lauryl sulfate, *etc.*). Other suitable pharmaceutically acceptable carriers for the compositions contemplated herein include, but are not limited to, water, salt solutions, alcohols, polyethylene glycols, gelatins, amyloses, magnesium stearates, talcs, silicic acids, viscous paraffins, hydroxymethylcelluloses, polyvinylpyrrolidones and the like.

Such carrier solutions also can contain buffers, diluents and other suitable additives. The term "buffer" as used herein refers to a solution or liquid whose chemical makeup neutralizes acids or bases without a significant change in pH. Examples of buffers contemplated herein include, but are not limited to, Dulbecco´s phosphate buffered saline (PBS), Ringer´s solution, 5% dextrose in water (D5W), normal/physiologic saline (0.9% NaCl).

The pharmaceutically acceptable carriers may be present in amounts sufficient to maintain a pH of the composition of about 7. Alternatively, the composition has a pH in a range from about 6.8 to about 7.4, *e.g.*, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, and 7.4. In still another embodiment, the composition has a pH of about 7.4.

Compositions contemplated herein may comprise a nontoxic pharmaceutically acceptable medium. The compositions may be a suspension. The term "suspension" as used herein refers to non-adherent conditions in which cells are not attached to a solid support. For example, cells maintained as a suspension may be stirred or agitated and are not adhered to a support, such as a culture dish.

In particular embodiments, compositions contemplated herein are formulated in a suspension, where the T cells modified with one or more polynucleotides encoding one or more DARIC components and/or engineered antigen receptors are dispersed within an

acceptable liquid medium or solution, *e.g.*, saline or serum-free medium, in an intravenous (IV) bag or the like. Acceptable diluents include, but are not limited to water, PlasmaLyte, Ringer´s solution, isotonic sodium chloride (saline) solution, serum-free cell culture medium, and medium suitable for cryogenic storage, *e.g.*, Cryostor® medium.

In certain embodiments, a pharmaceutically acceptable carrier is substantially free of natural proteins of human or animal origin, and suitable for storing a composition comprising a population of modified T cells. The therapeutic composition is intended to be administered into a human patient, and thus is substantially free of cell culture components such as bovine serum albumin, horse serum, and fetal bovine serum.

In some embodiments, compositions are formulated in a pharmaceutically acceptable cell culture medium. Such compositions are suitable for administration to human subjects. In particular embodiments, the pharmaceutically acceptable cell culture medium is a serum free medium.

Serum-free medium has several advantages over serum containing medium, including a simplified and better-defined composition, a reduced degree of contaminants, elimination of a potential source of infectious agents, and lower cost. In various embodiments, the serum-free medium is animal-free, and may optionally be protein-free. Optionally, the medium may contain biopharmaceutically acceptable recombinant proteins. "Animal-free" medium refers to medium wherein the components are derived from non-animal sources. Recombinant proteins replace native animal proteins in animal-free medium and the nutrients are obtained from synthetic, plant or microbial sources. "Protein-free" medium, in contrast, is defined as substantially free of protein.

Illustrative examples of serum-free media used in particular compositions includes, but is not limited to, QBSF-60 (Quality Biological, Inc.), StemPro-34 (Life Technologies), and X-VIVO 10.

In one embodiment, the compositions comprising modified T cells are formulated in PlasmaLyte.

In various embodiments, compositions comprising modified T cells are formulated in a cryopreservation medium. For example, cryopreservation media with cryopreservation agents may be used to maintain a high cell viability outcome post-thaw.

Illustrative examples of cryopreservation media used in particular compositions includes, but is not limited to, CryoStor CS10, CryoStor CS5, and CryoStor CS2.

In one embodiment, the compositions are formulated in a solution comprising 50:50 PlasmaLyte A to CryoStor CS10.

In particular embodiments, the composition is substantially free of mycoplasma, endotoxin, and microbial contamination. By "substantially free" with respect to endotoxin is meant that there is less endotoxin per dose of cells than is allowed by the FDA for a biologic, which is a total endotoxin of 5 EU/kg body weight per day, which for an average 70 kg person is 350 EU per total dose of cells. In particular embodiments, compositions contemplated herein contain about 0.5 EU/mL to about 5.0 EU/mL, or about 0.5 EU/mL, 1.0 EU/mL, 1.5 EU/mL, 2.0 EU/mL, 2.5 EU/mL, 3.0 EU/mL, 3.5 EU/mL, 4.0 EU/mL, 4.5 EU/mL, or 5.0 EU/mL.

In particular embodiments, formulation of pharmaceutically-acceptable carrier solutions is well-known to those of skill in the art, as is the development of suitable dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens, including *e.g.*, enteral and parenteral, *e.g.*, intravascular, intravenous, intrarterial, intraosseously, intraventricular, intracerebral, intracranial, intraspinal, intrathecal, and intramedullary administration and formulation. It would be understood by the skilled artisan that particular embodiments contemplated herein may comprise other formulations, such as those that are well known in the pharmaceutical art, and are described, for example, in *Remington: The Science and Practice of Pharmacy*, volume I and volume II. 22nd Edition. Edited by Loyd V. Allen Jr. Philadelphia, PA: Pharmaceutical Press; 2012, which is incorporated by reference herein, in its entirety.

In particular embodiments, compositions comprise an amount of immune effector cells that express one or more DARIC components contemplated herein. In particular embodiments, compositions comprise an amount of immune effector cells that express an engineered antigen receptor and one or more DARIC components contemplated herein. As used herein, the term "amount" refers to "an amount effective" or "an effective amount" of cells comprising one or more DARIC components contemplated herein, *etc.*, to achieve a

beneficial or desired prophylactic or therapeutic result in the presence of a bridging factor, including clinical results.

A "prophylactically effective amount" refers to an amount of cells comprising one or more DARIC components contemplated herein, *etc.*, effective to achieve the desired prophylactic result in the presence of a bridging factor. Typically but not necessarily, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount is less than the therapeutically effective amount.

A "therapeutically effective amount" refers to an amount of cells comprising one or more DARIC components contemplated herein that is effective to "treat" a subject (*e.g.,* a patient) in the presence of a bridging factor. When a therapeutic amount is indicated, the precise amount of the compositions to be administered, cells, bridging factor, etc, can be determined by a physician with consideration of individual differences in age, weight, tumor size, extent of infection or metastasis, and condition of the patient (subject).

It can generally be stated that a pharmaceutical composition comprising the immune effector cells described herein may be administered at a dosage of 10^2 to 10^{10} cells/kg body weight, preferably 10^5 to 10^6 cells/kg body weight, including all integer values within those ranges. The number of cells will depend upon the ultimate use for which the composition is intended as will the type of cells included therein. For uses provided herein, the cells are generally in a volume of a liter or less, can be 500 mLs or less, even 250 mLs or 100 mLs or less. Hence the density of the desired cells is typically greater than 10^6 cells/ml and generally is greater than 10^7 cells/ml, generally 10^8 cells/ml or greater. The clinically relevant number of immune cells can be apportioned into multiple infusions that cumulatively equal or exceed 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, or 10^{12} cells. In some embodiments, particularly since all the infused cells will be redirected to a particular target antigen, lower numbers of cells, in the range of 10^6/kilogram (10^6-10^{11} per patient) may be administered.

If desired, the treatment may also include administration of mitogens (*e.g.,* PHA) or lymphokines, cytokines, and/or chemokines (*e.g.,* IFN-γ, IL-2, IL-12, TNF-alpha, IL-18, and TNF-beta, GM-CSF, IL-4, IL-13, Flt3-L, RANTES, MIP1α, *etc.*) as described herein to enhance induction of the immune response.

Generally, compositions comprising the cells activated and expanded as described herein may be utilized in the treatment and prevention of diseases that arise in individuals who are immunocompromised. In particular, compositions contemplated herein are used in the treatment of cancer. In particular embodiments, the immune effector cells may be administered either alone, or as a pharmaceutical composition in combination with carriers, diluents, excipients, and/or with other components such as IL-2 or other cytokines or cell populations.

In particular embodiments, pharmaceutical compositions comprise an amount of genetically modified T cells, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients.

In particular embodiments, pharmaceutical compositions comprise an amount of bridging factor, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients.

In a particular embodiment, compositions comprise an effective amount of immune effector cells comprising one or more DARIC components contemplated herein, alone or in combination with a bridging factor and/or one or more therapeutic agents, such as radiation therapy, chemotherapy, transplantation, immunotherapy, hormone therapy, photodynamic therapy, *etc.* The compositions may also be administered in combination with antibiotics. Such therapeutic agents may be accepted in the art as a standard treatment for a particular disease state as described herein, such as a particular cancer. Exemplary therapeutic agents contemplated include cytokines, growth factors, steroids, NSAIDs, DMARDs, anti-inflammatories, chemotherapeutics, radiotherapeutics, therapeutic antibodies, or other active and ancillary agents.

In a particular embodiment, a composition comprising an effective amount of immune effector cells comprising one or more DARIC components contemplated herein is administered to a subject, and a composition comprising an effective amount of a bridging factor is administered to the subject, before, during, in combination with or subsequently to the cellular composition, and optionally repetitively administered to the subject.

In certain embodiments, compositions comprising immune effector cells comprising one or more DARIC components contemplated herein may be administered in conjunction with any number of chemotherapeutic agents.

A variety of other therapeutic agents may be used in conjunction with the compositions described herein. In one embodiment, the composition comprising immune effector cells comprising one or more DARIC components contemplated herein is administered with an anti-inflammatory agent. Anti-inflammatory agents or drugs include, but are not limited to, steroids and glucocorticoids (including betamethasone, budesonide, dexamethasone, hydrocortisone acetate, hydrocortisone, hydrocortisone, methylprednisolone, prednisolone, prednisone, triamcinolone), nonsteroidal anti-inflammatory drugs (NSAIDS) including aspirin, ibuprofen, naproxen, methotrexate, sulfasalazine, leflunomide, anti-TNF medications, cyclophosphamide and mycophenolate.

Illustrative examples of therapeutic antibodies suitable for combination treatment with the modified T cells comprising one or more DARIC components contemplated herein, include but are not limited to, atezolizumab, avelumab, bavituximab, bevacizumab (avastin), bivatuzumab, blinatumomab, conatumumab, daratumumab, duligotumab, dacetuzumab, dalotuzumab, durvalumab, elotuzumab (HuLuc63), gemtuzumab, ibritumomab, indatuximab, inotuzumab, ipilimumab, lorvotuzumab, lucatumumab, milatuzumab, moxetumomab, nivolumab, ocaratuzumab, ofatumumab, pembrolizumab, rituximab, siltuximab, teprotumumab, and ublituximab.

In certain embodiments, the compositions described herein are administered in conjunction with a cytokine. By "cytokine" as used herein is meant a generic term for proteins released by one cell population that act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones.

I. THERAPEUTIC METHODS

Immune effector cells modified to express a DARIC and/or an engineered antigen receptor contemplated herein provide improved methods of adoptive immunotherapy for use in the prevention, treatment, and amelioration of, or for preventing, treating, or

ameliorating at least one symptom associated with, a cancer, GVHD, an infectious disease, an autoimmune disease, an inflammatory disease, or an immunodeficiency.

In particular embodiments, immune effector cells modified to express a DARIC provide improved methods of adoptive immunotherapy to fine-tune the safety and efficacy of a cytotoxic response against target cells, *e.g.*, tumor cells, expressing target antigens while decreasing the risk of on-target antigen, off-target cell cytotoxicity (recognizing the target antigen on a normal, non-target cell).

In particular embodiments, a method of preventing, treating, or ameliorating at least one symptom of a cancer, GVHD, an infectious disease, an autoimmune disease, an inflammatory disease, or an immunodeficiency comprises administering the subject an effective amount of modified immune effector cells or T cells comprising one or more components of a DARIC and an engineered TCR, CAR, or other therapeutic transgene to redirect the cells to a target cell. The genetically modified cells are a more efficacious and safe cellular immunotherapy by virtue of transducing a chemically regulatable immunostimulatory signal.

In particular embodiments, one or more immune effector cells, *e.g.*, T cells, are modified to express both a DARIC binding component and a DARIC signaling component. In this case, the modified cells are administered to a subject in need thereof and home to the target cells via the interaction of the DARIC binding component expressed on the immune effector cell and the target antigen expressed on the target cell. A bridging factor is administered to the subject before the modified cells, about the same time as the modified cells, or after the modified cells have been administered to the subject. In the presence of the bridging factor, a ternary complex forms between the DARIC binding component, the bridging factor, and the DARIC signaling component. Upon formation of the ternary complex, the DARIC transduces an immunostimulatory signal to the immune effector cell that in turn, elicits a cytotoxic response from the immune effector cell against the target cell.

In various embodiments, immune effector cells comprising a DARIC and/or an engineered antigen receptor fine-tune the safety and efficacy of a cytotoxic response against target cells using a dual targeting strategy wherein one or more target cells express one or more

target antigens recognized by the engineered antigen receptor and one or more target antigens recognized by the DARIC.

In particular embodiments, one or more immune effector cells, *e.g.,* T cells, are modified to express both the DARIC binding component and the DARIC signaling component and an engineered antigen receptor, *e.g.,* a CAR. In this case, the modified cells are administered to a subject in need thereof and home to the target cells via the interaction of the DARIC binding component and the CAR, both of which are expressed on the immune effector cell, and the target antigens expressed on the target cell. Interaction of the CAR with a target antigen on the target cell may elicit a cytotoxic response from the immune effector cell against the target cell. A bridging factor is administered to the subject before the modified cells, about the same time as the modified cells, or after the modified cells have been administered to the subject. In the presence of the bridging factor, a ternary complex forms between the DARIC binding component, the bridging factor, and the DARIC signaling component. Upon formation of the ternary complex, the DARIC transduces an immunostimulatory signal to the immune effector cell that in turn, elicits or augments a cytotoxic response from the immune effector cell against the target cell. In particular embodiments, DARIC activation can be induced in cases where remission or regression is incomplete and the condition relapses or becomes refractory to treatment.

In particular preferred embodiments, the specificity of a primary T cell is redirected to tumor or cancer cells that express one or more target antigens by genetically modifying a T cell, *e.g.,* a primary T cell, with one or more DARIC components.

In particular preferred embodiments, the specificity of a primary T cell is redirected to tumor or cancer cells that express a target antigen recognized by an engineered antigen receptor and a target antigen recognized by a DARIC.

In particular embodiments, the modified immune effector cells contemplated herein are used in the treatment of solid tumors or cancers.

In particular embodiments, the modified immune effector cells contemplated herein are used in the treatment of solid tumors or cancers including, but not limited to: adrenal cancer, adrenocortical carcinoma, anal cancer, appendix cancer, astrocytoma, atypical teratoid/rhabdoid tumor, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancer,

brain/CNS cancer, breast cancer, bronchial tumors, cardiac tumors, cervical cancer, cholangiocarcinoma, chondrosarcoma, chordoma, colon cancer, colorectal cancer, craniopharyngioma, ductal carcinoma in situ (DCIS) endometrial cancer, ependymoma, esophageal cancer, esthesioneuroblastoma, Ewing's sarcoma, extracranial germ cell tumor, extragonadal germ cell tumor, eye cancer, fallopian tube cancer, fibrous histiosarcoma, fibrosarcoma, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumors, gastrointestinal stromal tumor (GIST), germ cell tumors, glioma, glioblastoma, head and neck cancer, hemangioblastoma, hepatocellular cancer, hypopharyngeal cancer, intraocular melanoma, kaposi sarcoma, kidney cancer, laryngeal cancer, leiomyosarcoma, lip cancer, liposarcoma, liver cancer, lung cancer, non-small cell lung cancer, lung carcinoid tumor, malignant mesothelioma, medullary carcinoma, medulloblastoma, menangioma, melanoma, Merkel cell carcinoma, midline tract carcinoma, mouth cancer, myxosarcoma, myelodysplastic syndrome, myeloproliferative neoplasms, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, oligodendroglioma, oral cancer, oral cavity cancer, oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, pancreatic islet cell tumors, papillary carcinoma, paraganglioma, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pinealoma, pituitary tumor, pleuropulmonary blastoma, primary peritoneal cancer, prostate cancer, rectal cancer, retinoblastoma, renal cell carcinoma, renal pelvis and ureter cancer, rhabdomyosarcoma, salivary gland cancer, sebaceous gland carcinoma, skin cancer, soft tissue sarcoma, squamous cell carcinoma, small cell lung cancer, small intestine cancer, stomach cancer, sweat gland carcinoma, synovioma, testicular cancer, throat cancer, thymus cancer, thyroid cancer, urethral cancer, uterine cancer, uterine sarcoma, vaginal cancer, vascular cancer, vulvar cancer, and Wilms Tumor.

In particular embodiments, the modified immune effector cells contemplated herein are used in the treatment of solid tumors or cancers including, without limitation, liver cancer, pancreatic cancer, lung cancer, breast cancer, bladder cancer, brain cancer, bone cancer, thyroid cancer, kidney cancer, or skin cancer.

In particular embodiments, the modified immune effector cells contemplated herein are used in the treatment of various cancers including but not limited to pancreatic, bladder, and lung.

In particular embodiments, the modified immune effector cells contemplated herein are used in the treatment of liquid cancers or hematological cancers.

In particular embodiments, the modified immune effector cells contemplated herein are used in the treatment of B-cell malignancies, including but not limited to: leukemias, lymphomas, and multiple myeloma.

In particular embodiments, the modified immune effector cells contemplated herein are used in the treatment of liquid cancers including, but not limited to leukemias, lymphomas, and multiple myelomas: acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), myeloblastic, promyelocytic, myelomonocytic, monocytic, erythroleukemia, hairy cell leukemia (HCL), chronic lymphocytic leukemia (CLL), and chronic myeloid leukemia (CML), chronic myelomonocytic leukemia (CMML) and polycythemia vera, Hodgkin lymphoma, nodular lymphocyte-predominant Hodgkin lymphoma, Burkitt lymphoma, small lymphocytic lymphoma (SLL), diffuse large B-cell lymphoma, follicular lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, mantle cell lymphoma, marginal zone lymphoma, mycosis fungoides, anaplastic large cell lymphoma, Sézary syndrome, precursor T-lymphoblastic lymphoma, multiple myeloma, overt multiple myeloma, smoldering multiple myeloma, plasma cell leukemia, non-secretory myeloma, IgD myeloma, osteosclerotic myeloma, solitary plasmacytoma of bone, and extramedullary plasmacytoma.

Preferred cells for use in the methods contemplated herein include autologous/autogeneic ("self") cells, preferably hematopoietic cells, more preferably T cells, and more preferably immune effector cells.

In particular embodiments, a method comprises administering a therapeutically effective amount of modified immune effector cells that express one or more DARIC components, and optionally an engineered antigen receptor, or a composition comprising the same, to a patient in need thereof, and also administering a bridging factor to the subject. In certain embodiments, the cells are used in the treatment of patients at risk for developing a cancer, GVHD, an infectious disease, an autoimmune disease, an inflammatory disease, or an immunodeficiency. Thus, particular embodiments comprise the treatment or prevention or amelioration of at least one symptom of a cancer, an infectious disease, an autoimmune disease, an inflammatory disease, or an immunodeficiency comprising administering to a

subject in need thereof, a therapeutically effective amount of the modified immune effector cells contemplated herein and a bridging factor.

In particular embodiments, a method comprises administering a therapeutically effective amount of modified immune effector cells that express a DARIC signaling component, and optionally an engineered antigen receptor, or a composition comprising the same, to a patient in need thereof, and also administering a DARIC binding component and a bridging factor, optionally where the DARIC binding component is bound to the bridging factor prior to administration, to the subject. In certain embodiments, the cells are used in the treatment of patients at risk for developing a cancer, GVHD, an infectious disease, an autoimmune disease, an inflammatory disease, or an immunodeficiency. Thus, particular embodiments comprise the treatment or prevention or amelioration of at least one symptom of a cancer, an infectious disease, an autoimmune disease, an inflammatory disease, or an immunodeficiency comprising administering to a subject in need thereof, a therapeutically effective amount of the modified immune effector cells that express an DARIC signaling component and optionally an engineered antigen receptor, a DARIC binding component, and a bridging factor.

The quantity and frequency of administration of modified immune effector cells, DARIC binding components, and/or bridging factor will be determined by such factors as the condition of the patient, and the type and severity of the patient's disease, although appropriate dosages and dose schedules may be determined by clinical trials.

In one illustrative embodiment, the effective amount of modified immune effector cells provided to a subject is at least 2 x 10^6 cells/kg, at least 3 x 10^6 cells/kg, at least 4 x 10^6 cells/kg, at least 5 x 10^6 cells/kg, at least 6 x 10^6 cells/kg, at least 7 x 10^6 cells/kg, at least 8 x 10^6 cells/kg, at least 9 x 10^6 cells/kg, or at least 10 x 10^6 cells/kg, or more cells/kg, including all intervening doses of cells.

In another illustrative embodiment, the effective amount of modified immune effector cells provided to a subject is about 2 x 10^6 cells/kg, about 3 x 10^6 cells/kg, about 4 x 10^6 cells/kg, about 5 x 10^6 cells/kg, about 6 x 10^6 cells/kg, about 7 x 10^6 cells/kg, about 8 x 10^6 cells/kg, about 9 x 10^6 cells/kg, or about 10 x 10^6 cells/kg, or more cells/kg, including all intervening doses of cells.

In another illustrative embodiment, the effective amount of modified immune effector cells provided to a subject is from about 2 x 10^6 cells/kg to about 10 x 10^6 cells/kg, about 3 x 10^6 cells/kg to about 10 x 10^6 cells/kg, about 4 x 10^6 cells/kg to about 10 x 10^6 cells/kg, about 5 x 10^6 cells/kg to about 10 x 10^6 cells/kg, 2 x 10^6 cells/kg to about 6 x 10^6 cells/kg, 2 x 10^6 cells/kg to about 7 x 10^6 cells/kg, 2 x 10^6 cells/kg to about 8 x 10^6 cells/kg, 3 x 10^6 cells/kg to about 6 x 10^6 cells/kg, 3 x 10^6 cells/kg to about 7 x 10^6 cells/kg, 3 x 10^6 cells/kg to about 8 x 10^6 cells/kg, 4 x 10^6 cells/kg to about 6 x 10^6 cells/kg, 4 x 10^6 cells/kg to about 7 x 10^6 cells/kg, 4 x 10^6 cells/kg to about 8 x 10^6 cells/kg, 5 x 10^6 cells/kg to about 6 x 10^6 cells/kg, 5 x 10^6 cells/kg to about 7 x 10^6 cells/kg, 5 x 10^6 cells/kg to about 8 x 10^6 cells/kg, or 6 x 10^6 cells/kg to about 8 x 10^6 cells/kg, including all intervening doses of cells.

One of ordinary skill in the art would recognize that multiple administrations of the compositions contemplated in particular embodiments may be required to effect the desired therapy. For example, a composition may be administered 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more times over a span of 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 1 year, 2 years, 5, years, 10 years, or more. Modified immune effector cells, DARIC components, and bridging factor may be administered in the same or different compositions; in one or more compositions at the same time; or more than one composition at different times. Modified immune effector cells, DARIC components, and bridging factor may be administered through the same route of administration or different routes.

In certain embodiments, it may be desirable to administer activated T cells to a subject and then subsequently redraw blood (or have an apheresis performed), activate T cells therefrom, and reinfuse the patient with these activated and expanded T cells. This process can be carried out multiple times every few weeks. In certain embodiments, T cells can be activated from blood draws of from 10cc to 400cc. In certain embodiments, T cells are activated from blood draws of 20cc, 30cc, 40cc, 50cc, 60cc, 70cc, 80cc, 90cc, 100cc, 150cc, 200cc, 250cc, 300cc, 350cc, or 400cc or more. Not to be bound by theory, using this multiple blood draw/multiple reinfusion protocol may serve to select out certain populations of T cells.

In one embodiment, a method of treating a subject diagnosed with a cancer, comprises removing immune effector cells from the subject, modifying the immune effector

cells by introducing one or more vectors encoding one or more DARIC components into the cell and producing a population of modified immune effector cells, and administering the population of modified immune effector cells to the same subject. In a preferred embodiment, the immune effector cells comprise T cells.

In one embodiment, a method of treating a subject diagnosed with a cancer, comprises removing immune effector cells from the subject, modifying the immune effector cells by introducing one or more vectors encoding an engineered antigen receptor and one or more DARIC components into the cell and producing a population of modified immune effector cells, and administering the population of modified immune effector cells to the same subject. In a preferred embodiment, the immune effector cells comprise T cells.

The methods for administering the cell compositions contemplated in particular embodiments include any method which is effective to result in reintroduction of *ex vivo* modified immune effector cells or reintroduction of modified progenitors of immune effector cells that upon introduction into a subject differentiate into mature immune effector cells. One method comprises modifying peripheral blood T cells *ex vivo* by introducing one or more vectors encoding an engineered antigen receptor and one or more DARIC components and returning the transduced cells into the subject.

The methods for administering the cell compositions contemplated in particular embodiments include any method which is effective to result in reintroduction of *ex vivo* modified immune effector cells or reintroduction of modified progenitors of immune effector cells that upon introduction into a subject differentiate into mature immune effector cells. One method comprises modifying peripheral blood T cells *ex vivo* by introducing one or more vectors encoding one or more DARIC components and returning the transduced cells into the subject.

All publications, patent applications, and issued patents cited in this specification are herein incorporated by reference as if each individual publication, patent application, or issued patent were specifically and individually indicated to be incorporated by reference.

Although the foregoing embodiments have been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings contemplated herein that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims. The following examples are provided by way of illustration only and not by way of limitation. Those of skill in the art will readily recognize a variety of noncritical parameters that could be changed or modified in particular embodiments to yield essentially similar results.

EXAMPLES

EXAMPLE 1

NKG2D DARIC BINDING COMPONENTS CONTAINING A COSTIMULATORY DOMAIN

Lentiviral vectors comprising NKG2D DARICs that have DARIC binding components comprising various costimulatory signaling domains were designed, constructed, and verified. Figure 2A. The costimulatory domains were obtained from TNFR2, OX40, CD27, HVEM (TNFRS14), GITR (TNFRS18) and DR3 (TNFRS25) proteins. See, e.g., SEQ ID NOs: 1 and 20-22.

Human PBMCs (1 x 10^6 cells/mL) were activated with soluble anti-CD3 and anti-CD28 antibodies (50 ng/mL) on day 0. After 24hrs. incubation, 1 x 10^6 cells were transduced with LVV encoding the DARICs. An additional untransduced sample was included as a control (UTD). The cells were washed and resuspended at 0.3 x 10^6 cells/mL) on day 3. The cells were cultured for expansion 7 additional days in T cell growth medium containing IL-2 (250 IU/mL). Medium was changed every other day.

The cells were counted and split to a defined density with every media exchange. After the 10-day expansion period, the T cells were counted and phenotyped using CD4 and CD8 antibody staining.

UTD T cells, NKG2D DARIC T cells, NKG2D.TNFR2 DARIC T cells, NKG2D.OX40 DARIC T cells, NKG2D.CD27 DARIC T cells, NKG2D.HVEM DARIC T cells, NKG2D.DR3 DARIC T cells, and NKG2D.GITR DARIC T cells displayed similar rates of *ex vivo* expansion. Figure 2B. The T cells were stained with anti-NKG2D antibody and DARIC binding component expression was quantified on $CD4^+$ T cells. Expression was comparable among the different NKG2D DARIC binding components. Figure 2C. Together, the data suggest that a DARIC binding component comprising a costimulatory domain does not alter *ex vivo* T cell expansion or expression.

UTD T cells, NKG2D DARIC T cells, NKG2D.TNFR2 DARIC T cells, NKG2D.OX40 DARIC T cells, NKG2D.CD27 DARIC T cells, NKG2D.HVEM DARIC T cells, NKG2D.DR3 DARIC T cells, and NKG2D.GITR DARIC T cells were co-cultured

with NKG2DL$^+$ HCT116 cells for 24 hrs in the presence or absence of rapamycin at 1:1 E:T ratio and cytokine production was analyzed by Qbead PlexScreen. DARIC binding domains comprising a costimulatory domain consistently boosted cytokine production when T cells were cultured with tumor cells in the presence of rapamycin. Figure 2D. All T cell samples produced negligible amounts of cytokines in the absence of rapamycin or NKG2DL$^+$ A549 cells. The NKG2D.TNFR2 DARIC architecture produced increased levels of cytokines compared to DARIC binding components that expressed other costimulatory domains. *Id.*

EXAMPLE 2

NKG2D DARIC.TNFR T CELLS ARE RESISTANT TO RAPAMYCIN-MEDIATED IMMUNOSUPPRESSION

Human PBMCs were activated, transduced and expanded as described in Example 1. Anti-EGFR CAR T cells, NKG2D DARIC T cells, NKG2D.TNFR2 DARIC T cells, and NKG2D.OX40 DARIC T cells were co-cultured with NKG2DL$^+$ A549 cells or NKG2DL$^+$ HT1080 cells at a 1:1 ratio, in vehicle, rapamycin, or the non-immunosuppressive rapalog AP21967.

The NKG2D DARIC T cells did not produce cytokines when co-cultured with tumor cells in the absence of dimerization drug. Figure 3A and 3B. There was robust cytokine production when NKG2D DARIC T cells were co-cultured with tumor cells in the presence of rapamycin and AP21967. *Id.* As expected, addition of rapamycin resulted in suppressed T cell activation and reduced cytokine production from anti-EGFR CAR T cells. *Id.* Similar immunosuppressive effects were observed for NKG2D DARIC T cells and NKG2D.OX40 DARIC T cells when comparing cytokine production in rapamycin and AP21967 co-cultures. Unexpectedly, NKG2D.TNFR2 DARIC T cells were resistant to immunosuppression when cultured in rapamycin. In some cases, there was even greater cytokine production in NKG2D.TNFR2 DARIC T cells co-cultured in the presence of rapamycin compared to AP21967. *Id.*

The cytokine production data was normalized using a ratio of AP21967 to rapamycin. Figure 3C. Using ratiometric analysis, rapamycin-mediated immunosuppression results in values greater than 1, whereas a value less than 1 suggests that rapamycin treatment has a neutral or synergistic effect on T cell activation. The anti-EGFR CAR T cells, NKG2D DARIC T cells, and NKG2D.OX40 DARIC T cells all had ratios greater than 1 for both A549- and HT1080-mediated cytokine production. *Id.* In contrast, NKG2D.TNFR2 DARIC T cells had ratios that were much lower than 1 for all cytokines and all target cell lines. These data suggest that inclusion of the TNFR2 costimulatory domain may partially alleviate rapamycin-mediated immunosuppression in NKG2D.TNFR2 DARIC T cells.

EXAMPLE 3

NKG2D DARIC BINDING COMPONENTS WITH TWO COSTIMULATORY DOMAINS

Lentiviral vectors encoding NKG2D DARIC binding components comprising single or dual costimulatory signaling domains were designed, constructed, and verified. Figure 4A. The costimulatory domains used for the DARIC binding components used in this Example were obtained from CD28, DAP10, OX40, or a combination of these domains.

Human PBMCs were activated, transduced and expanded as described in Example 1. Anti-EGFR CAR T cells, NKG2D DARIC T cells, NKG2D.DAP10 DARIC T cells, NKG2D.CD28 DARIC T cells, NKG2D.CD28.DAP10 DARIC T cells, NKG2D.DAP10.OX40 DARIC T cells, and NKG2D.OX40.DAP10 DARIC T cells displayed similar rates of *ex vivo* expansion and the NKG2D DARICs had comparable expression levels compared to the parental NKG2D DARIC.

Anti-EGFR CAR T cells, NKG2D DARIC T cells, NKG2D.DAP10 DARIC T cells, NKG2D.CD28 DARIC T cells, NKG2D.CD28.DAP10 DARIC T cells, NKG2D.DAP10.OX40 DARIC T cells, and NKG2D.OX40.DAP10 DARIC T cells were co-cultured with $NKG2DL^+$ A549 cells for 24 hrs in the presence or absence of rapamycin at a 1:1 E:T ratio and cytokine production was analyzed by Qbead PlexScreen. DARIC

binding components comprising a CD28 costimulatory domain, DAP10 costimulatory domain, or CD28 costimulatory domain and DAP10 costimulatory domain had minimal impact on cytokine production. Figure 4B. In addition, DARIC binding components comprising a DAP10 costimulatory domain with or without an OX40 costimulatory domain (in either orientation) did not result in altered cytokine production. Figure 4C.

EXAMPLE 4

NKG2D DARICs COMPRISING ICOS DOMAINS

Lentiviral vectors encoding NKG2D DARIC architectures comprising an ICOS transmembrane domain and/or costimulatory domain were designed, constructed, and verified. Figure 5A. DmrA is FKBP12; DmrB is FKBP12 F36V; and DmrC is FRB (2021-2113) T2098L.

Human PBMCs were activated, transduced and expanded as described in Example 1. Anti-EGFR CAR T cells, NKG2D DARIC T cells were used as controls. The various groups of DARIC T cells displayed similar rates of *ex vivo* expansion, similar CD4:CD8 ratios, and had comparable expression levels compared to the parental NKG2D DARIC.

Anti-EGFR CAR T cells and DARIC T cells were co-cultured with NKG2DL$^+$ A549 cells at a 1:1 E:T ratio for 24 hrs. in the presence or absence of AP21967 and cytokine production was analyzed by Qbead PlexScreen. DARIC binding components comprising an ICOS transmembrane domain or costimulatory domain alone or in combination with DAP10 had minimal impact on cytokine production. In contrast DARIC signaling components comprising an ICOS transmembrane domain or costimulatory domain significantly reduced cytokine production compared to the NKG2D DARIC control T cells. Figure 5B and Figure 5C.

EXAMPLE 5

DUAL TARGETING DARIC PLATFORM

A lentiviral vector comprising a DARIC signaling component (FRB T2098L-CD8α TM-CD137-CD3ζ), an NKG2D.TNFR2 DARIC binding component, and a CD19 DARIC

binding component (anti-CD19 scFV-FKBP12-CD4 TM) was designed, constructed, and verified. Figure 6A.

Human PBMCs were activated, transduced and expanded as described in Example 4. UTD T cells, NKG2D.TNFR2 DARIC T cells, CD19 DARIC T cells, and NKG2D/CD19 DARIC dual targeting T cells were stained with either anti-NKG2D antibodies or recombinant CD19-Fc protein. The NKG2D DARIC binding component and CD19 DARIC binding component had similar expression levels in both DARIC single targeting and DARIC dual targeting T cells. Figures 6B and 6C.

UTD T cells, NKG2D.TNFR2 DARIC T cells, CD19 DARIC T cells, and NKG2D/CD19 DARIC dual targeting T cells were co-cultured with $NKG2DL^{+}$ A549 cells, an $NKG2DL^{neg}$ mouse B cell line A20, and A20 cells stably expressing CD19 (A20-hCD19) at 1:1 E:T ratio for 24 hrs. with or without AP21967. Cytokine production was measured from culture supernatants using a Qbead assay kit. Negligible cytokine production was observed in the absence of AP21967 or rapamycin. NKG2D/CD19-DARIC dual targeting T cells produced GM-CSF when cultured with both A549 and A20-CD19 cells. NKG2D.TNFR2 DARIC T cells and CD19 DARIC T cells produced cytokines when co-cultured with target cells expressing the cognate ligand. Figure 6D.

EXAMPLE 6

CD19 DARIC BINDING COMPONENTS CONTAINING A COSTIMULATORY DOMAIN

CD19 DARIC binding and signaling components were designed, constructed, and verified. A CD19 DARIC lentiviral vector was constructed comprising an MNDU3 promoter operably linked to a polynucleotide encoding: a DARIC signaling component (CD8α-signal peptide, an FRB variant (T82L), a CD8α transmembrane domain, an intracellular 4-1BB costimulatory domain, and a CD3ζsignaling domain); a P2A sequence; and a DARIC binding component (an Igκ-signal peptide, an anti-CD19 scFv binding domain, a G4S linker, an FKBP12 domain, a CD4 transmembrane domain and an OX40 costimulatory domain or TNFR2 costimulatory domain). Figure 7A; SEQ ID NO: 23.

Human PBMCs were activated, transduced and expanded as described in Example 1. UTD T cells, anti-CD19 CAR T cells, CD19 DARIC T cells, CD19.OX40 DARIC T cells, and CD19.TNFR2 DARIC T cells displayed similar rates of *ex vivo* expansion. The T cells were stained with recombinant CD19-Fc protein and anti-CD19 scFv expression was quantified on $CD4^+$ T cells. Expression was comparable among the different CD19 CAR and DARIC binding components. Figure 7B.

UTD T cells, anti-CD19 CAR T cells, CD19 DARIC T cells, CD19.OX40 DARIC T cells, and CD19.TNFR2 DARIC T cells were co-cultured with $CD19^+$ Nalm6 (Figure 7C) or $CD19^+$ Jeko-1 (Figure 7D) tumor cell lines at 1:1 E:T ratio for 24 hrs. with or without rapamycin. Cytokine production was measured from culture supernatants using a Qbead assay kit. CD19.OX40 DARIC T cells, and CD19.TNFR2 DARIC T cells showed consistent increases in cytokine production in the presence of rapamycin compared to anti-CD19 CAR T cells and CD19 DARIC T cells. Figure 7C and 7D.

EXAMPLE 7

CD33 DARIC BINDING COMPONENTS CONTAINING A COSTIMULATORY DOMAIN

CD33 DARIC binding and signaling components were designed, constructed, and verified. CD33 DARIC lentiviral vectors were constructed comprising an MNDU3 promoter operably linked to a polynucleotide encoding: a DARIC signaling component (CD8α-signal peptide, an FRB variant (T82L), a CD8α transmembrane domain, an intracellular 4-1BB costimulatory domain, and a CD3ζsignaling domain); a P2A sequence; and a DARIC binding component (an Igκ-signal peptide, one of two an anti-CD3 scFv binding domains, a G4S linker, an FKBP12 domain, a CD4 transmembrane domain and an OX40 costimulatory domain or TNFR2 costimulatory domain). Figure 8A; SEQ ID NOs: 28 and 29.

Human PBMCs were activated, transduced and expanded as described in Example 1. UTD T cells, anti-CD33 (scFv-1) CAR T cells, anti-CD33 (scFv-2) CAR T cells, CD33 (scFv-1) DARIC T cells, CD33 (scFv-2) DARIC T cells, CD33.OX40 (scFv-1) DARIC T cells, CD33.OX40 (scFv-2) DARIC T cells, CD33.TNFR2 (scFv-1) DARIC T cells, and

CD33.TNFR2 (scFv-2) DARIC T cells displayed similar rates of *ex vivo* expansion. The T cells were stained with recombinant CD33-Fc protein and anti-CD33 scFv expression was quantified on $CD4^+$ T cells. Expression was comparable among the different CD33 DARIC binding components. Figure 8B.

UTD T cells, anti-CD33 (scFv-1) CAR T cells, anti-CD33 (scFv-2) CAR T cells, CD33 (scFv-1) DARIC T cells, CD33 (scFv-2) DARIC T cells, CD33.OX40 (scFv-1) DARIC T cells, CD33.OX40 (scFv-2) DARIC T cells, CD33.TNFR2 (scFv-1) DARIC T cells, and CD33.TNFR2 (scFv-2) DARIC T cells were co-incubated with $CD33^+$ THP-1 or $CD33^+$ Molm-1 tumor cell lines at 1:1 E:T ratio for 24 hrs. with or without rapamycin. Cytokine production was measured from culture supernatants using a Qbead assay kit. CD33 DARICs comprising binding components with a costimulatory domain showed consistent increases in cytokine production in the presence of rapamycin compared to anti-CD33 CAR T cells and CD33 DARIC T cells. Figure 8C.

In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

CLAIMS

1. A non-natural cell comprising:

(a) a first polypeptide comprising: a first multimerization domain polypeptide or variant thereof; a first transmembrane domain; a first costimulatory domain; and/or a primary signaling domain; and

(b) a second polypeptide comprising: an extracellular binding domain; a second multimerization domain polypeptide or variant thereof; a second transmembrane domain; and a second costimulatory domain;

wherein a bridging factor promotes the formation of a polypeptide complex on the non-natural cell surface with the bridging factor associated with and disposed between the first and second multimerization domains.

2. The non-natural cell of claim 1, wherein the first and second multimerization domains are different.

3. The non-natural cell of claim 1 or claim 2, wherein the first and second costimulatory domains are different.

4. The non-natural cell of any one of claims 1 to 3, wherein the first multimerization domain and the second multimerization domain associate with a bridging factor selected from the group consisting of: rapamycin or a rapalog thereof, coumermycin or a derivative thereof, gibberellin or a derivative thereof, abscisic acid (ABA) or a derivative thereof, methotrexate or a derivative thereof, cyclosporin A or a derivative thereof, FK506/cyclosporin A (FKCsA) or a derivative thereof, and trimethoprim (Tmp)-synthetic ligand for FK506 binding protein (FKBP) (SLF) or a derivative thereof.

5. The non-natural cell of any one of claims 1 to 4, wherein the first multimerization domain and the second multimerization domain are a pair selected from the group consisting of:

FKBP and FKBP-rapamycin binding (FRB), FKBP and calcineurin, FKBP and cyclophilin, FKBP and bacterial dihydrofolate reductase (DHFR), calcineurin and cyclophilin, and PYR1-like 1 (PYL1) and abscisic acid insensitive 1 (ABI1).

6. The non-natural cell of any one of claims 1 to 5, wherein the first multimerization domain comprises an FKBP polypeptide or variant thereof, and the second multimerization domain comprises an FRB polypeptide or variant thereof.

7. The non-natural cell of any one of claims 1 to 5, wherein the first multimerization domain comprises an FRB polypeptide or variant thereof, and the second multimerization domain comprises an FKBP polypeptide or variant thereof.

8. The non-natural cell of any one of claims 1 to 7, wherein the bridging factor is selected from the group consisting of: AP21967, sirolimus, everolimus, novolimus, pimecrolimus, ridaforolimus, tacrolimus, temsirolimus, umirolimus, and zotarolimus.

9. The non-natural cell of any one of claims 1 to 8, wherein the first and second multimerization domains are selected from FRB T2098L and FKBP12; and the bridging factor is sirolimus or AP21967.

10. The non-natural cell of any one of claims 1 to 9, wherein the first transmembrane domain and the second transmembrane domain are independently selected from a polypeptide selected from the group consisting of: alpha, beta, gamma, or delta chain of the T-cell receptor, CD3δ, CD3ε, CD3γ, CD3ζ, CD4, CD5, CD8α, CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD 134, CD137, CD152, CD154, CD278, amnionless (AMN), and programmed cell death 1 (PDCD1).

11. The non-natural cell of any one of claims 1 to 10, wherein the first transmembrane domain and the second transmembrane domain are independently selected from

the group consisting of: a CD4 transmembrane domain, a CD8α transmembrane domain, and an AMN transmembrane domain.

12. The non-natural cell of any one of claims 1 to 11, wherein the first transmembrane domain and the second transmembrane domain are different.

13. The non-natural cell of any one of claims 1 to 12, wherein the costimulatory domain and/or the primary signaling domain comprise an immunoreceptor tyrosine activation motif (ITAM).

14. The non-natural cell of any one of claims 1 to 13, wherein the first and second costimulatory domain are independently selected from a costimulatory molecule selected from the group consisting of: Toll-like receptor 1 (TLR1), TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, caspase recruitment domain family member 11 (CARD11), CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD94, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), DNAX-Activation Protein 10 (DAP10), Linker for activation of T-cells family member 1 (LAT), SH2 Domain-Containing Leukocyte Protein Of 76 kD (SLP76), T cell receptor associated transmembrane adaptor 1 (TRAT1), TNFR2, TNFRS14, TNFRS18, TNFRS25, and zeta chain of T cell receptor associated protein kinase 70 (ZAP70).

15. The non-natural cell of any one of claims 1 to 14, wherein the first costimulatory domain is isolated from a costimulatory molecule selected from the group consisting of: CD28, CD134, and CD137 and the second costimulatory domain is isolated from CD28, CD278, TNFRS14, TNFRS18, TNFRS25, OX40 or TNFR2.

16. The non-natural cell of any one of claims 1 to 15, wherein the first costimulatory domain is isolated from CD137 and the second costimulatory domain is isolated from OX40 or TNFR2.

17. The non-natural cell of any one of claims 1 to 16, wherein the first costimulatory domain is isolated from CD137 and the second costimulatory domain is isolated from OX40.

18. The non-natural cell of any one of claims 1 to 16, wherein the first costimulatory domain is isolated from CD137 and the second costimulatory domain is isolated from TNFR2.

19. The non-natural cell of any one of claims 1 to 18, wherein the primary signaling domain isolated from a polypeptide selected from the group consisting of: FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d.

20. The non-natural cell of any one of claims 1 to 19, wherein the primary signaling domain is isolated from a CD3ζ polypeptide.

21. The non-natural cell of any one of claims 1 to 20, wherein the extracellular binding domain comprises an antibody or antigen binding fragment thereof, a receptor ectodomain, or a ligand.

22. The non-natural cell of any one of claims 1 to 21, wherein the extracellular binding domain comprises an antibody or antigen binding fragment thereof selected from the group consisting of: a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a $F(ab')_2$ fragment, a bispecific Fab dimer (Fab2), a trispecific Fab trimer (Fab3), an Fv, an single chain Fv protein ("scFv"), a bis-scFv, $(scFv)_2$, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody).

23. The non-natural cell of any one of claims 1 to 22, wherein the extracellular binding domain comprises a humanized antibody or antigen binding fragment thereof.

24. The non-natural cell of any one of claims 1 to 22, wherein the extracellular binding domain comprises a human antibody or antigen binding fragment thereof.

25. The non-natural cell of any one of claims 1 to 24, wherein the extracellular binding domain comprises an scFv.

26. The non-natural cell of any one of claims 1 to 23, wherein the extracellular binding domain comprises one or more camelid VHH antibodies.

27. The non-natural cell of any one of claims 1 to 26, wherein the extracellular binding domain binds an antigen selected from the group consisting of: tumor associated antigens (TAA), tumor specific antigens (TSA), NKG2D ligands, γδ T cell receptor (TCR) ligands, and αβ TCR ligands.

28. The non-natural cell of any one of claims 1 to 27, wherein the extracellular binding domain binds an antigen selected from the group consisting of: alpha folate receptor (FRα), $\alpha_v\beta_6$ integrin, B cell maturation antigen (BCMA), B7-H3 (CD276), B7-H6, carbonic anhydrase IX (CAIX), CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, carcinoembryonic antigen (CEA), claudin 6, (CLDN6), claudin 18 isoform 2 (CLDN18.2), C-type lectin-like molecule-1 (CLL-1), CD2 subset 1 (CS-1), chondroitin sulfate proteoglycan 4 (CSPG4), cutaneous T cell lymphoma-associated antigen 1 (CTAGE1), delta like canonical Notch ligand 3 (DLL3), epidermal growth factor receptor (EGFR), epidermal growth factor receptor variant III (EGFRvIII), epithelial glycoprotein 2 (EGP2), epithelial glycoprotein 40 (EGP40), epithelial cell adhesion molecule (EPCAM), ephrin type-A receptor 2 (EPHA2), erb-b2 receptor tyrosine kinase 4 (ERBB4), fibroblast activation protein (FAP), Fc Receptor Like 5 (FCRL5), fetal acetylcholinesterase receptor (AchR), ganglioside G2 (GD2), ganglioside G3 (GD3), Glypican-3 (GPC3), EGFR family including ErbB2 (HER2), HER2 p95, IL-10Rα, IL-13Rα2, Kappa, cancer/testis antigen 2 (LAGE-1A), Lambda, Lewis-Y (LeY), L1 cell adhesion molecule (L1-

CAM), melanoma antigen gene (MAGE)-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGEA10, melanoma antigen recognized by T cells 1 (MelanA or MART1), Mesothelin (MSLN), MUC1, MUC16, MHC class I chain related proteins A (MICA), MHC class I chain related proteins B (MICB), neural cell adhesion molecule (NCAM), cancer/testis antigen 1 (NY-ESO-1), polysialic acid; placenta-specific 1 (PLAC1), preferentially expressed antigen in melanoma (PRAME), prostate stem cell antigen (PSCA), prostate-specific membrane antigen (PSMA), receptor tyrosine kinase-like orphan receptor 1 (ROR1), synovial sarcoma, X breakpoint 2 (SSX2), Survivin, tumor associated glycoprotein 72 (TAG72), tumor endothelial marker 1 (TEM1/CD248), tumor endothelial marker 7-related (TEM7R), trophoblast glycoprotein (TPBG), UL16-binding protein (ULBP) 1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, vascular endothelial growth factor receptor 2 (VEGFR2), and Wilms tumor 1 (WT-1).

29. A non-natural cell comprising:

(a) a first polypeptide comprising: an FK506 binding protein (FKBP) multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain; and

(b) a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP-rapamycin binding (FRB) multimerization domain polypeptide or variant thereof; a second transmembrane domain; and an OX40 costimulatory domain;

wherein a bridging factor promotes the formation of a polypeptide complex on the non-natural cell surface with the bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

30. A non-natural cell comprising:

(a) a first polypeptide comprising: an FKBP multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain; and

(b) a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FRB multimerization domain polypeptide or variant thereof; a second transmembrane domain; and a TNFR2 costimulatory domain;

wherein a bridging factor promotes the formation of a polypeptide complex on the non-natural cell surface with the bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

31. A non-natural cell comprising:

(a) a first polypeptide comprising: an FRB multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain; and

(b) a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP multimerization domain polypeptide or variant thereof; a second transmembrane domain; and an OX40 costimulatory domain;

wherein a bridging factor promotes the formation of a polypeptide complex on the non-natural cell surface with the bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

32. A non-natural cell comprising:

(a) a first polypeptide comprising: an FRB multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain; and

(b) a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP multimerization domain polypeptide or variant thereof; a second transmembrane domain; and a TNFR2 costimulatory domain;

wherein a bridging factor promotes the formation of a polypeptide complex on the non-natural cell surface with the bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

33. The non-natural cell of any one of claims 29 to 32, wherein the bridging factor is selected from the group consisting of: AP21967, sirolimus, everolimus, novolimus, pimecrolimus, ridaforolimus, tacrolimus, temsirolimus, umirolimus, and zotarolimus.

34. The non-natural cell of any one of claims 29 to 33, wherein the FRB multimerization domain is FRB T2098L; the FKBP multimerization domain is FKBP12; and the bridging factor is sirolimus or AP21967.

35. The non-natural cell of any one of claims 29 to 34, wherein the first transmembrane domain and the second transmembrane domain are independently selected from a polypeptide selected from the group consisting of: alpha, beta, gamma, or delta chain of the T-cell receptor, CD3δ, CD3ε, CD3γ, CD3ζ, CD4, CD5, CD8α, CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD 134, CD137, CD152, CD154, AMN, and PDCD1.

36. The non-natural cell of any one of claims 29 to 35, wherein the first transmembrane domain and the second transmembrane domain are independently selected from a polypeptide selected from the group consisting of: a CD4 transmembrane domain and a CD8α transmembrane domain.

37. The non-natural cell of any one of claims 29 to 36, wherein the costimulatory domain and/or the primary signaling domain comprise an immunoreceptor tyrosine activation motif (ITAM).

38. The non-natural cell of any one of claims 29 to 37, wherein the costimulatory domain is isolated from a costimulatory molecule selected from the group consisting of: Toll-like receptor 1 (TLR1), TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, caspase recruitment domain family member 11 (CARD11), CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD94, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), DNAX-

Activation Protein 10 (DAP10), Linker for activation of T-cells family member 1 (LAT), SH2 Domain-Containing Leukocyte Protein Of 76 kD (SLP76), T cell receptor associated transmembrane adaptor 1 (TRAT1), TNFR2, TNFRS14, TNFRS18, TNFRS25, and zeta chain of T cell receptor associated protein kinase 70 (ZAP70).

39. The non-natural cell of any one of claims 29 to 38, wherein the costimulatory domain is isolated from a costimulatory molecule selected from the group consisting of: CD28, CD134, and CD137.

40. The non-natural cell of any one of claims 29 to 39, wherein the costimulatory domain is isolated from a CD137 costimulatory molecule.

41. The non-natural cell of any one of claims 29 to 40, wherein the primary signaling domain isolated from a polypeptide selected from the group consisting of: FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d.

42. The non-natural cell of any one of claims 29 to 41, wherein the primary signaling domain is isolated from a CD3ζ polypeptide.

43. The non-natural cell of any one of claims 29 to 42, wherein the antibody or antigen binding fragment thereof selected is from the group consisting of: a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a $F(ab')_2$ fragment, a bispecific Fab dimer (Fab2), a trispecific Fab trimer (Fab3), an Fv, an single chain Fv protein ("scFv"), a bis-scFv, $(scFv)_2$, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody).

44. The non-natural cell of any one of claims 29 to 43, wherein the antibody or antigen binding fragment thereof is human or humanized.

45. The non-natural cell of any one of claims 29 to 44, wherein the antibody or antigen binding fragment thereof comprises an scFv or one or more camelid VHH antibodies.

46. The non-natural cell of any one of claims 29 to 45, wherein the antibody or antigen binding fragment thereof binds an antigen selected from the group consisting of: tumor associated antigens (TAA), tumor specific antigens (TSA), NKG2D ligands, γδ T cell receptor (TCR) ligands, and αβ TCR ligands.

47. The non-natural cell of any one of claims 29 to 46, wherein the antibody or antigen binding fragment thereof binds an antigen selected from the group consisting of: FRα, $\alpha_v\beta_6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, CEA, CLDN6, CLDN18.2, CLL-1, CS-1, CSPG4, CTAGE1, DLL3, EGFR, EGFRvIII, EGP2, EGP40, EPCAM, EPHA2, ERBB4, FAP, FCRL5, AchR, GD2, GD3, GPC3, HER2, HER2 p95, IL-10Rα, IL-13Rα2, Kappa, LAGE-1A, Lambda, LeY, L1-CAM, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGEA10, MelanA or MART1, SLN), MUC1, MUC16, MICA, MICB, NCAM, NY-ESO-1, PLAC1, PRAME, PSCA, PSMA, ROR1, SSX2, Survivin, TAG72, TEM1/CD248, TEM7R, TPBG, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, VEGFR2, and WT-1.

48. The non-natural cell of any one of claims 29 to 47, wherein the antibody or antigen binding fragment thereof binds BCMA, B7-H3, CD19, CD20, CD22, CD33, CD79A, CD79B, and/or EGFRvIII.

49. The non-natural cell of any one of claims 29 to 48, wherein the antibody or antigen binding fragment thereof binds BCMA.

50. The non-natural cell of any one of claims 29 to 48, wherein the antibody or antigen binding fragment thereof binds CD19.

51. The non-natural cell of any one of claims 29 to 48, wherein the antibody or antigen binding fragment thereof binds CD20 or CD22.

52. The non-natural cell of any one of claims 29 to 48, wherein the antibody or antigen binding fragment thereof binds B7-H3.

53. The non-natural cell of any one of claims 29 to 48, wherein the antibody or antigen binding fragment thereof binds CD33.

54. The non-natural cell of any one of claims 29 to 48, wherein the antibody or antigen binding fragment thereof binds CD79A.

55. The non-natural cell of any one of claims 29 to 48, wherein the antibody or antigen binding fragment thereof binds CD79B.

56. The non-natural cell of any one of claims 29 to 48, wherein the antibody or antigen binding fragment thereof binds EGFRvIII.

57. A non-natural cell comprising:

(a) a first polypeptide comprising: an FKBP12 multimerization domain polypeptide or variant thereof; a CD8α transmembrane domain; a CD137 costimulatory domain; and a CD3ζ primary signaling domain; and

(b) a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FRB T2098L multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and a TNFR2 costimulatory domain;

wherein a bridging factor promotes the formation of a polypeptide complex on the non-natural cell surface with the bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

58. A non-natural cell comprising:

(a) a first polypeptide comprising: an FKBP12 multimerization domain polypeptide or variant thereof; a CD8α transmembrane domain; a CD137 costimulatory domain; and a CD3ζ primary signaling domain; and

(b) a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FRB T2098L multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and an OX40 costimulatory domain;

wherein a bridging factor promotes the formation of a polypeptide complex on the non-natural cell surface with the bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

59. A non-natural cell comprising:

(a) a first polypeptide comprising: an FRB T2098L multimerization domain polypeptide or variant thereof; a CD8α transmembrane domain; a CD137 costimulatory domain; and a CD3ζ primary signaling domain; and

(b) a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP12 multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and a TNFR2 costimulatory domain;

wherein a bridging factor promotes the formation of a polypeptide complex on the non-natural cell surface with the bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

60. A non-natural cell comprising:

(a) a first polypeptide comprising: an FRB T2098L multimerization domain polypeptide or variant thereof; a CD8α transmembrane domain; a CD137 costimulatory domain; and a CD3ζ primary signaling domain; and

(b) a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP12 multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and an OX40 costimulatory domain;

wherein a bridging factor promotes the formation of a polypeptide complex on the non-natural cell surface with the bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

61. The non-natural cell of any one of claims 57 to 60, wherein the bridging factor is AP21967 or sirolimus.

62. The non-natural cell of any one of claims 57 to 61, wherein the antibody or antigen binding fragment thereof selected is from the group consisting of: a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a $F(ab')_2$ fragment, a bispecific Fab dimer (Fab2), a trispecific Fab trimer (Fab3), an Fv, an single chain Fv protein ("scFv"), a bis-scFv, $(scFv)_2$, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody).

63. The non-natural cell of any one of claims 57 to 62, wherein the antibody or antigen binding fragment thereof is human or humanized.

64. The non-natural cell of any one of claims 57 to 63, wherein the antibody or antigen binding fragment thereof comprises an scFv or one or more camelid VHH antibodies.

65. The non-natural cell of any one of claims 57 to 64, wherein the antibody or antigen binding fragment thereof binds an antigen selected from the group consisting of: tumor associated antigens (TAA), tumor specific antigens (TSA), NKG2D ligands, γδ T cell receptor (TCR) ligands, and αβ TCR ligands.

66. The non-natural cell of any one of claims 57 to 65, wherein the antibody or antigen binding fragment thereof binds an antigen selected from the group consisting of: FRα, $\alpha_v\beta_6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171,

CEA, CLDN6, CLDN18.2, CLL-1, CS-1, CSPG4, CTAGE1, DLL3, EGFR, EGFRvIII, EGP2, EGP40, EPCAM, EPHA2, ERBB4, FAP, FCRL5, AchR, GD2, GD3, GPC3, HER2, HER2 p95, IL-10Rα, IL-13Rα2, Kappa, LAGE-1A, Lambda, LeY, L1-CAM, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGEA10, MelanA or MART1, SLN), MUC1, MUC16, MICA, MICB, NCAM, NY-ESO-1, PLAC1, PRAME, PSCA, PSMA, ROR1, SSX2, Survivin, TAG72, TEM1/CD248, TEM7R, TPBG, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, VEGFR2, and WT-1.

67. The non-natural cell of any one of claims 57 to 66, wherein the antibody or antigen binding fragment thereof binds BCMA, B7-H3, CD19, CD20, CD22, CD33, CD79A, CD79B, and/or EGFRvIII.

68. The non-natural cell of any one of claims 57 to 67, wherein the antibody or antigen binding fragment thereof binds BCMA.

69. The non-natural cell of any one of claims 57 to 67, wherein the antibody or antigen binding fragment thereof binds CD19.

70. The non-natural cell of any one of claims 57 to 67, wherein the antibody or antigen binding fragment thereof binds CD20 or CD22.

71. The non-natural cell of any one of claims 57 to 67, wherein the antibody or antigen binding fragment thereof binds B7H3.

72. The non-natural cell of any one of claims 57 to 67, wherein the antibody or antigen binding fragment thereof binds CD33.

73. The non-natural cell of any one of claims 57 to 67, wherein the antibody or antigen binding fragment thereof binds CD79A.

74. The non-natural cell of any one of claims 57 to 67, wherein the antibody or antigen binding fragment thereof binds CD79B.

75. The non-natural cell of any one of claims 57 to 67, wherein the antibody or antigen binding fragment thereof binds EGFRvIII, optionally wherein the antibody is EGFR806 or an antigen binding fragment thereof.

76. The non-natural cell of any one of claims 1 to 75, wherein the multimerization domains localize extracellularly when of the first polypeptide and the second polypeptide are expressed.

77. The non-natural cell of any one of claims 1 to 76, wherein the cell is a hematopoietic cell.

78. The non-natural cell of any one of claims 1 to 76, wherein the cell is a T cell.

79. The non-natural cell of any one of claims 1 to 76, wherein the cell is a $CD3^+$, $CD4^+$, and/or $CD8^+$ cell.

80. The non-natural cell of any one of claims 1 to 76, wherein the cell is an immune effector cell.

81. The non-natural cell of any one of claims 1 to 76, wherein the cell is a cytotoxic T lymphocyte (CTL), a tumor infiltrating lymphocyte (TIL), or a helper T cell.

82. The non-natural cell of any one of claims 1 to 76, wherein the cell is a natural killer (NK) cell or natural killer T (NKT) cell.

83. The non-natural cell of any one of claims 1 to 82, wherein the source of the cell is peripheral blood mononuclear cells, bone marrow, lymph nodes tissue, cord blood, thymus issue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, or tumors.

84. A fusion polypeptide comprising:

(a) a first polypeptide comprising: a first multimerization domain polypeptide or variant thereof; a first transmembrane domain; a first costimulatory domain; and/or a primary signaling domain; and

(b) a polypeptide cleavage signal; and

(c) a second polypeptide comprising: an extracellular binding domain; a second multimerization domain polypeptide or variant thereof; a second transmembrane domain; and a second costimulatory domain.

85. The fusion polypeptide of claim 84, wherein the first and second multimerization domains are different.

86. The fusion polypeptide of claim 84 or claim 85, wherein the first and second costimulatory domains are different.

87. The fusion polypeptide of any one of claims 84 to 86, wherein the first multimerization domain and the second multimerization domain associate with a bridging factor selected from the group consisting of: rapamycin or a rapalog thereof, coumermycin or a derivative thereof, gibberellin or a derivative thereof, abscisic acid (ABA) or a derivative thereof, methotrexate or a derivative thereof, cyclosporin A or a derivative thereof, FK506/cyclosporin A (FKCsA) or a derivative thereof, and trimethoprim (Tmp)-synthetic ligand for FK506 binding protein (FKBP) (SLF) or a derivative thereof.

88. The fusion polypeptide of any one of claims 84 to 87, wherein the first multimerization domain and the second multimerization domain are a pair selected from the

group consisting of: FKBP and FKBP-rapamycin binding (FRB), FKBP and calcineurin, FKBP and cyclophilin, FKBP and bacterial dihydrofolate reductase (DHFR), calcineurin and cyclophilin, and PYR1-like 1 (PYL1) and abscisic acid insensitive 1 (ABI1).

89. The fusion polypeptide of any one of claims 84 to 88, wherein the first multimerization domain comprises an FKBP polypeptide or variant thereof, and the second multimerization domain comprises an FRB polypeptide or variant thereof.

90. The fusion polypeptide of any one of claims 84 to 88, wherein the first multimerization domain comprises an FRB polypeptide or variant thereof, and the second multimerization domain comprises an FKBP polypeptide or variant thereof.

91. The fusion polypeptide of any one of claims 84 to 90, wherein the bridging factor is selected from the group consisting of: AP21967, sirolimus, everolimus, novolimus, pimecrolimus, ridaforolimus, tacrolimus, temsirolimus, umirolimus, and zotarolimus.

92. The fusion polypeptide of any one of claims 84 to 91, wherein the first and second multimerization domains are selected from FRB T2098L and FKBP12; and the bridging factor is sirolimus or AP21967.

93. The fusion polypeptide of any one of claims 84 to 92, wherein the first transmembrane domain and the second transmembrane domain are independently selected from a polypeptide selected from the group consisting of: alpha, beta, gamma, or delta chain of the T-cell receptor, CD3δ, CD3ε, CD3γ, CD3ζ, CD4, CD5, CD8α, CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD 134, CD137, CD152, CD154, CD278, amnionless (AMN), and programmed cell death 1 (PDCD1).

94. The fusion polypeptide of any one of claims 84 to93, wherein the first transmembrane domain and the second transmembrane domain are independently selected from

the group consisting of: a CD4 transmembrane domain, a CD8α transmembrane domain, and an AMN transmembrane domain.

95. The fusion polypeptide of any one of claims 84 to 94, wherein the first transmembrane domain and the second transmembrane domain are different.

96. The fusion polypeptide of any one of claims 84 to 95, wherein the costimulatory domain and/or the primary signaling domain comprise an immunoreceptor tyrosine activation motif (ITAM).

97. The fusion polypeptide of any one of claims 84 to 96, wherein the first and second costimulatory domain are independently selected from a costimulatory molecule selected from the group consisting of: Toll-like receptor 1 (TLR1), TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, caspase recruitment domain family member 11 (CARD11), CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD94, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), DNAX-Activation Protein 10 (DAP10), Linker for activation of T-cells family member 1 (LAT), SH2 Domain-Containing Leukocyte Protein Of 76 kD (SLP76), T cell receptor associated transmembrane adaptor 1 (TRAT1), TNFR2, TNFRS14, TNFRS18, TNFRS25, and zeta chain of T cell receptor associated protein kinase 70 (ZAP70).

98. The fusion polypeptide of any one of claims 84 to 97, wherein the first costimulatory domain is isolated from a costimulatory molecule selected from the group consisting of: CD28, CD134, and CD137 and the second costimulatory domain is isolated from CD28, CD278, TNFRS14, TNFRS18, TNFRS25, OX40 or TNFR2.

99. The fusion polypeptide of any one of claims 84 to 98, wherein the first costimulatory domain is isolated from CD137 and the second costimulatory domain is isolated from OX40 or TNFR2.

100. The fusion polypeptide of any one of claims 84 to 99, wherein the first costimulatory domain is isolated from CD137 and the second costimulatory domain is isolated from OX40.

101. The fusion polypeptide of any one of claims 84 to 99, wherein the first costimulatory domain is isolated from CD137 and the second costimulatory domain is isolated from TNFR2.

102. The fusion polypeptide of any one of claims 84 to 101, wherein the primary signaling domain isolated from a polypeptide selected from the group consisting of: FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d.

103. The fusion polypeptide of any one of claims 84 to 102, wherein the primary signaling domain is isolated from a CD3ζ polypeptide.

104. The fusion polypeptide of any one of claims 84 to 103, wherein the extracellular binding domain comprises an antibody or antigen binding fragment thereof, a receptor ectodomain, or a ligand.

105. The fusion polypeptide of any one of claims 84 to 104, wherein the extracellular binding domain comprises an antibody or antigen binding fragment thereof selected from the group consisting of: a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a $F(ab')_2$ fragment, a bispecific Fab dimer (Fab2), a trispecific Fab trimer (Fab3), an Fv, an single chain Fv protein ("scFv"), a bis-scFv, $(scFv)_2$, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody).

106. The fusion polypeptide of any one of claims 84 to 105, wherein the extracellular binding domain comprises a humanized antibody or antigen binding fragment thereof.

107. The fusion polypeptide of any one of claims 84 to 105, wherein the extracellular binding domain comprises a human antibody or antigen binding fragment thereof.

108. The fusion polypeptide of any one of claims 84 to 107, wherein the extracellular binding domain comprises an scFv.

109. The fusion polypeptide of any one of claims 84 to 107, wherein the extracellular binding domain comprises one or more camelid VHH antibodies.

110. The fusion polypeptide of any one of claims 84 to 109, wherein the extracellular binding domain binds an antigen selected from the group consisting of: tumor associated antigens (TAA), tumor specific antigens (TSA), NKG2D ligands, γδ T cell receptor (TCR) ligands, and αβ TCR ligands.

111. The fusion polypeptide of any one of claims 84 to 110, wherein the extracellular binding domain binds an antigen selected from the group consisting of: FRα, $\alpha_v\beta_6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, CEA, CLDN6, CLDN18.2, CLL-1, CS-1, CSPG4, CTAGE1, DLL3, EGFR, EGFRvIII, EGP2, EGP40, EPCAM, EPHA2, ERBB4, FAP, FCRL5, AchR, GD2, GD3, GPC3, HER2, HER2 p95, IL-10Rα, IL-13Rα2, Kappa, LAGE-1A, Lambda, LeY, L1-CAM, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGEA10, MelanA or MART1, SLN), MUC1, MUC16, MICA, MICB, NCAM, NY-ESO-1, PLAC1, PRAME, PSCA, PSMA, ROR1, SSX2, Survivin, TAG72, TEM1/CD248, TEM7R, TPBG, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, VEGFR2, and WT-1.

112. A fusion polypeptide comprising:

(a) a first polypeptide comprising: a first multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain;

(b) a polypeptide cleavage signal; and

(c) a second polypeptide comprising: an extracellular binding domain; a second multimerization domain polypeptide or variant thereof; a second transmembrane domain; and an OX40 costimulatory domain.

113. A fusion polypeptide comprising:

(a) a first polypeptide comprising: a first multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain;

(b) a polypeptide cleavage signal; and

(c) a second polypeptide comprising: an extracellular binding domain; a second multimerization domain polypeptide or variant thereof; a second transmembrane domain; and a TNFR2 costimulatory domain.

114. The fusion polypeptide of claim 112 or claim 113, wherein the first and second multimerization domains are the same.

115. The fusion polypeptide of claim 112 or claim 113, wherein the first and second multimerization domains are different.

116. The fusion polypeptide of claim 112 to 115, wherein the first multimerization domain and the second multimerization domain associate with a bridging factor selected from the group consisting of: rapamycin or a rapalog thereof, coumermycin or a derivative thereof, gibberellin or a derivative thereof, ABA or a derivative thereof, methotrexate or a derivative thereof, cyclosporin A or a derivative thereof, FKCsA or a derivative thereof, and SLF or a derivative thereof.

117. The fusion polypeptide of claim 112 to 116, wherein the first multimerization domain and the second multimerization domain are a pair selected from the group consisting of: FKBP and FRB, FKBP and calcineurin, FKBP and cyclophilin, FKBP and DHFR, calcineurin and cyclophilin, and PYL1 and ABI1.

118. The fusion polypeptide of claim 112 to 117, wherein the first multimerization domain comprises an FKBP polypeptide or variant thereof, and the second multimerization domain comprises an FRB polypeptide or variant thereof.

119. The fusion polypeptide of claim 112 to 117, wherein the first multimerization domain comprises an FRB polypeptide or variant thereof, and the second multimerization domain comprises an FKBP polypeptide or variant thereof.

120. The fusion polypeptide of claim 112 to 119, wherein the first transmembrane domain and the second transmembrane domain are independently selected from a polypeptide selected from the group consisting of: alpha, beta, gamma, or delta chain of the T-cell receptor, CD3δ, CD3ε, CD3γ, CD3ζ, CD4, CD5, CD8α, CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD 134, CD137, CD152, CD154, AMN, and PDCD1.

121. The fusion polypeptide of claim 112 to 120, wherein the first transmembrane domain and the second transmembrane domain are independently selected from the group consisting of: a CD4 transmembrane domain and a CD8α transmembrane domain.

122. The fusion polypeptide of claim 112 to 121, wherein the first transmembrane domain and the second transmembrane domain are the same.

123. The fusion polypeptide of claim 112 to 121, wherein the first transmembrane domain and the second transmembrane domain are different.

124. The fusion polypeptide of claim 112 to 123, wherein the costimulatory domain and/or the primary signaling domain comprise an immunoreceptor tyrosine activation motif (ITAM).

125. The fusion polypeptide of claim 112 to 124, wherein the costimulatory domain is isolated from a costimulatory molecule selected from the group consisting of: TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, CARD11, CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD94, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), DAP10, LAT, SLP76, TRAT1, TNFR2, TNFRS14, TNFRS18, TNFRS25, and ZAP70.

126. The fusion polypeptide of claim 112 to 125, wherein the costimulatory domain is isolated from a costimulatory molecule selected from the group consisting of: CD28, CD134, and CD137.

127. The fusion polypeptide of claim 112 to 126, wherein the costimulatory domain is isolated from a CD137 costimulatory molecule.

128. The fusion polypeptide of claim 112 to 127, wherein the primary signaling domain isolated from a polypeptide selected from the group consisting of: FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d.

129. The fusion polypeptide of claim 112 to 128, wherein the primary signaling domain is isolated from a CD3ζ polypeptide.

130. The fusion polypeptide of claim 112 to 129, wherein the extracellular binding domain comprises an antibody or antigen binding fragment thereof, a receptor ectodomain, or a ligand.

131. The fusion polypeptide of claim 112 to 130, wherein the extracellular binding domain comprises an antibody or antigen binding fragment thereof selected from the group consisting of: a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a $F(ab')_2$ fragment, a bispecific Fab dimer (Fab2), a trispecific Fab trimer (Fab3), an Fv, an single chain Fv protein ("scFv"), a bis-scFv, $(scFv)_2$, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody).

132. The fusion polypeptide of claim 112 to 131, wherein the extracellular binding domain comprises a humanized antibody or antigen binding fragment thereof.

133. The fusion polypeptide of claim 112 to 131, wherein the extracellular binding domain comprises a human antibody or antigen binding fragment thereof.

134. The fusion polypeptide of claim 112 to 133, wherein the extracellular binding domain comprises an scFv.

135. The fusion polypeptide of claim 112 to 131, wherein the extracellular binding domain comprises one or more camelid VHH antibodies.

136. The fusion polypeptide of claim 112 to 135, wherein the extracellular binding domain binds an antigen selected from the group consisting of: tumor associated antigens (TAA), tumor specific antigens (TSA), NKG2D ligands, γδ T cell receptor (TCR) ligands, and αβ TCR ligands.

137. The fusion polypeptide of claim 112 to 136, wherein the extracellular binding domain binds an antigen selected from the group consisting of: FRα, $\alpha_v\beta_6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, CEA, CLDN6, CLDN18.2,

CLL-1, CS-1, CSPG4, CTAGE1, DLL3, EGFR, EGFRvIII, EGP2, EGP40, EPCAM, EPHA2, ERBB4, FAP, FCRL5, AchR, GD2, GD3, GPC3, HER2, HER2 p95, IL-10Rα, IL-13Rα2, Kappa, LAGE-1A, Lambda, LeY, L1-CAM, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGEA10, MelanA or MART1, SLN), MUC1, MUC16, MICA, MICB, NCAM, NY-ESO-1, PLAC1, PRAME, PSCA, PSMA, ROR1, SSX2, Survivin, TAG72, TEM1/CD248, TEM7R, TPBG, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, VEGFR2, and WT-1.

138. A fusion polypeptide comprising:

(a) a first polypeptide comprising: an FKBP multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain;

(b) a polypeptide cleavage signal; and

(c) a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FRB multimerization domain polypeptide or variant thereof; a second transmembrane domain; and an OX40 costimulatory domain.

139. A fusion polypeptide comprising:

(a) a first polypeptide comprising: an FKBP multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain;

(b) a polypeptide cleavage signal; and

(c) a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FRB multimerization domain polypeptide or variant thereof; a second transmembrane domain; and a TNFR2 costimulatory domain.

140. A fusion polypeptide comprising:

(a) a first polypeptide comprising: an FRB multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain;

(b) a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP multimerization domain polypeptide or variant thereof; a second transmembrane domain; and an OX40 costimulatory domain.

141. A fusion polypeptide comprising:

(a) a first polypeptide comprising: an FRB multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain;

(b) a polypeptide cleavage signal; and

(c) a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP multimerization domain polypeptide or variant thereof; a second transmembrane domain; and a TNFR2 costimulatory domain.

142. The fusion polypeptide of any one of claims 138 to 141, wherein the first transmembrane domain and the second transmembrane domain are independently selected from a polypeptide selected from the group consisting of: alpha, beta, gamma, or delta chain of the T-cell receptor, CD3δ, CD3ε, CD3γ, CD3ζ, CD4, CD5, CD8α, CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD 134, CD137, CD152, CD154, AMN, and PDCD1.

143. The fusion polypeptide of any one of claims 138 to 142, wherein the first transmembrane domain and the second transmembrane domain are independently selected from a polypeptide selected from the group consisting of: a CD4 transmembrane domain and a CD8α transmembrane domain.

144. The fusion polypeptide of any one of claims 138 to 143, wherein the costimulatory domain and/or the primary signaling domain comprise an immunoreceptor tyrosine activation motif (ITAM).

145. The fusion polypeptide of any one of claims 138 to 144, wherein the costimulatory domain is isolated from a costimulatory molecule selected from the group consisting of: TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, CARD11, CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD94, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), DAP10, LAT, SLP76, TRAT1, TNFR2, TNFRS14, TNFRS18, TNFRS25,and ZAP70.

146. The fusion polypeptide of any one of claims 138 to 145, wherein the costimulatory domain is isolated from a costimulatory molecule selected from the group consisting of: CD28, CD134, and CD137.

147. The fusion polypeptide of any one of claims 138 to 146, wherein the costimulatory domain is isolated from a CD137 costimulatory molecule.

148. The fusion polypeptide of any one of claims 138 to 147, wherein the primary signaling domain isolated from a polypeptide selected from the group consisting of: FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d.

149. The fusion polypeptide of any one of claims 138 to 148, wherein the primary signaling domain is isolated from a CD3ζ polypeptide.

150. The fusion polypeptide of any one of claims 138 to 149, wherein the antibody or antigen binding fragment thereof selected is from the group consisting of: a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a $F(ab')_2$ fragment, a bispecific Fab dimer (Fab2), a trispecific Fab trimer (Fab3), an Fv, an single chain Fv protein ("scFv"), a bis-scFv, $(scFv)_2$, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody).

151. The fusion polypeptide of any one of claims 138 to 150, wherein the antibody or antigen binding fragment thereof is human or humanized.

152. The fusion polypeptide of any one of claims 138 to 150, wherein the antibody or antigen binding fragment thereof comprises an scFv or one or more camelid VHH antibodies.

153. The fusion polypeptide of any one of claims 138 to 152, wherein the antibody or antigen binding fragment thereof binds an antigen selected from the group consisting of: tumor associated antigens (TAA), tumor specific antigens (TSA), NKG2D ligands, γδ T cell receptor (TCR) ligands, and αβ TCR ligands.

154. The fusion polypeptide of any one of claims 138 to 153, wherein the antibody or antigen binding fragment thereof binds an antigen selected from the group consisting of: FRα, $\alpha_v\beta_6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, CEA, CLDN6, CLDN18.2, CLL-1, CS-1, CSPG4, CTAGE1, DLL3, EGFR, EGFRvIII, EGP2, EGP40, EPCAM, EPHA2, ERBB4, FAP, FCRL5, AchR, GD2, GD3, GPC3, HER2, HER2 p95, IL-10Rα, IL-13Rα2, Kappa, LAGE-1A, Lambda, LeY, L1-CAM, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGEA10, MelanA or MART1, SLN), MUC1, MUC16, MICA, MICB, NCAM, NY-ESO-1, PLAC1, PRAME, PSCA, PSMA, ROR1, SSX2, Survivin, TAG72, TEM1/CD248, TEM7R, TPBG, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, VEGFR2, and WT-1.

155. The fusion polypeptide of any one of claims 138 to 154, wherein the antibody or antigen binding fragment thereof binds BCMA, B7-H3, CD19, CD20, CD22, CD33, CD79A, CD79B, and/or EGFRvIII.

156. The fusion polypeptide of any one of claims 138 to 155, wherein the antibody or antigen binding fragment thereof binds BCMA.

157. The fusion polypeptide of any one of claims 138 to 155, wherein the antibody or antigen binding fragment thereof binds CD19.

158. The fusion polypeptide of any one of claims 138 to 155, wherein the antibody or antigen binding fragment thereof binds CD20 or CD22.

159. The fusion polypeptide of any one of claims 138 to 155, wherein the antibody or antigen binding fragment thereof binds B7-H3.

160. The fusion polypeptide of any one of claims 138 to 155, wherein the antibody or antigen binding fragment thereof binds CD33.

161. The fusion polypeptide of any one of claims 138 to 155, wherein the antibody or antigen binding fragment thereof binds CD79A.

162. The fusion polypeptide of any one of claims 138 to 155, wherein the antibody or antigen binding fragment thereof binds CD79B.

163. The fusion polypeptide of any one of claims 138 to 155, wherein the antibody or antigen binding fragment thereof binds EGFRvIII.

164. A fusion polypeptide comprising:

(a) a first polypeptide comprising: an FKBP12 multimerization domain polypeptide or variant thereof; a CD8α transmembrane domain; a CD137 costimulatory domain; and a CD3ζ primary signaling domain;

(b) a polypeptide cleavage signal; and

(c) a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FRB T2098L multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and a TNFR2 costimulatory domain.

165. A fusion polypeptide comprising:

(a) a first polypeptide comprising: an FKBP12 multimerization domain polypeptide or variant thereof; a CD8α transmembrane domain; a CD137 costimulatory domain; and a CD3ζ primary signaling domain;

(b) a polypeptide cleavage signal; and

(c) a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FRB T2098L multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and an OX40 costimulatory domain.

166. A fusion polypeptide comprising:

(a) a first polypeptide comprising: an FRB T2098L multimerization domain polypeptide or variant thereof; a CD8α transmembrane domain; a CD137 costimulatory domain; and a CD3ζ primary signaling domain;

(b) a polypeptide cleavage signal; and

(c) a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP12 multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and a TNFR2 costimulatory domain.

167. A fusion polypeptide comprising:

(a) a first polypeptide comprising: an FRB T2098L multimerization domain polypeptide or variant thereof; a CD8α transmembrane domain; a CD137 costimulatory domain; and a CD3ζ primary signaling domain;

(b) a polypeptide cleavage signal; and

(c) a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP12 multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and an OX40 costimulatory domain.

168. The fusion polypeptide of any one of claims 164 to 167, wherein the antibody or antigen binding fragment thereof selected is from the group consisting of: a Camel Ig, a Llama

Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a $F(ab')_2$ fragment, a bispecific Fab dimer (Fab2), a trispecific Fab trimer (Fab3), an Fv, an single chain Fv protein ("scFv"), a bis-scFv, $(scFv)_2$, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody).

169. The fusion polypeptide of any one of claims 164 to 168, wherein the antibody or antigen binding fragment thereof is human or humanized.

170. The fusion polypeptide of any one of claims 164 to 169, wherein the antibody or antigen binding fragment thereof comprises an scFv or one or more camelid VHH antibodies.

171. The fusion polypeptide of any one of claims 164 to 170, wherein the antibody or antigen binding fragment thereof binds an antigen selected from the group consisting of: tumor associated antigens (TAA), tumor specific antigens (TSA), NKG2D ligands, γδ T cell receptor (TCR) ligands, and αβ TCR ligands.

172. The fusion polypeptide of any one of claims 164 to 171, wherein the antibody or antigen binding fragment thereof binds an antigen selected from the group consisting of: FRα, $\alpha_v\beta_6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, CEA, CLDN6, CLDN18.2, CLL-1, CS-1, CSPG4, CTAGE1, DLL3, EGFR, EGFRvIII, EGP2, EGP40, EPCAM, EPHA2, ERBB4, FAP, FCRL5, AchR, GD2, GD3, GPC3, HER2, HER2 p95, IL-10Rα, IL-13Rα2, Kappa, LAGE-1A, Lambda, LeY, L1-CAM, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGEA10, MelanA or MART1, SLN), MUC1, MUC16, MICA, MICB, NCAM, NY-ESO-1, PLAC1, PRAME, PSCA, PSMA, ROR1, SSX2, Survivin, TAG72, TEM1/CD248, TEM7R, TPBG, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, VEGFR2, and WT-1.

173. The fusion polypeptide of any one of claims 164 to 172, wherein the antibody or antigen binding fragment thereof binds BCMA, B7-H3, CD19, CD20, CD22, CD33, CD79A, CD79B, and/or EGFRvIII.

174. The fusion polypeptide of any one of claims 164 to 173, wherein the antibody or antigen binding fragment thereof binds BCMA.

175. The fusion polypeptide of any one of claims 164 to 173, wherein the antibody or antigen binding fragment thereof binds CD19.

176. The fusion polypeptide of any one of claims 164 to 173, wherein the antibody or antigen binding fragment thereof binds CD20 or CD22.

177. The fusion polypeptide of any one of claims 164 to 173, wherein the antibody or antigen binding fragment thereof binds B7-H3.

178. The fusion polypeptide of any one of claims 164 to 173, wherein the antibody or antigen binding fragment thereof binds CD33.

179. The fusion polypeptide of any one of claims 164 to 173, wherein the antibody or antigen binding fragment thereof binds CD79A.

180. The fusion polypeptide of any one of claims 164 to 173, wherein the antibody or antigen binding fragment thereof binds CD79B.

181. The fusion polypeptide of any one of claims 164 to 173, wherein the antibody or antigen binding fragment thereof binds EGFRvIII, optionally wherein the antibody is EGFR806 or an antigen binding fragment thereof.

182. The fusion polypeptide of any one of claims 164 to 181, wherein the multimerization domains localize extracellularly when of the first polypeptide and the second polypeptide are expressed.

183. The fusion polypeptide of any one of claims 84 to 182, wherein the polypeptide cleavage signal is a viral self-cleaving polypeptide.

184. The fusion polypeptide of any one of claims 84 to 183, wherein the polypeptide cleavage signal is a viral self-cleaving 2A polypeptide.

185. The fusion polypeptide of any one of claims 84 to 184, wherein the polypeptide cleavage signal is a viral self-cleaving polypeptide selected from the group consisting of: a foot-and-mouth disease virus (FMDV) (F2A) peptide, an equine rhinitis A virus (ERAV) (E2A) peptide, a Thosea asigna virus (TaV) (T2A) peptide, a porcine teschovirus-1 (PTV-1) (P2A) peptide, a Theilovirus 2A peptide, and an encephalomyocarditis virus 2A peptide.

186. A polynucleotide encoding the first or the second polypeptide of any one of claims 1 to 83 or the fusion polypeptide of any one of claims 84 to 185.

187. A cDNA encoding the first or the second polypeptide of any one of claims 1 to 83 or the fusion polypeptide of any one of claims 84 to 185.

188. An RNA encoding the first or the second polypeptide of any one of claims 1 to 83 or the fusion polypeptide of any one of claims 84 to 185.

189. A vector comprising the polynucleotide of any one of claims 186 to 188.

190. The vector of claim 189, wherein the vector is an expression vector.

191. The vector of claim 189, wherein the vector is a transposon.

192. The vector of claim 191, wherein the vector is a piggyBAC transposon or a Sleeping Beauty transposon.

193. The vector of claim 189, wherein the vector is a viral vector.

194. The vector of claim 193, wherein the vector is an adenoviral vector, an adeno-associated viral (AAV) vector, a herpes virus vector, a vaccinia virus vector, or a retroviral vector.

195. The vector of claim 194, wherein the retroviral vector is a lentiviral vector.

196. The vector of claim 195, wherein the lentiviral vector is selected from the group consisting of: human immunodeficiency virus 1 (HIV-1); human immunodeficiency virus 2 (HIV-2), visna-maedi virus (VMV) virus; caprine arthritis-encephalitis virus (CAEV); equine infectious anemia virus (EIAV); feline immunodeficiency virus (FIV); bovine immune deficiency virus (BIV); and simian immunodeficiency virus (SIV).

197. A composition comprising the non-natural cell of any one of claims 1 to 83, the fusion polypeptide of any one of claims 84 to 185, the polynucleotide of any one of claims 186 to 188, or the vector of any one of claims 189 to 196.

198. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the non-natural cell of any one of claims 1 to 83, the fusion polypeptide of any one of claims 84 to 185, the polynucleotide of any one of claims 186 to 188, or the vector of any one of claims 189 to 196.

199. A method of treating a subject in need thereof comprising administering the subject an effective amount of the composition of claim 197 or claim 198.

200. A method of treating, preventing, or ameliorating at least one symptom of a cancer, infectious disease, autoimmune disease, inflammatory disease, and immunodeficiency, or condition associated therewith, comprising administering to the subject an effective amount of the composition of claim 197 or claim 198.

201. A method of treating a solid cancer comprising administering to the subject an effective amount of the composition of claim 197 or claim 198.

202. The method of claim 201, wherein the solid cancer comprises liver cancer, pancreatic cancer, lung cancer, breast cancer, ovarian cancer, prostate cancer, testicular cancer, bladder cancer, brain cancer, sarcoma, head and neck cancer, bone cancer, thyroid cancer, kidney cancer, or skin cancer.

203. The method of claim 201 or claim 202, wherein the solid cancer is a pancreatic cancer, a lung cancer, or a breast cancer.

204. A method of treating a hematological malignancy comprising administering to the subject an effective amount of the composition of claim 197 or claim 198.

205. The method of claim 204, wherein the hematological malignancy is a leukemia, lymphoma, or multiple myeloma.

206. A polypeptide complex comprising:

(a) a first polypeptide comprising: a first multimerization domain polypeptide or variant thereof; a first transmembrane domain; a first costimulatory domain; and/or a primary signaling domain; and

(b) a second polypeptide comprising: an extracellular binding domain; a second multimerization domain polypeptide or variant thereof; a second transmembrane domain; and a second costimulatory domain; and

(c) a bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

207. The polypeptide complex of claim 206, wherein the first and second multimerization domains are different.

208. The polypeptide complex of claim 206 or claim 207, wherein the first and second costimulatory domains are different.

209. The polypeptide complex of any one of claims 206 to 208, wherein the first multimerization domain and the second multimerization domain associate with a bridging factor selected from the group consisting of: rapamycin or a rapalog thereof, coumermycin or a derivative thereof, gibberellin or a derivative thereof, abscisic acid (ABA) or a derivative thereof, methotrexate or a derivative thereof, cyclosporin A or a derivative thereof, FK506/cyclosporin A (FKCsA) or a derivative thereof, and trimethoprim (Tmp)-synthetic ligand for FK506 binding protein (FKBP) (SLF) or a derivative thereof.

210. The polypeptide complex of any one of claims 206 to 209, wherein the first multimerization domain and the second multimerization domain are a pair selected from the group consisting of: FKBP and FKBP-rapamycin binding (FRB), FKBP and calcineurin, FKBP and cyclophilin, FKBP and bacterial dihydrofolate reductase (DHFR), calcineurin and cyclophilin, and PYR1-like 1 (PYL1) and abscisic acid insensitive 1 (ABI1).

211. The polypeptide complex of any one of claims 206 to 210, wherein the first multimerization domain comprises an FKBP polypeptide or variant thereof, and the second multimerization domain comprises an FRB polypeptide or variant thereof.

212. The polypeptide complex of any one of claims 206 to 211, wherein the first multimerization domain comprises an FRB polypeptide or variant thereof, and the second multimerization domain comprises an FKBP polypeptide or variant thereof.

213. The polypeptide complex of any one of claims 206 to 212, wherein the bridging factor is selected from the group consisting of: AP21967, sirolimus, everolimus, novolimus, pimecrolimus, ridaforolimus, tacrolimus, temsirolimus, umirolimus, and zotarolimus.

214. The polypeptide complex of any one of claims 206 to 213, wherein the first and second multimerization domains are selected from FRB T2098L and FKBP12; and the bridging factor is sirolimus or AP21967.

215. The polypeptide complex of any one of claims 206 to 214, wherein the first transmembrane domain and the second transmembrane domain are independently selected from a polypeptide selected from the group consisting of: alpha, beta, gamma, or delta chain of the T-cell receptor, CD3δ, CD3ε, CD3γ, CD3ζ, CD4, CD5, CD8α, CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD 134, CD137, CD152, CD154, CD278, amnionless (AMN), and programmed cell death 1 (PDCD1).

216. The polypeptide complex of any one of claims 206 to 215, wherein the first transmembrane domain and the second transmembrane domain are independently selected from the group consisting of: a CD4 transmembrane domain, a CD8α transmembrane domain, and an AMN transmembrane domain.

217. The polypeptide complex of any one of claims 206 to 216, wherein the first transmembrane domain and the second transmembrane domain are different.

218. The polypeptide complex of any one of claims 206 to 217, wherein the costimulatory domain and/or the primary signaling domain comprise an immunoreceptor tyrosine activation motif (ITAM).

219. The polypeptide complex of any one of claims 206 to 218, wherein the first and second costimulatory domain are independently selected from a costimulatory molecule selected from the group consisting of: Toll-like receptor 1 (TLR1), TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, caspase recruitment domain family member 11 (CARD11), CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD94, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), DNAX-Activation Protein 10 (DAP10), Linker for activation of T-cells family member 1 (LAT), SH2 Domain-Containing Leukocyte Protein Of 76 kD (SLP76), T cell receptor associated transmembrane adaptor 1 (TRAT1), TNFR2, TNFRS14, TNFRS18, TNFRS25, and zeta chain of T cell receptor associated protein kinase 70 (ZAP70).

220. The polypeptide complex of any one of claims 206 to 219, wherein the first costimulatory domain is isolated from a costimulatory molecule selected from the group consisting of: CD28, CD134, and CD137 and the second costimulatory domain is isolated from CD28, CD278, TNFRS14, TNFRS18, TNFRS25, OX40 or TNFR2.

221. The polypeptide complex of any one of claims 206 to 220, wherein the first costimulatory domain is isolated from CD137 and the second costimulatory domain is isolated from OX40 or TNFR2.

222. The polypeptide complex of any one of claims 206 to 221, wherein the first costimulatory domain is isolated from CD137 and the second costimulatory domain is isolated from OX40.

223. The polypeptide complex of any one of claims 206 to 221 wherein the first costimulatory domain is isolated from CD137 and the second costimulatory domain is isolated from TNFR2.

224. The polypeptide complex of any one of claims 206 to 223, wherein the primary signaling domain isolated from a polypeptide selected from the group consisting of: FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d.

225. The polypeptide complex of any one of claims 206 to 224, wherein the primary signaling domain is isolated from a CD3ζ polypeptide.

226. The polypeptide complex of any one of claims 206 to 225, wherein the extracellular binding domain comprises an antibody or antigen binding fragment thereof, a receptor ectodomain, or a ligand.

227. The polypeptide complex of any one of claims 206 to 226, wherein the extracellular binding domain comprises an antibody or antigen binding fragment thereof selected from the group consisting of: a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a $F(ab')_2$ fragment, a bispecific Fab dimer (Fab2), a trispecific Fab trimer (Fab3), an Fv, an single chain Fv protein ("scFv"), a bis-scFv, $(scFv)_2$, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody).

228. The polypeptide complex of any one of claims 206 to 227, wherein the extracellular binding domain comprises a humanized antibody or antigen binding fragment thereof.

229. The polypeptide complex of any one of claims 206 to 227, wherein the extracellular binding domain comprises a human antibody or antigen binding fragment thereof.

230. The polypeptide complex of any one of claims 206 to 229, wherein the extracellular binding domain comprises an scFv.

231. The polypeptide complex of any one of claims 206 to 229, wherein the extracellular binding domain comprises one or more camelid VHH antibodies.

232. The polypeptide complex of any one of claims 206 to 231, wherein the extracellular binding domain binds an antigen selected from the group consisting of: tumor associated antigens (TAA), tumor specific antigens (TSA), NKG2D ligands, γδ T cell receptor (TCR) ligands, and αβ TCR ligands.

233. The polypeptide complex of any one of claims 206 to 232, wherein the extracellular binding domain binds an antigen selected from the group consisting of: FRα, $\alpha_v\beta_6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, CEA, CLDN6, CLDN18.2, CLL-1, CS-1, CSPG4, CTAGE1, DLL3, EGFR, EGFRvIII, EGP2, EGP40, EPCAM, EPHA2, ERBB4, FAP, FCRL5, AchR, GD2, GD3, GPC3, HER2, HER2 p95, IL-10Rα, IL-13Rα2, Kappa, LAGE-1A, Lambda, LeY, L1-CAM, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGEA10, MelanA or MART1, SLN), MUC1, MUC16, MICA, MICB, NCAM, NY-ESO-1, PLAC1, PRAME, PSCA, PSMA, ROR1, SSX2, Survivin, TAG72, TEM1/CD248, TEM7R, TPBG, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, VEGFR2, and WT-1.

234. A polypeptide complex comprising:

(a) a first polypeptide comprising: a first multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain;

(b) a second polypeptide comprising: an extracellular binding domain; a second multimerization domain polypeptide or variant thereof; a second transmembrane domain; and an OX40 costimulatory domain; and

(c) a bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

235. A polypeptide complex comprising:

(a) a first polypeptide comprising: a first multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain;

(b) a second polypeptide comprising: an extracellular binding domain; a second multimerization domain polypeptide or variant thereof; a second transmembrane domain; and a TNFR2 costimulatory domain; and

(c) a bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

236. The polypeptide complex of claim 234 or claim 235, wherein the first and second multimerization domains are the same.

237. The polypeptide complex of claim 234 or claim 236, wherein the first and second multimerization domains are different.

238. The polypeptide complex of any one of claims 234 to 237, wherein the first multimerization domain and the second multimerization domain associate with a bridging factor selected from the group consisting of: rapamycin or a rapalog thereof, coumermycin or a derivative thereof, gibberellin or a derivative thereof, ABA or a derivative thereof, methotrexate or a derivative thereof, cyclosporin A or a derivative thereof, FKCsA or a derivative thereof, and SLF or a derivative thereof.

239. The polypeptide complex of any one of claims 234 to 238, wherein the first multimerization domain and the second multimerization domain are a pair selected from the group consisting of: FKBP and FRB, FKBP and calcineurin, FKBP and cyclophilin, FKBP and DHFR, calcineurin and cyclophilin, and PYL1 and ABI1.

240. The polypeptide complex of any one of claims 234 to 239, wherein the first multimerization domain comprises an FKBP polypeptide or variant thereof, and the second multimerization domain comprises an FRB polypeptide or variant thereof.

241. The polypeptide complex of any one of claims 234 to 239, wherein the first multimerization domain comprises an FRB polypeptide or variant thereof, and the second multimerization domain comprises an FKBP polypeptide or variant thereof.

242. The polypeptide complex of any one of claims 234 to 241, wherein the bridging factor is selected from the group consisting of: AP21967, sirolimus, everolimus, novolimus, pimecrolimus, ridaforolimus, tacrolimus, temsirolimus, umirolimus, and zotarolimus.

243. The polypeptide complex of any one of claims 234 to 242, wherein the first and second multimerization domains are selected from FRB T2098L and FKBP12; and the bridging factor is sirolimus or AP21967.

244. The polypeptide complex of any one of claims 234 to 243, wherein the first transmembrane domain and the second transmembrane domain are independently selected from a polypeptide selected from the group consisting of: alpha, beta, gamma, or delta chain of the T-cell receptor, CD3δ, CD3ε, CD3γ, CD3ζ, CD4, CD5, CD8α, CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD 134, CD137, CD152, CD154, AMN, and PDCD1.

245. The polypeptide complex of any one of claims 234 to 244, wherein the first transmembrane domain and the second transmembrane domain are independently selected from the group consisting of: a CD4 transmembrane domain and a CD8α transmembrane domain.

246. The polypeptide complex of any one of claims 234 to 245, wherein the first transmembrane domain and the second transmembrane domain are the same.

247. The polypeptide complex of any one of claims 234 to 245, wherein the first transmembrane domain and the second transmembrane domain are different.

248. The polypeptide complex of any one of claims 234 to 247, wherein the costimulatory domain and/or the primary signaling domain comprise an immunoreceptor tyrosine activation motif (ITAM).

249. The polypeptide complex of any one of claims 234 to 248, wherein the costimulatory domain is isolated from a costimulatory molecule selected from the group consisting of: TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, CARD11, CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD94, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), DAP10, LAT, SLP76, TRAT1, TNFR2, and ZAP70.

250. The polypeptide complex of any one of claims 234 to 249, wherein the costimulatory domain is isolated from a costimulatory molecule selected from the group consisting of: CD28, CD134, and CD137.

251. The polypeptide complex of any one of claims 234 to 250, wherein the costimulatory domain is isolated from a CD137 costimulatory molecule.

252. The polypeptide complex of any one of claims 234 to 251, wherein the primary signaling domain isolated from a polypeptide selected from the group consisting of: FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d.

253. The polypeptide complex of any one of claims 234 to 252, wherein the primary signaling domain is isolated from a CD3ζ polypeptide.

254. The polypeptide complex of any one of claims 234 to 253, wherein the extracellular binding domain comprises an antibody or antigen binding fragment thereof, a receptor ectodomain, or a ligand.

255. The polypeptide complex of any one of claims 234 to 254, wherein the extracellular binding domain comprises an antibody or antigen binding fragment thereof selected from the group consisting of: a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a $F(ab')_2$ fragment, a bispecific Fab dimer (Fab2), a trispecific Fab trimer (Fab3), an Fv, an single chain Fv protein ("scFv"), a bis-scFv, $(scFv)_2$, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody).

256. The polypeptide complex of any one of claims 234 to 255, wherein the extracellular binding domain comprises a humanized antibody or antigen binding fragment thereof.

257. The polypeptide complex of any one of claims 234 to 255, wherein the extracellular binding domain comprises a human antibody or antigen binding fragment thereof.

258. The polypeptide complex of any one of claims 234 to 257, wherein the extracellular binding domain comprises an scFv.

259. The polypeptide complex of any one of claims 234 to 255, wherein the extracellular binding domain comprises one or more camelid VHH antibodies.

260. The polypeptide complex of any one of claims 234 to 259, wherein the extracellular binding domain binds an antigen selected from the group consisting of: tumor associated antigens (TAA), tumor specific antigens (TSA), NKG2D ligands, γδ T cell receptor (TCR) ligands, and αβ TCR ligands.

261. The polypeptide complex of any one of claims 234 to 260, wherein the extracellular binding domain binds an antigen selected from the group consisting of: FRα, $\alpha_v\beta_6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, CEA, CLDN6, CLDN18.2, CLL-1, CS-1, CSPG4, CTAGE1, DLL3, EGFR, EGFRvIII, EGP2, EGP40, EPCAM, EPHA2, ERBB4, FAP, FCRL5, AchR, GD2, GD3, GPC3, HER2, HER2 p95, IL-10Rα, IL-13Rα2, Kappa, LAGE-1A, Lambda, LeY, L1-CAM, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGEA10, MelanA or MART1, SLN), MUC1, MUC16, MICA, MICB, NCAM, NY-ESO-1, PLAC1, PRAME, PSCA, PSMA, ROR1, SSX2, Survivin, TAG72, TEM1/CD248, TEM7R, TPBG, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, VEGFR2, and WT-1.

262. A polypeptide complex comprising:

(a) a first polypeptide comprising: an FKBP multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain;

(b) a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FRB multimerization domain polypeptide or variant thereof; a second transmembrane domain; and an OX40 costimulatory domain; and

(c) a bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

263. A polypeptide complex comprising:

(a) a first polypeptide comprising: an FKBP multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain;

(b) a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FRB multimerization domain polypeptide or variant thereof; a second transmembrane domain; and a TNFR2 costimulatory domain; and

(c) a bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

264. A polypeptide complex comprising:

(a) a first polypeptide comprising: an FRB multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain;

(b) a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP multimerization domain polypeptide or variant thereof; a second transmembrane domain; and an OX40 costimulatory domain; and

(c) a bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

265. A polypeptide complex comprising:

(a) a first polypeptide comprising: an FRB multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain;

(b) a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP multimerization domain polypeptide or variant thereof; a second transmembrane domain; and a TNFR2 costimulatory domain; and

(c) a bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

266. The polypeptide complex of any one of claims 262 to 265, wherein the bridging factor is selected from the group consisting of: AP21967, sirolimus, everolimus, novolimus, pimecrolimus, ridaforolimus, tacrolimus, temsirolimus, umirolimus, and zotarolimus.

267. The polypeptide complex of any one of claims 262 to 266, wherein the FRB multimerization domain is FRB T2098L; the FKBP multimerization domain is FKBP12; and the bridging factor is sirolimus or AP21967.

268. The polypeptide complex of any one of claims 262 to 267, wherein the first transmembrane domain and the second transmembrane domain are independently selected from a polypeptide selected from the group consisting of: alpha, beta, gamma, or delta chain of the T-cell receptor, CD3δ, CD3ε, CD3γ, CD3ζ, CD4, CD5, CD8α, CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD 134, CD137, CD152, CD154, AMN, and PDCD1.

269. The polypeptide complex of any one of claims 262 to 268, wherein the first transmembrane domain and the second transmembrane domain are independently selected from a polypeptide selected from the group consisting of: a CD4 transmembrane domain and a CD8α transmembrane domain.

270. The polypeptide complex of any one of claims 262 to 269, wherein the costimulatory domain and/or the primary signaling domain comprise an immunoreceptor tyrosine activation motif (ITAM).

271. The polypeptide complex of any one of claims 262 to 270, wherein the costimulatory domain is isolated from a costimulatory molecule selected from the group consisting of: TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, CARD11, CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD94, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), DAP10, LAT, SLP76, TRAT1, TNFR2, and ZAP70.

272. The polypeptide complex of any one of claims 262 to 271, wherein the costimulatory domain is isolated from a costimulatory molecule selected from the group consisting of: CD28, CD134, and CD137.

273. The polypeptide complex of any one of claims 262 to 272, wherein the costimulatory domain is isolated from a CD137 costimulatory molecule.

274. The polypeptide complex of any one of claims 262 to 273, wherein the primary signaling domain isolated from a polypeptide selected from the group consisting of: FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d.

275. The polypeptide complex of any one of claims 262 to 274, wherein the primary signaling domain is isolated from a CD3ζ polypeptide.

276. The polypeptide complex of any one of claims 262 to 275, wherein the antibody or antigen binding fragment thereof selected is from the group consisting of: a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a $F(ab')_2$ fragment, a bispecific Fab dimer (Fab2), a trispecific Fab trimer (Fab3), an Fv, an single chain Fv protein ("scFv"), a bis-scFv, $(scFv)_2$, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody).

277. The polypeptide complex of any one of claims 262 to 276, wherein the antibody or antigen binding fragment thereof is human or humanized.

278. The polypeptide complex of any one of claims 262 to 277, wherein the antibody or antigen binding fragment thereof comprises an scFv or one or more camelid VHH antibodies.

279. The polypeptide complex of any one of claims 262 to 278, wherein the antibody or antigen binding fragment thereof binds an antigen selected from the group consisting of:

tumor associated antigens (TAA), tumor specific antigens (TSA), NKG2D ligands, γδ T cell receptor (TCR) ligands, and αβ TCR ligands.

280. The polypeptide complex of any one of claims 262 to 279, wherein the antibody or antigen binding fragment thereof binds an antigen selected from the group consisting of: FRα, $\alpha_v\beta_6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, CEA, CLDN6, CLDN18.2, CLL-1, CS-1, CSPG4, CTAGE1, DLL3, EGFR, EGFRvIII, EGP2, EGP40, EPCAM, EPHA2, ERBB4, FAP, FCRL5, AchR, GD2, GD3, GPC3, HER2, HER2 p95, IL-10Rα, IL-13Rα2, Kappa, LAGE-1A, Lambda, LeY, L1-CAM, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGEA10, MelanA or MART1, SLN), MUC1, MUC16, MICA, MICB, NCAM, NY-ESO-1, PLAC1, PRAME, PSCA, PSMA, ROR1, SSX2, Survivin, TAG72, TEM1/CD248, TEM7R, TPBG, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, VEGFR2, and WT-1.

281. The polypeptide complex of any one of claims 262 to 280, wherein the antibody or antigen binding fragment thereof binds BCMA, B7-H3, CD19, CD20, CD22, CD33, CD79A, CD79B, and/or EGFRvIII.

282. The polypeptide complex of any one of claims 262 to 281, wherein the antibody or antigen binding fragment thereof binds BCMA.

283. The polypeptide complex of any one of claims 262 to 281, wherein the antibody or antigen binding fragment thereof binds CD19.

284. The polypeptide complex of any one of claims 262 to 281, wherein the antibody or antigen binding fragment thereof binds CD20 or CD22.

285. The polypeptide complex of any one of claims 262 to 281, wherein the antibody or antigen binding fragment thereof binds B7-H3.

286. The polypeptide complex of any one of claims 262 to 281, wherein the antibody or antigen binding fragment thereof binds CD33.

287. The polypeptide complex of any one of claims 262 to 281, wherein the antibody or antigen binding fragment thereof binds CD79A.

288. The polypeptide complex of any one of claims 262 to 281, wherein the antibody or antigen binding fragment thereof binds CD79B.

289. The polypeptide complex of any one of claims 262 to 281, wherein the antibody or antigen binding fragment thereof binds EGFRvIII.

290. A polypeptide complex comprising:

(a) a first polypeptide comprising: an FKBP12 multimerization domain polypeptide or variant thereof; a CD8α transmembrane domain; a CD137 costimulatory domain; and a CD3ζ primary signaling domain;

(b) a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FRB T2098L multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and a TNFR2 costimulatory domain; and

(c) a bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

291. A polypeptide complex comprising:

(a) a first polypeptide comprising: an FKBP12 multimerization domain polypeptide or variant thereof; a CD8α transmembrane domain; a CD137 costimulatory domain; and a CD3ζ primary signaling domain;

(b) a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FRB T2098L multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and an OX40 costimulatory domain; and

(c) a bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

292. A polypeptide complex comprising:

(a) a first polypeptide comprising: an FRB T2098L multimerization domain polypeptide or variant thereof; a CD8α transmembrane domain; a CD137 costimulatory domain; and a CD3ζ primary signaling domain;

(b) a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP12 multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and a TNFR2 costimulatory domain; and

(c) a bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

293. A polypeptide complex comprising:

(a) a first polypeptide comprising: an FRB T2098L multimerization domain polypeptide or variant thereof; a CD8α transmembrane domain; a CD137 costimulatory domain; and a CD3ζ primary signaling domain;

(b) a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP12 multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and an OX40 costimulatory domain; and

(c) a bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.

294. The polypeptide complex of any one of claims 290 to 293, wherein the bridging factor is AP21967 or sirolimus.

295. The polypeptide complex of any one of claims 290 to 294, wherein the antibody or antigen binding fragment thereof selected is from the group consisting of: a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a $F(ab')_2$ fragment, a bispecific Fab dimer (Fab2), a trispecific Fab trimer (Fab3), an Fv, an single chain Fv protein ("scFv"), a bis-scFv, $(scFv)_2$, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody).

296. The polypeptide complex of any one of claims 290 to 295, wherein the antibody or antigen binding fragment thereof is human or humanized.

297. The polypeptide complex of any one of claims 290 to 296, wherein the antibody or antigen binding fragment thereof comprises an scFv or one or more camelid VHH antibodies.

298. The polypeptide complex of any one of claims 290 to 297, wherein the antibody or antigen binding fragment thereof binds an antigen selected from the group consisting of: tumor associated antigens (TAA), tumor specific antigens (TSA), NKG2D ligands, γδ T cell receptor (TCR) ligands, and αβ TCR ligands.

299. The polypeptide complex of any one of claims 290 to 298, wherein the antibody or antigen binding fragment thereof binds an antigen selected from the group consisting of: FRα, $\alpha_v\beta_6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD16, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, CEA, CLDN6, CLDN18.2, CLL-1, CS-1, CSPG4, CTAGE1, DLL3, EGFR, EGFRvIII, EGP2, EGP40, EPCAM, EPHA2, ERBB4, FAP, FCRL5, AchR, GD2, GD3, GPC3, HER2, HER2 p95, IL-10Rα, IL-13Rα2, Kappa, LAGE-1A, Lambda, LeY, L1-CAM, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGEA10, MelanA or MART1, SLN), MUC1, MUC16, MICA, MICB, NCAM, NY-ESO-1, PLAC1, PRAME, PSCA, PSMA, ROR1, SSX2, Survivin, TAG72, TEM1/CD248, TEM7R, TPBG, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, VEGFR2, and WT-1.

300. The polypeptide complex of any one of claims 290 to 299, wherein the antibody or antigen binding fragment thereof binds BCMA, B7-H3, CD19, CD20, CD22, CD33, CD79A, CD79B, and/or EGFRvIII.

301. The polypeptide complex of any one of claims 290 to 300, wherein the antibody or antigen binding fragment thereof binds BCMA.

302. The polypeptide complex of any one of claims 290 to 300, wherein the antibody or antigen binding fragment thereof binds CD19.

303. The polypeptide complex of any one of claims 290 to 300, wherein the antibody or antigen binding fragment thereof binds CD20 or CD22.

304. The polypeptide complex of any one of claims 290 to 300, wherein the antibody or antigen binding fragment thereof binds B7-H3.

305. The polypeptide complex of any one of claims 290 to 300, wherein the antibody or antigen binding fragment thereof binds CD33.

306. The polypeptide complex of any one of claims 290 to 300, wherein the antibody or antigen binding fragment thereof binds CD79A.

307. The polypeptide complex of any one of claims 290 to 300, wherein the antibody or antigen binding fragment thereof binds CD79B.

308. The polypeptide complex of any one of claims 290 to 300, wherein the antibody or antigen binding fragment thereof binds EGFRvIII, optionally wherein the antibody is EGFR806 or an antigen binding fragment thereof.

309. The polypeptide complex of any one of claims 290 to 300, wherein the multimerization domains localize extracellularly when of the first polypeptide and the second polypeptide are expressed.

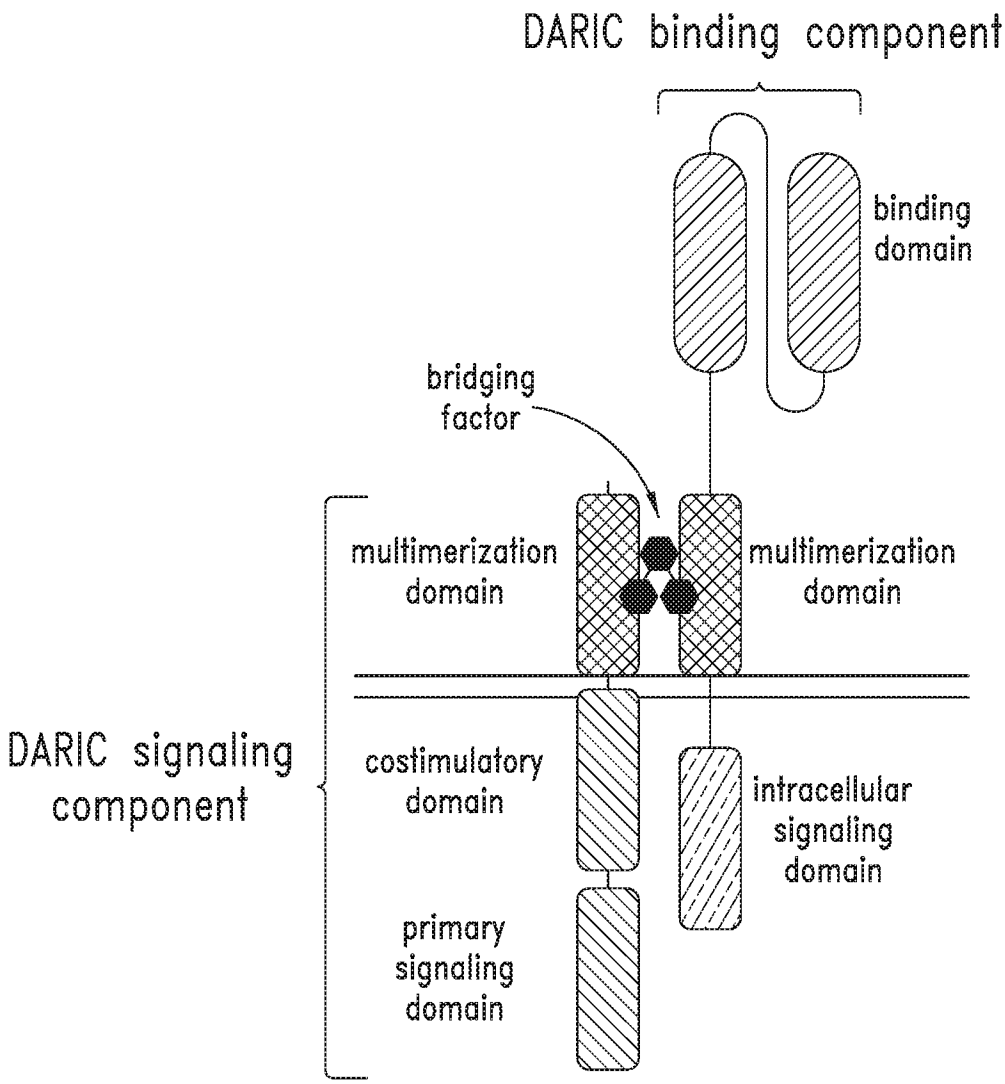

DARIC binding component
binding domain
bridging factor
multimerization domain
multimerization domain
DARIC signaling component
costimulatory domain
intracellular signaling domain
primary signaling domain

FIG. 1

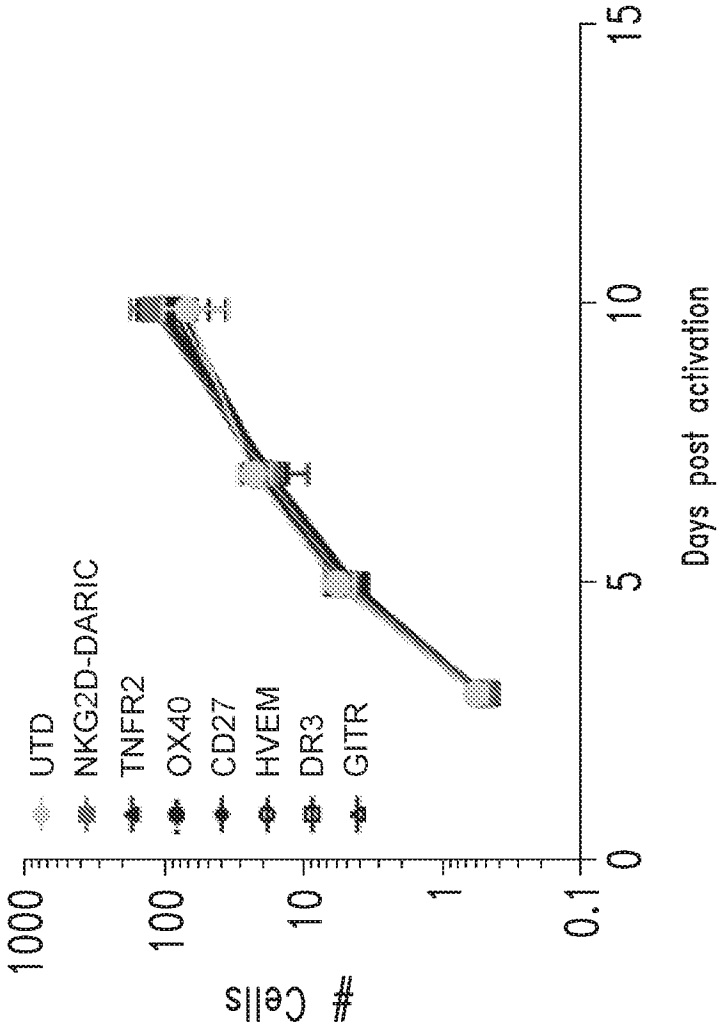

Cells
1000
100
10
1
0.1
0
5
10
15
Days post activation
UTD
NKG2D-DARIC
TNFR2
OX40
CD27
HVEM
DR3
GITR

FIG. 2B

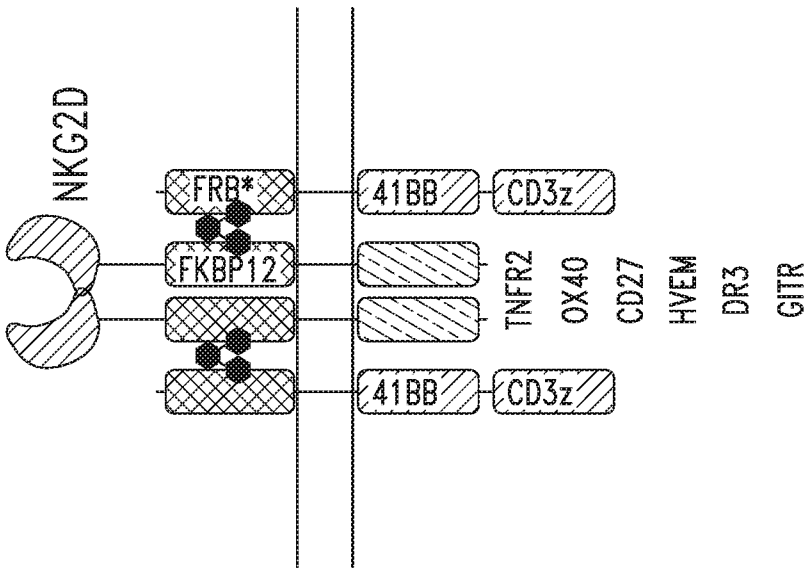

NKG2D
FRB*
FKBP12
41BB
CD3z
41BB
CD3z
TNFR2
OX40
CD27
HVEM
DR3
GITR

FIG. 2A

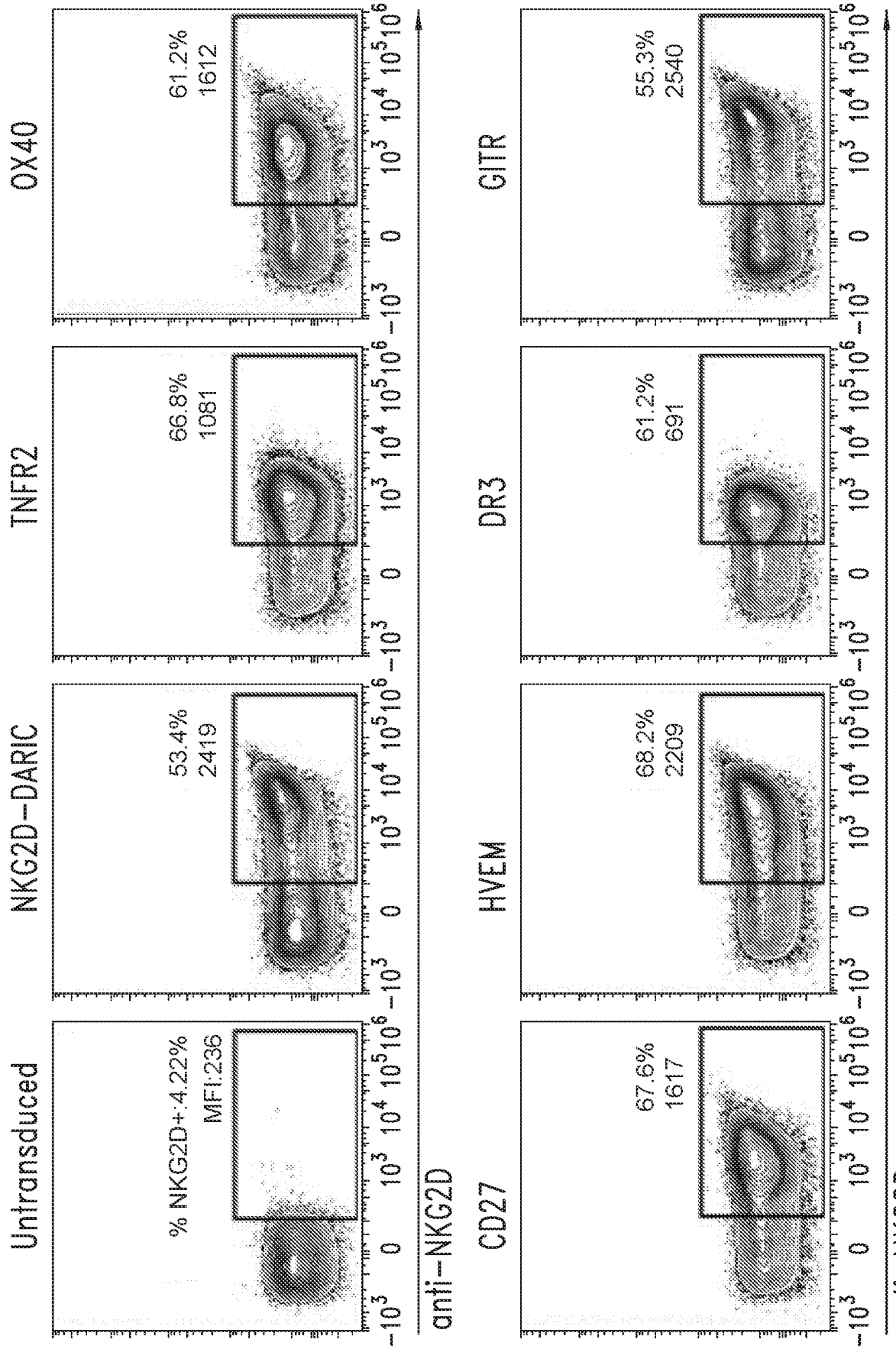

Untransduced
NKG2D-DARIC
TNFR2
OX40
% NKG2D+: 4.22%
MFI:236
53.4%
2419
66.8%
1081
61.2%
1612
anti-NKG2D
CD27
HVEM
DR3
GITR
67.6%
1617
68.2%
2209
61.2%
691
55.3%
2540
anti-NKG2D

FIG. 2C

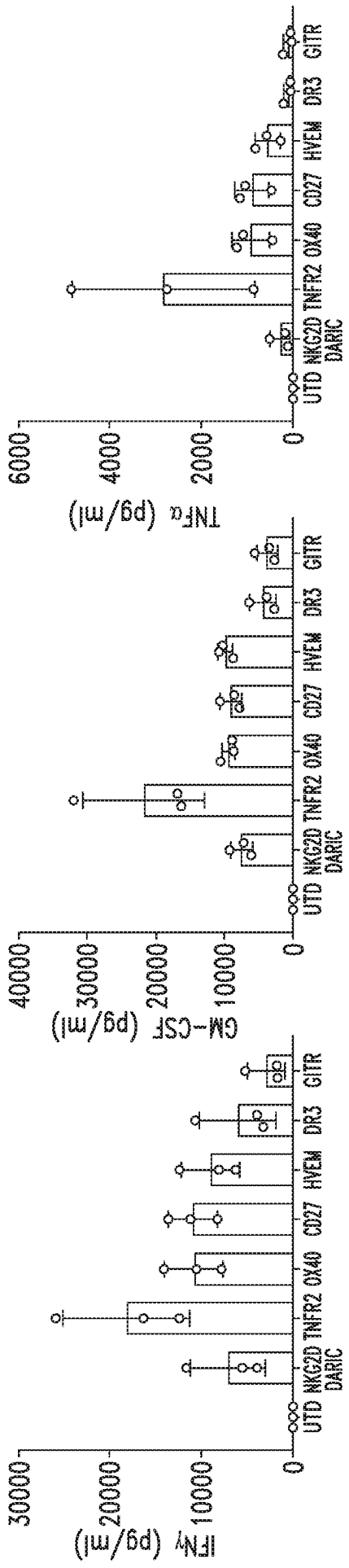

IFNγ (pg/ml)
30000
20000
10000
0
GM-CSF (pg/ml)
40000
30000
20000
10000
0
TNFα (pg/ml)
6000
4000
2000
0
UTD
NKG2D DARIC
TNFR2
OX40
CD27
HVEM
DR3
GITR

FIG. 2D

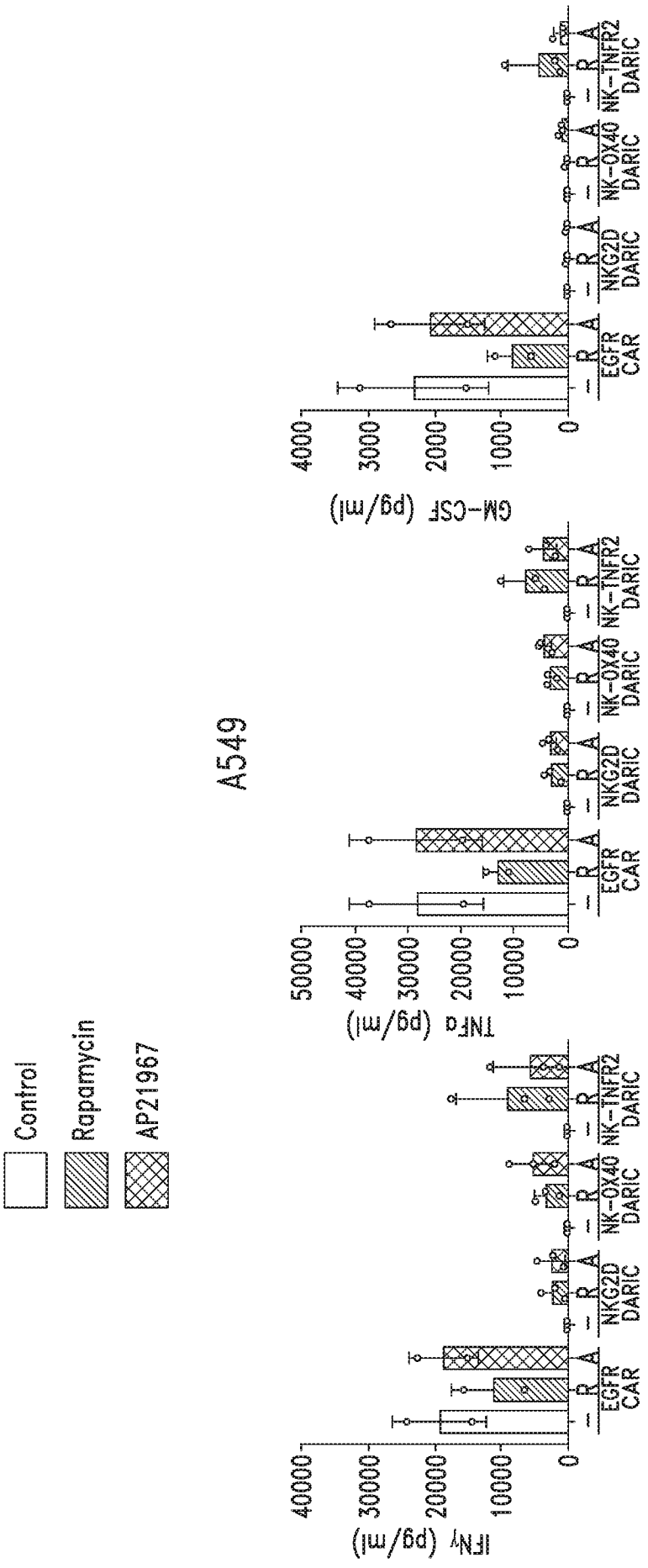

A549
Control
Rapamycin
AP21967
IFNγ (pg/ml)
0
10000
20000
30000
40000
TNFα (pg/ml)
0
10000
20000
30000
40000
50000
GM-CSF (pg/ml)
0
1000
2000
3000
4000
– RA
EGFR CAR
– RA
NKG2D DARIC
– RA
NK-0X40 DARIC
– RA
NK-TNFR2 DARIC

FIG. 3A

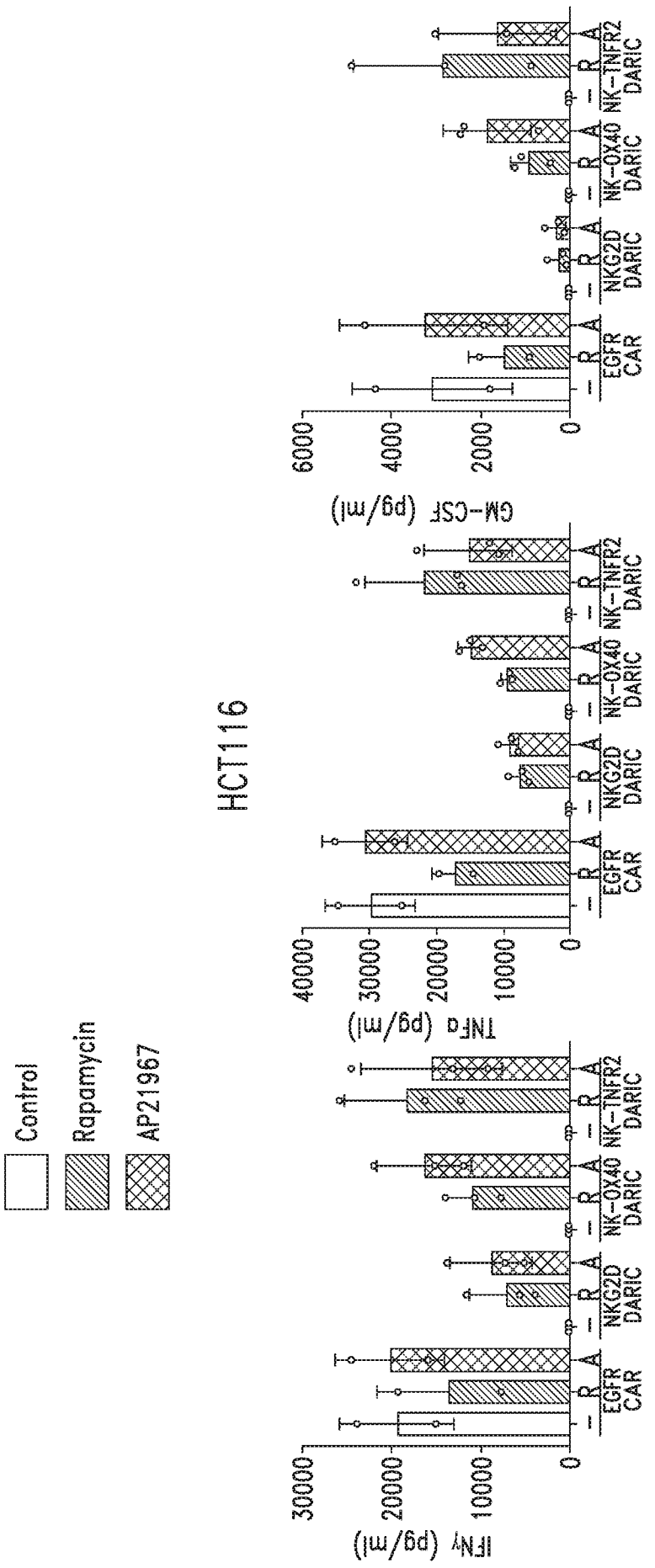

HCT116
Control
Rapamycin
AP21967
IFNγ (pg/ml)
TNFα (pg/ml)
GM-CSF (pg/ml)
- RA
EGFR CAR
NKG2D DARIC
NK-0X40 DARIC
NK-TNFR2 DARIC

FIG. 3B

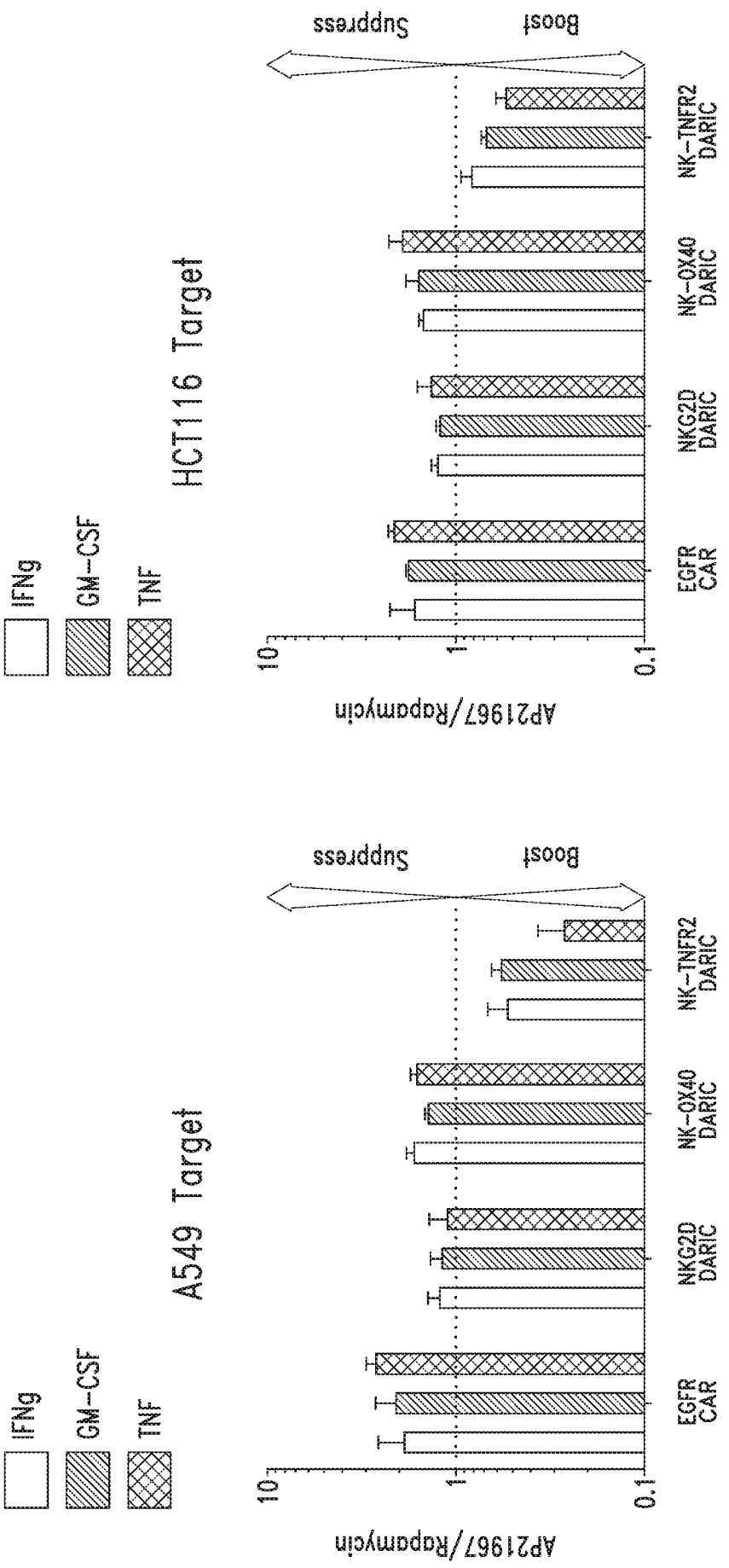
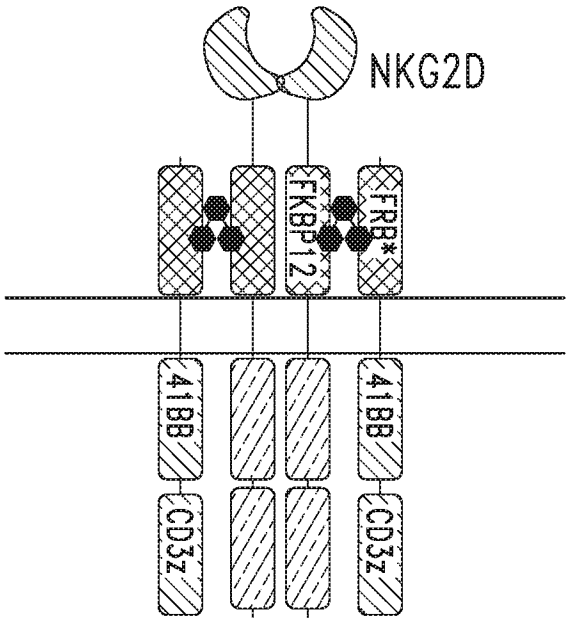

IFNg
GM-CSF
TNF
HCT116 Target
A549 Target
Boost
Suppress
10
1
0.1
AP21967/Rapamycin
EGFR CAR
NKG2D DARIC
NK-OX40 DARIC
NK-TNFR2 DARIC

FIG. 3C



DAP10
CD28
CD28+DAP10
DAP10+OX40
OX40+DAP10

FIG. 4A

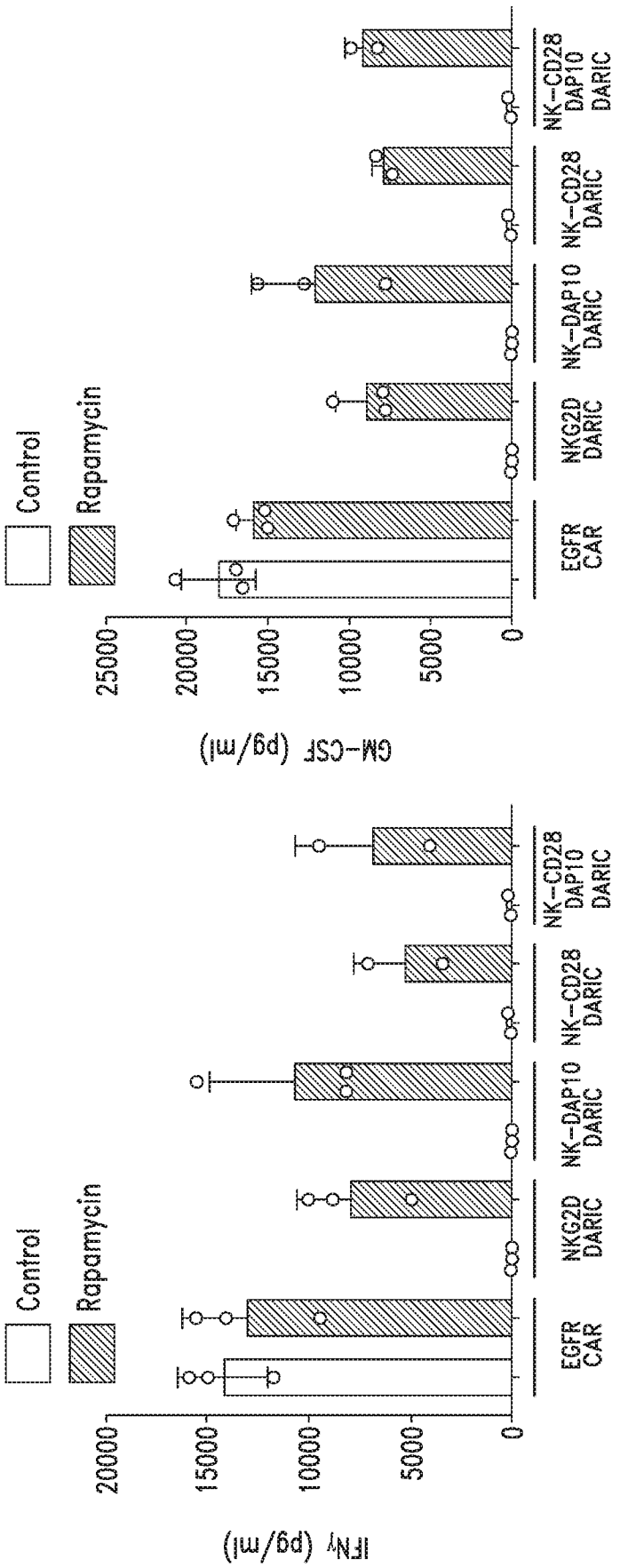

Control
Rapamycin
IFNγ (pg/ml)
20000
15000
10000
5000
0
EGFR CAR
NKG2D DARIC
NK-DAP10 DARIC
NK-CD28 DARIC
NK-CD28 DAP10 DARIC
GM-CSF (pg/ml)
25000
20000
15000
10000
5000
0
EGFR CAR
NKG2D DARIC
NK-DAP10 DARIC
NK-CD28 DARIC
NK-CD28 DAP10 DARIC

FIG. 4B

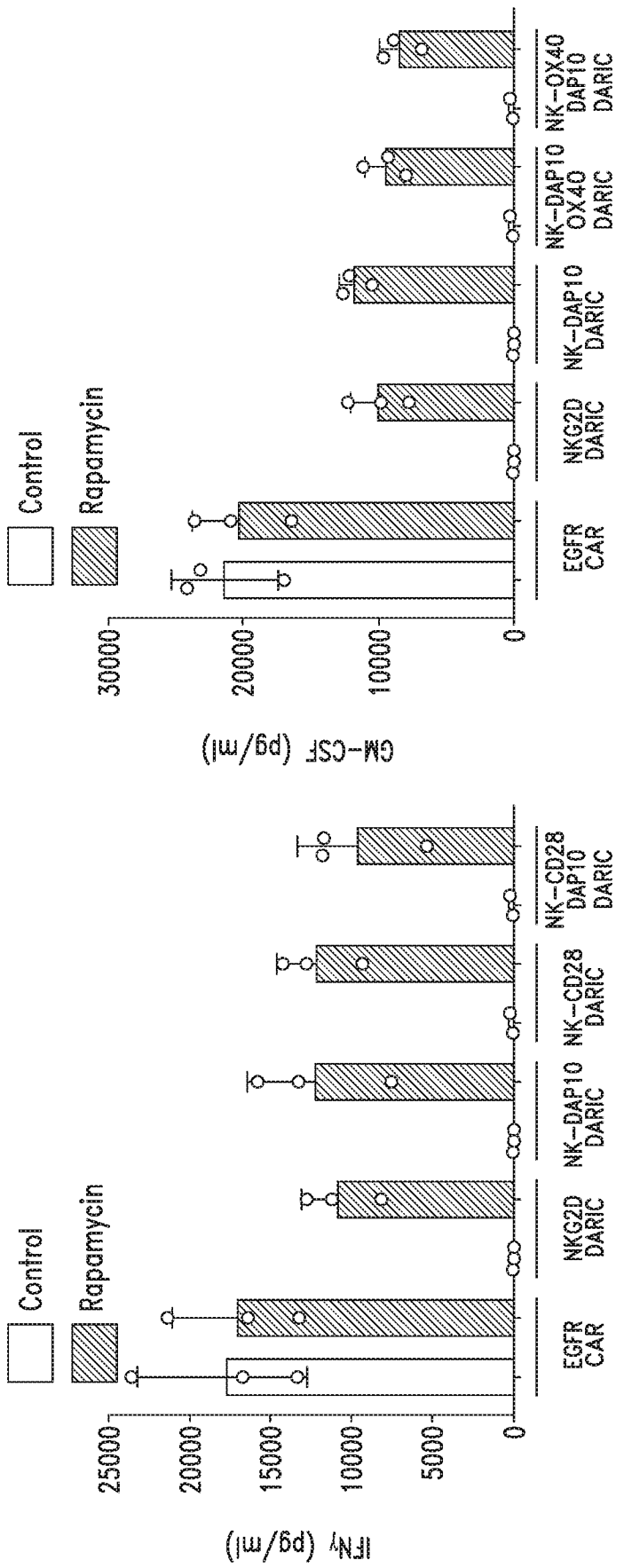

Control
Rapamycin
IFNγ (pg/ml)
25000
20000
15000
10000
5000
0
EGFR CAR
NKG2D DARIC
NK-DAP10 DARIC
NK-CD28 DARIC
NK-CD28 DAP10 DARIC
GM-CSF (pg/ml)
30000
20000
10000
0
NK-DAP10 OX40 DARIC
NK-OX40 DAP10 DARIC

FIG. 4C

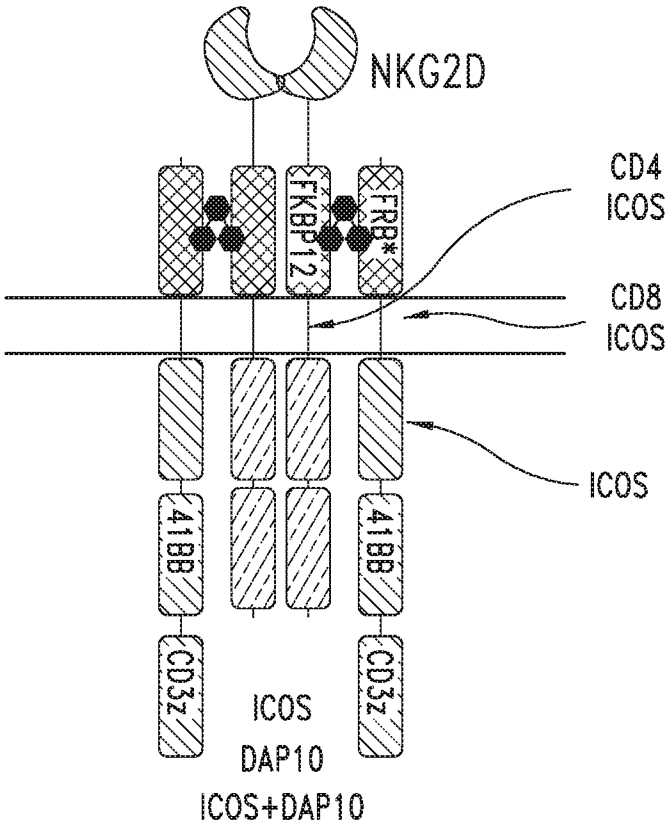

NKG2D
FRB*
FKBP12
CD4
ICOS
CD8
ICOS
ICOS
41BB
41BB
CD3z
CD3z
ICOS
DAP10
ICOS+DAP10

FIG. 5A

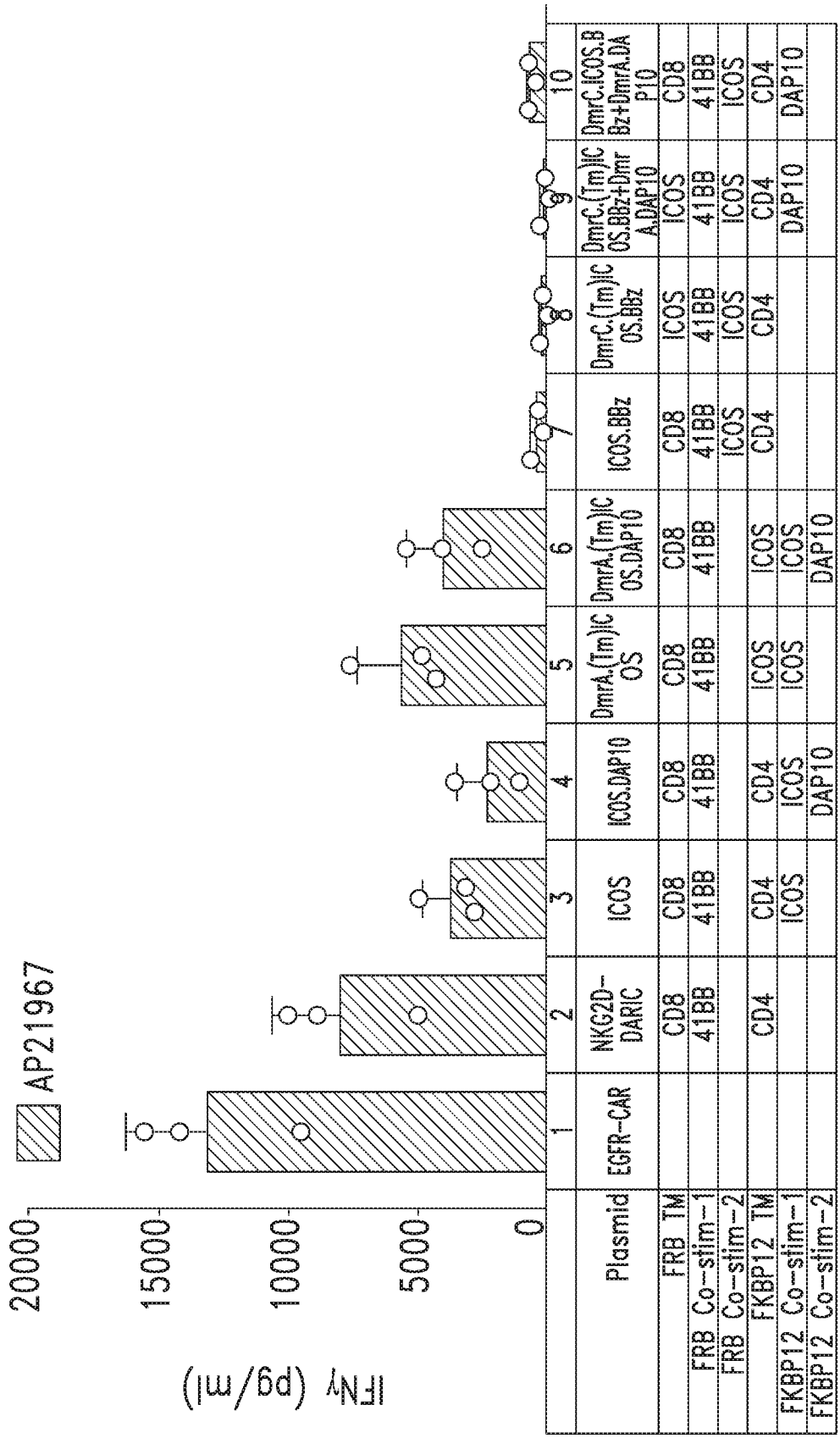

IFNγ (pg/ml)
20000
15000
10000
5000
0
AP21967
Plasmid
EGFR-CAR
NKG2D-DARIC
ICOS
ICOS.DAP10
DmrA.(Tm)IC OS
DmrA.(Tm)IC OS.DAP10
ICOS.BBz
DmrC.(Tm)IC OS.BBz
DmrC.(Tm)IC OS.BBz+DmrA.DAP10
DmrC.ICOS.BBz+DmrA.DAP10
FRB TM
FRB Co-stim-1
FRB Co-stim-2
FKBP12 TM
FKBP12 Co-stim-1
FKBP12 Co-stim-2
CD8
41BB
CD4
ICOS
DAP10

FIG. 5B

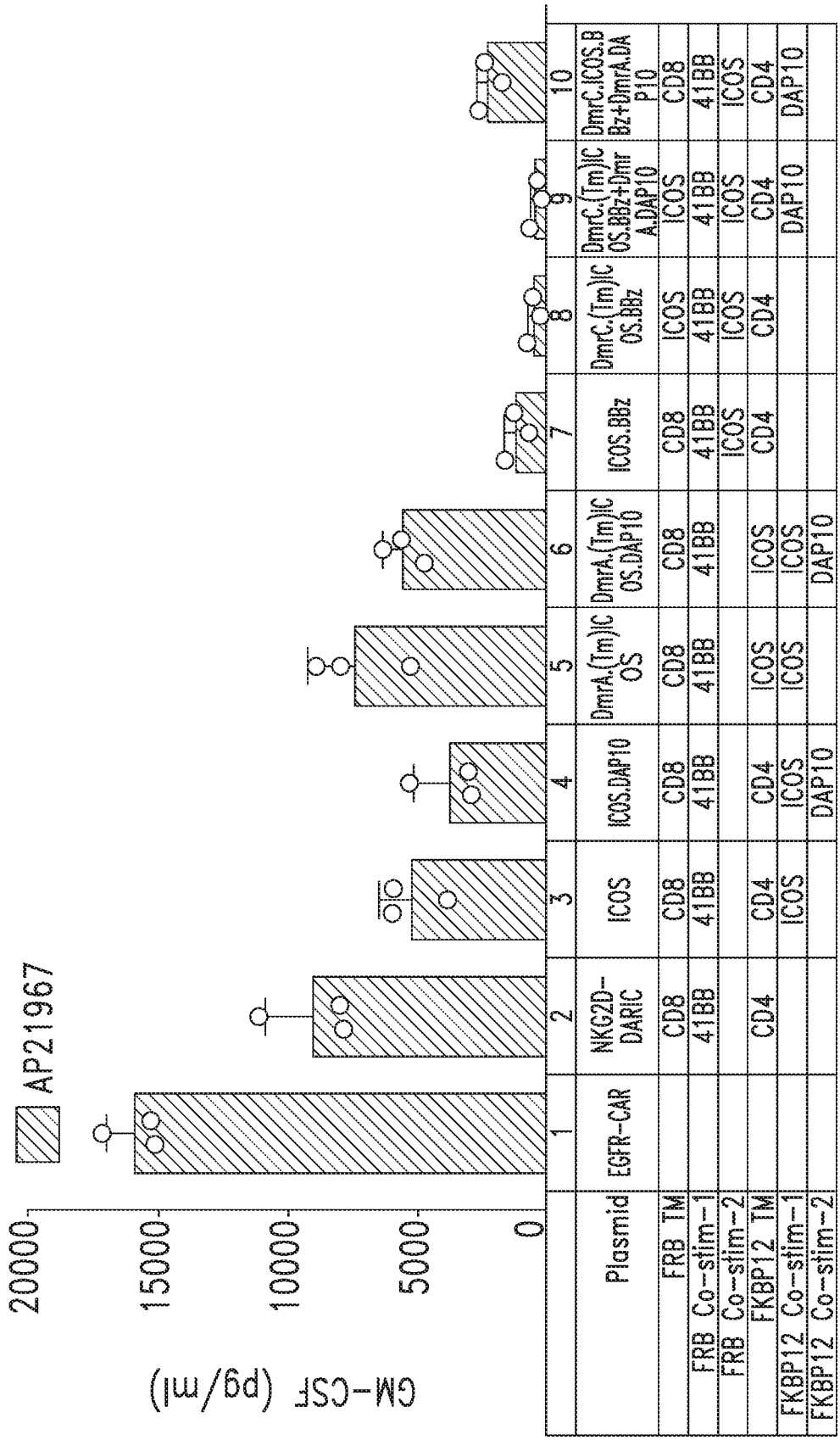

GM-CSF (pg/ml)
20000
15000
10000
5000
0
AP21967
Plasmid
EGFR-CAR
NKG2D-DARIC
ICOS
ICOS.DAP10
DmrA.(Tm)ICOS
DmrA.(Tm)ICOS.DAP10
ICOS.BBz
DmrC.(Tm)ICOS.BBz
DmrC.(Tm)ICOS.BBz+DmrA.DAP10
DmrC.ICOS.BBz+DmrA.DAP10
FRB TM
FRB Co-stim-1
FRB Co-stim-2
FKBP12 TM
FKBP12 Co-stim-1
FKBP12 Co-stim-2
CD8
41BB
CD4
ICOS
DAP10

FIG. 5C

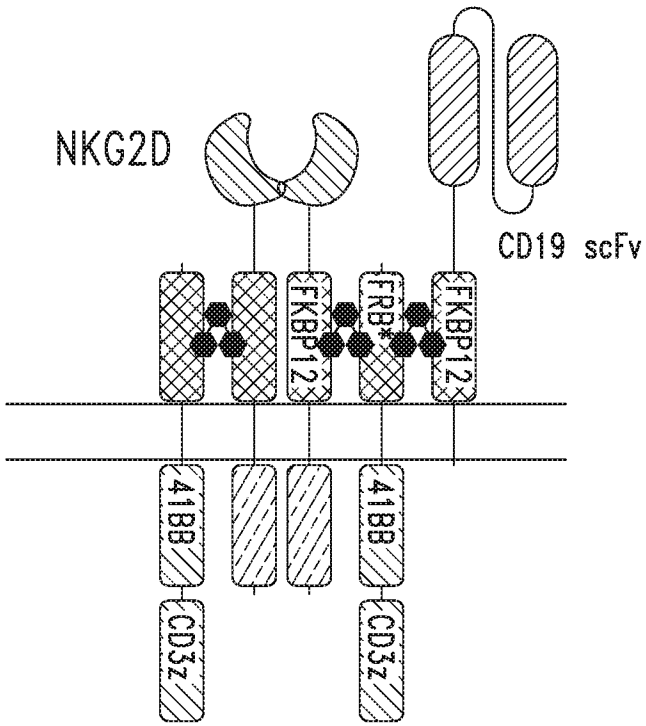


FIG. 6A

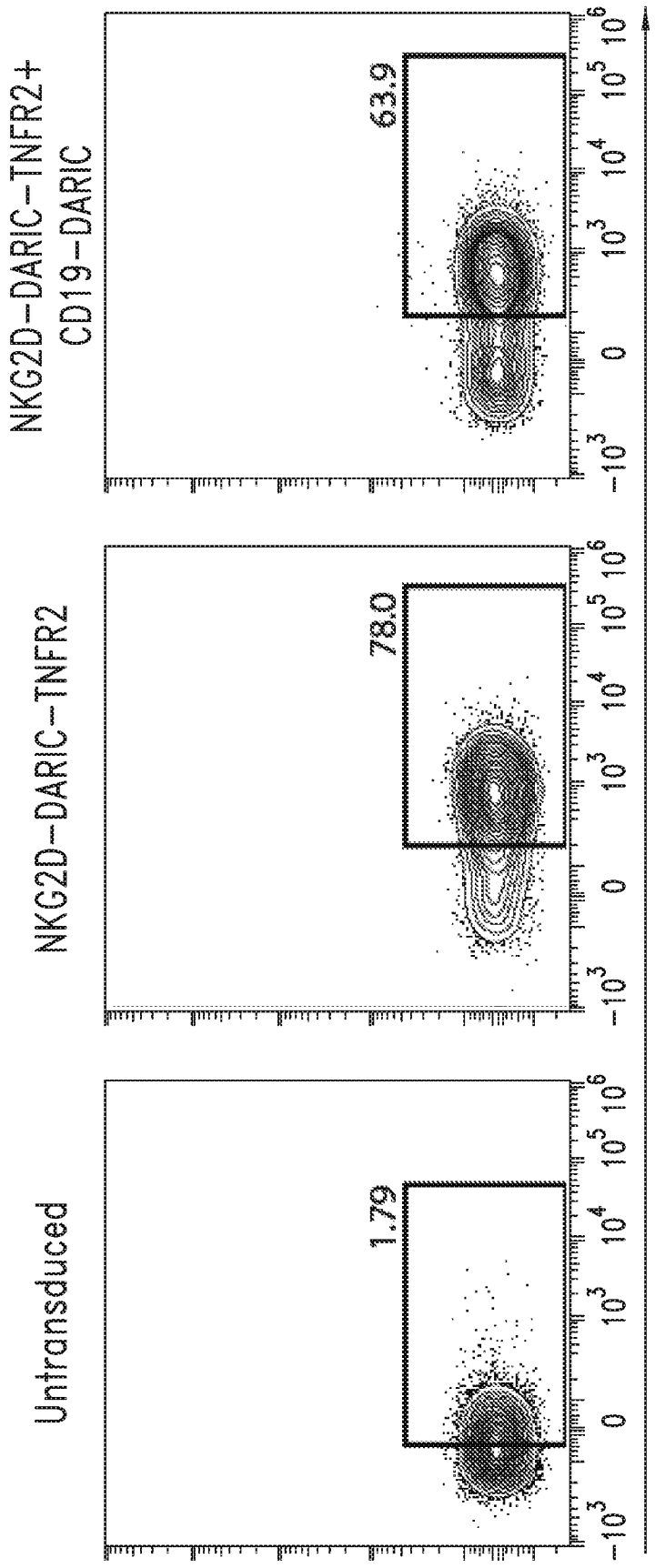

Untransduced
1.79
NKG2D-DARIC-TNFR2
78.0
NKG2D-DARIC-TNFR2+
CD19-DARIC
63.9
anti-NKG2D
-10^3
0
10^3
10^4
10^5
10^6

FIG. 6B

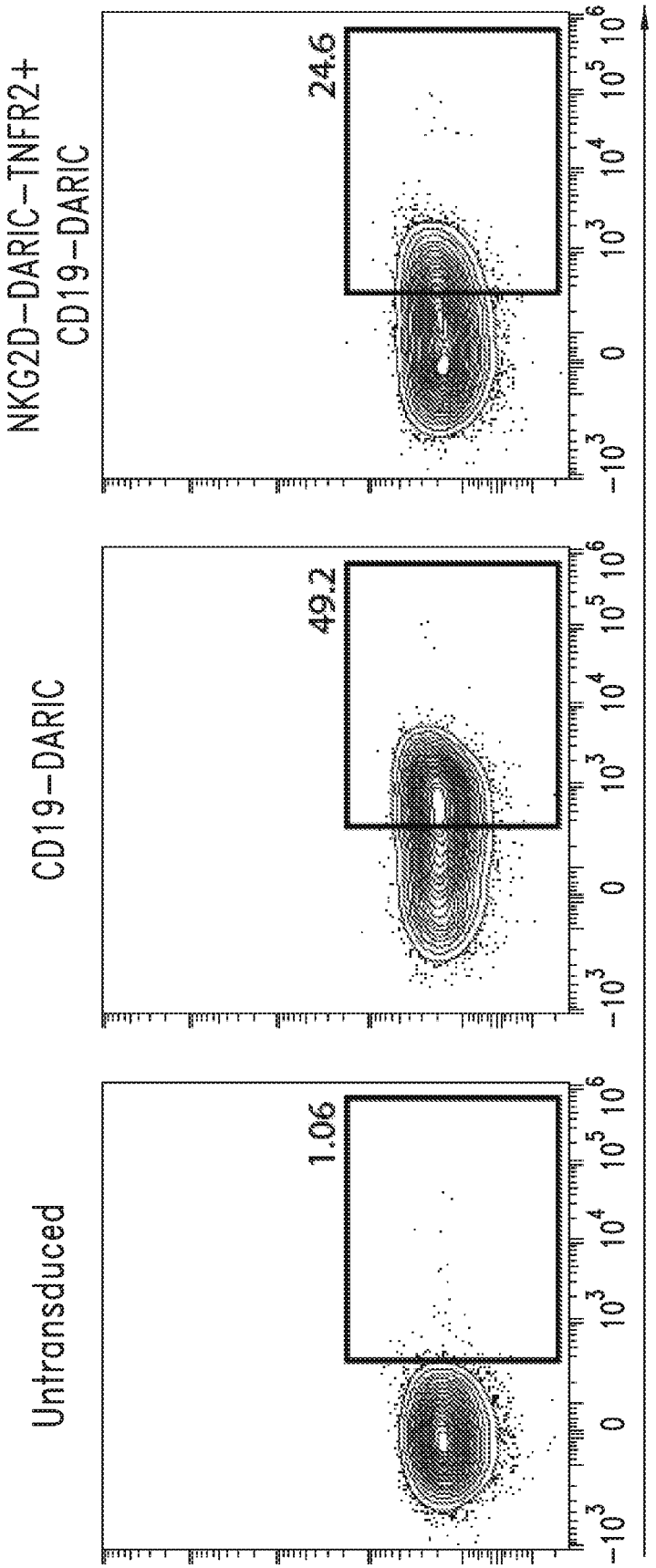

Untransduced
1.06
CD19-DARIC
49.2
NKG2D-DARIC-TNFR2+
CD19-DARIC
24.6
CD19-Fc
-10^3
0
10^3
10^4
10^5
10^6

FIG. 6C

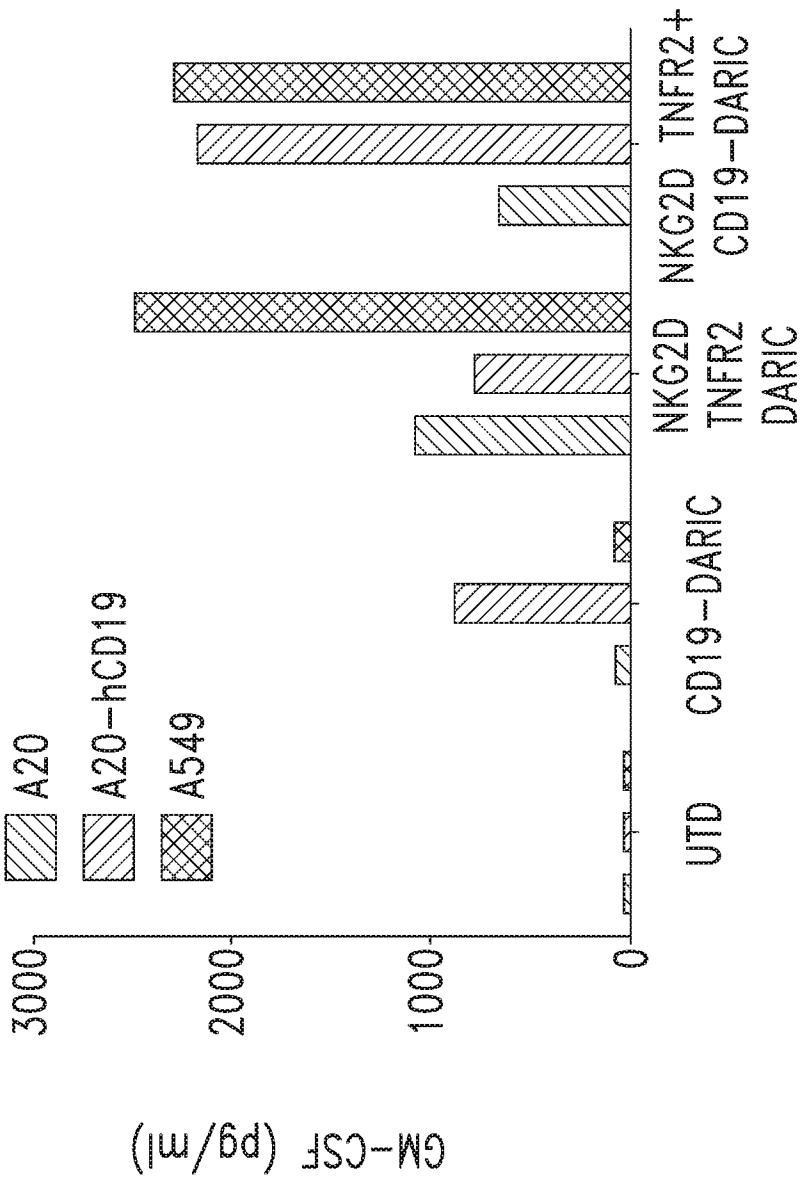

GM-CSF (pg/ml)
3000
2000
1000
0
A20
A20-hCD19
A549
UTD
CD19-DARIC
NKG2D TNFR2 DARIC
NKG2D TNFR2+ CD19-DARIC

FIG. 6D

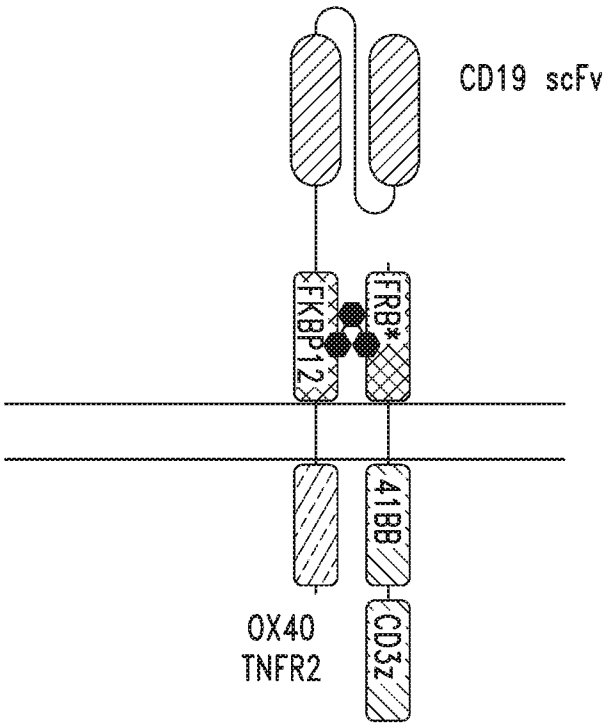


FIG. 7A

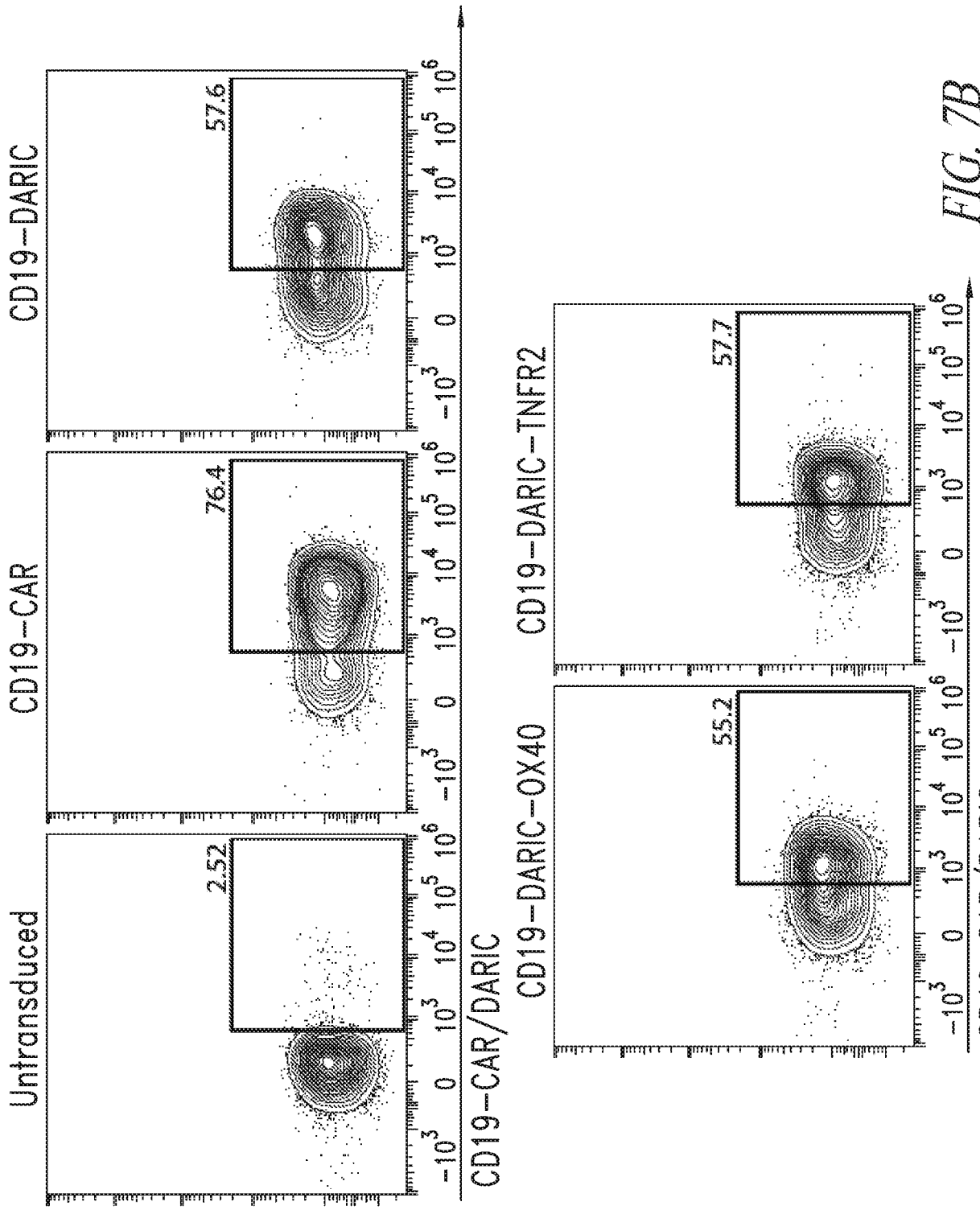

Untransduced
CD19–CAR
CD19–DARIC
CD19–DARIC–OX40
CD19–DARIC–TNFR2
2.52
76.4
57.6
55.2
57.7
CD19–CAR/DARIC
CD19–CAR/DARIC

FIG. 7B

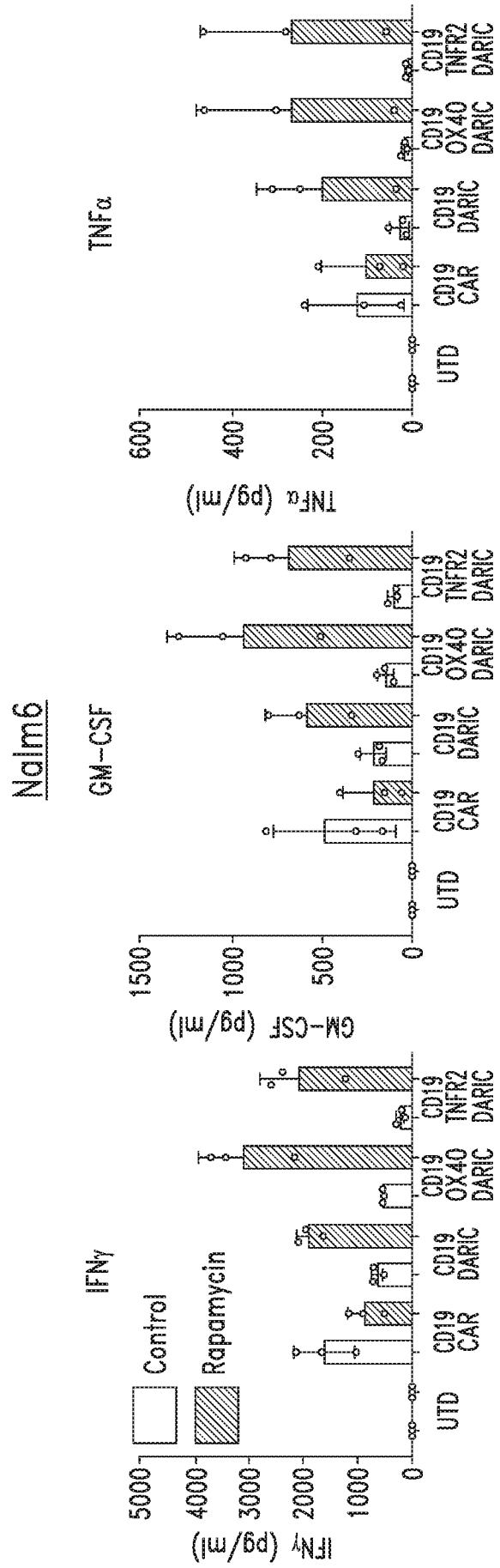

Nalm6
IFNγ
GM-CSF
TNFα
Control
Rapamycin
IFNγ (pg/ml)
GM-CSF (pg/ml)
TNFα (pg/ml)
0
1000
2000
3000
4000
5000
500
1500
200
400
600
UTD
CD19 CAR
CD19 DARIC
CD19 OX40 DARIC
CD19 TNFR2 DARIC

FIG. 7C

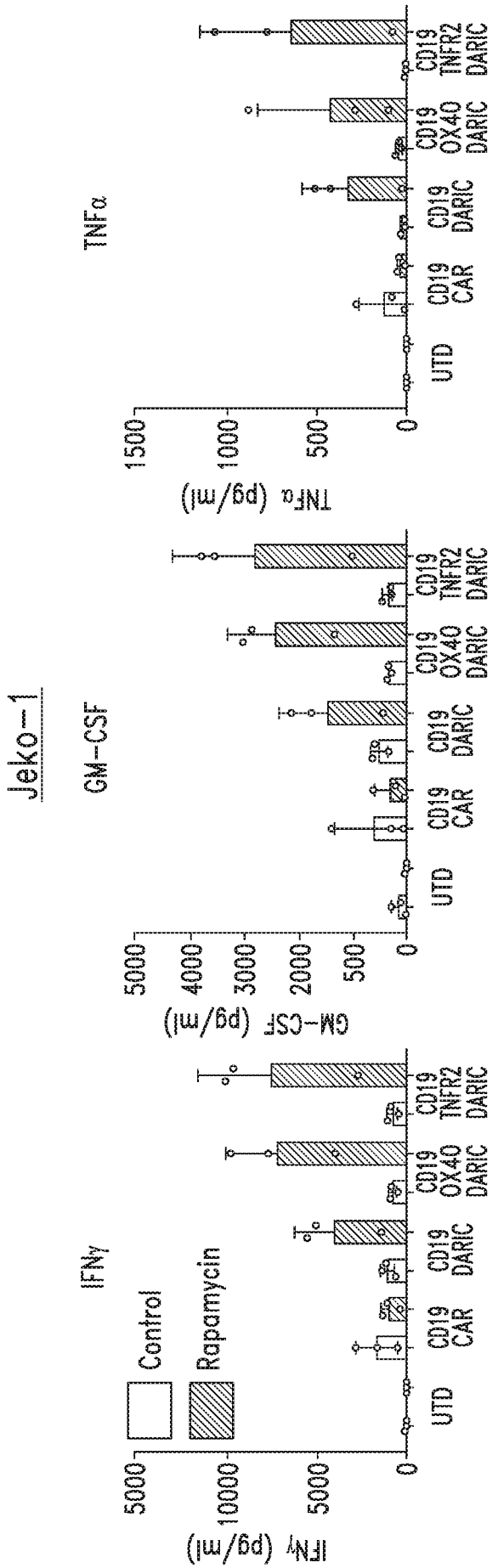

Jeko-1
IFNγ
GM-CSF
TNFα
IFNγ (pg/ml)
GM-CSF (pg/ml)
TNFα (pg/ml)
Control
Rapamycin
UTD
CD19 CAR
CD19 DARIC
CD19 OX40 DARIC
CD19 TNFR2 DARIC

FIG. 7D

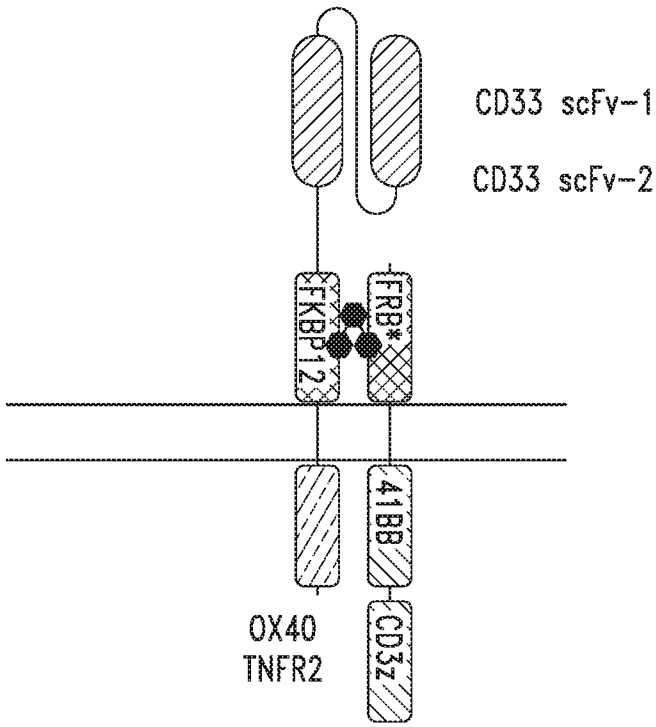


FIG. 8A

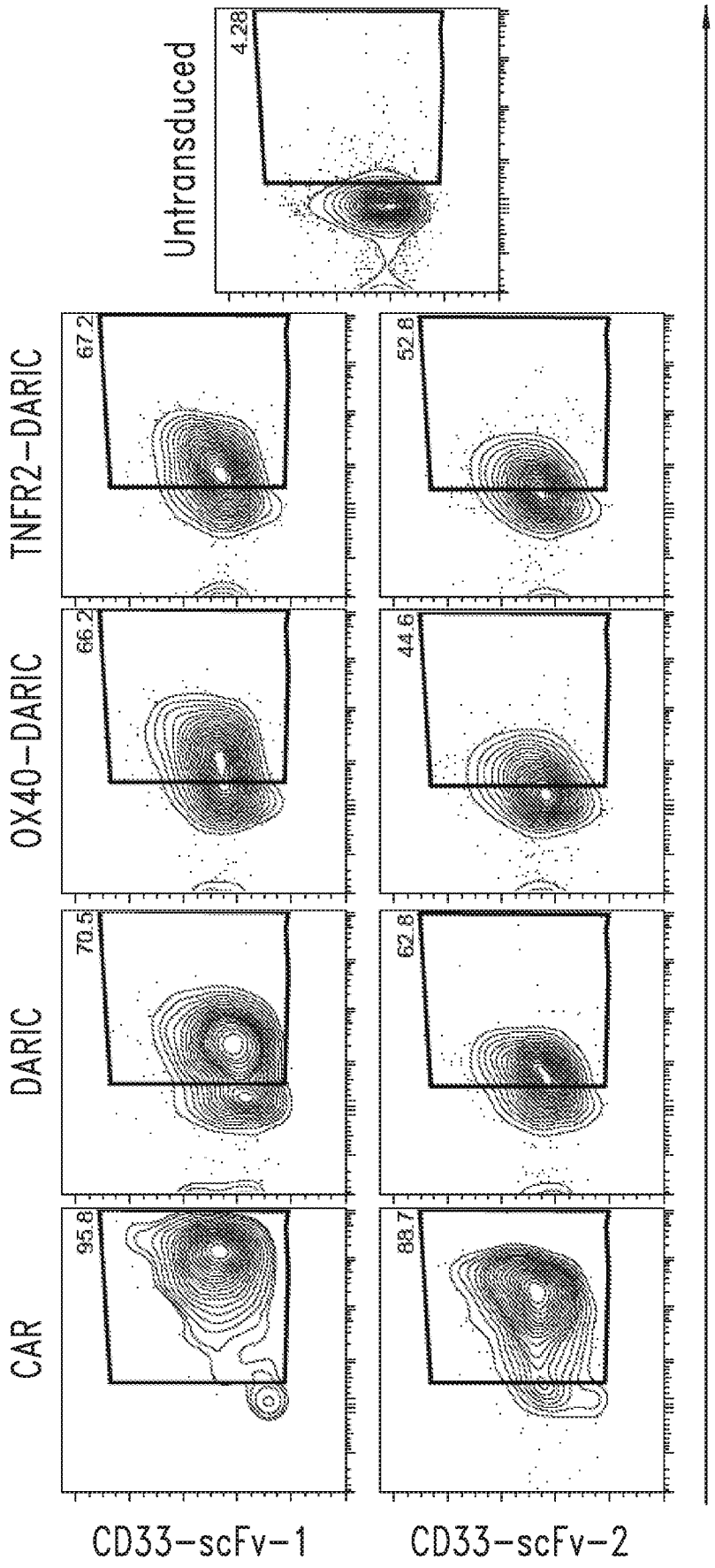
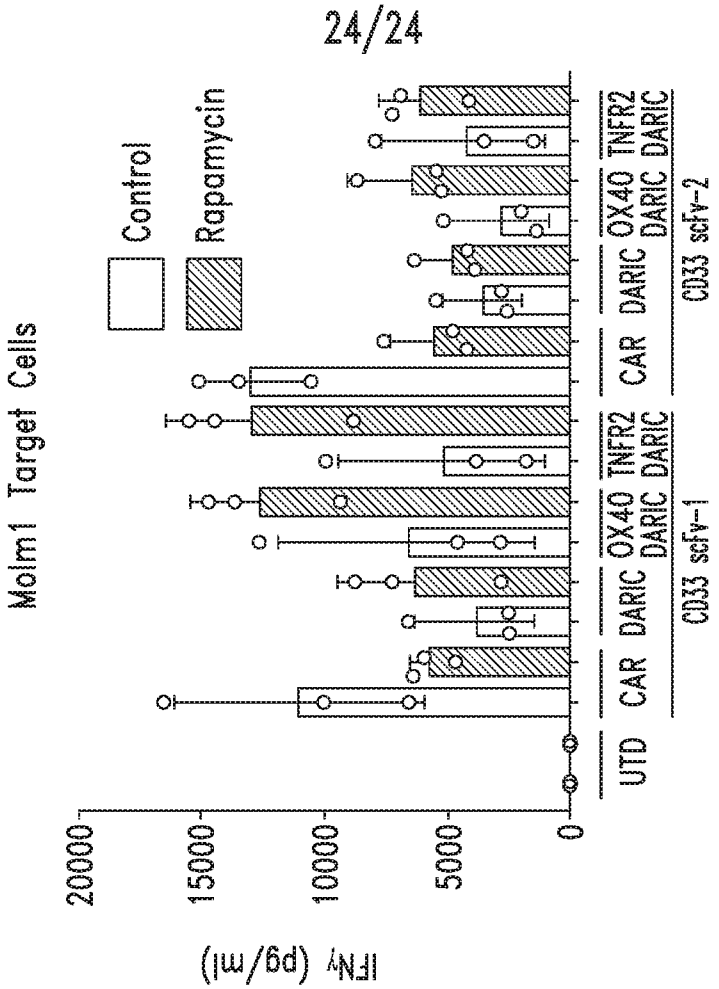

Untransduced
4.28
CAR
DARIC
OX40-DARIC
TNFR2-DARIC
95.8
70.5
66.2
67.2
88.7
62.8
44.6
52.8
CD33-scFv-1
CD33-scFv-2
CD33-Fc

FIG. 8B


Molm1 Target Cells
Control
Rapamycin
IFNγ (pg/ml)
20000
15000
10000
5000
0
UTD
CAR
DARIC
OX40 DARIC
TNFR2 DARIC
CD33 scFv-1
CAR
DARIC
OX40 DARIC
TNFR2 DARIC
CD33 scFv-2

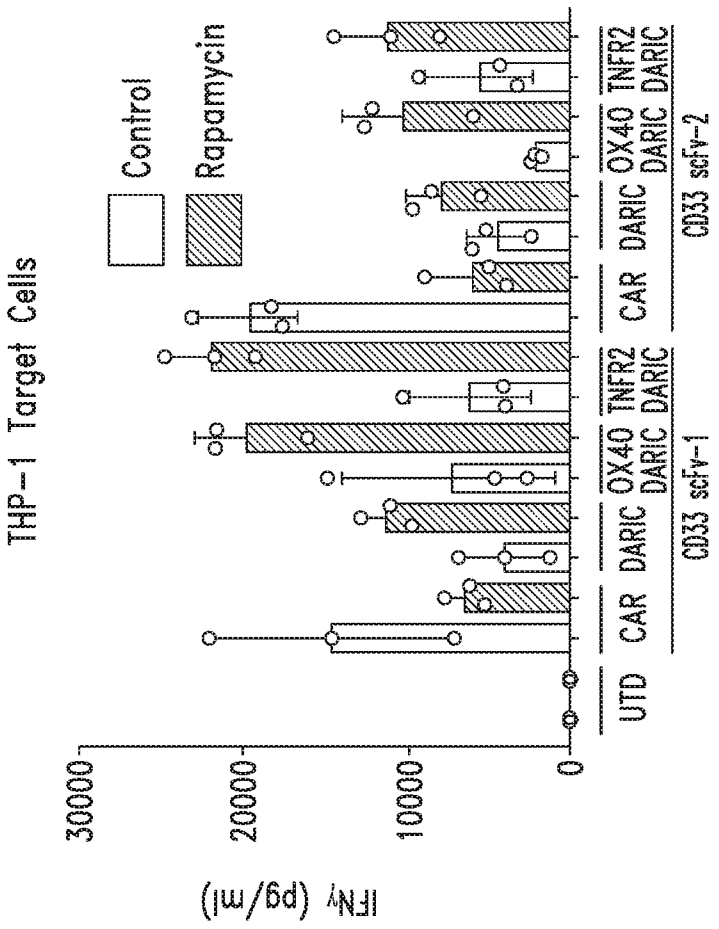

THP-1 Target Cells
Control
Rapamycin
IFNγ (pg/ml)
30000
20000
10000
0
UTD
CAR
DARIC
OX40 DARIC
TNFR2 DARIC
CD33 scFv-1
CAR
DARIC
OX40 DARIC
TNFR2 DARIC
CD33 scFv-2

FIG. 8C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/66223

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C07K 14/705; C07K 14/725; C07K 19/00; C07K 16/28; C07K 16/30; C07K 16/08 (2020.01)

CPC - C07K 14/705; C07K 70503; C07K 2319/70; C07K 16/2876; C07K 19/00; C07K 2317/622; C07K 2319/03

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2017/180993 A1 (BLUEBIRD BIO, INC.) 19 October 2017 (19.10.2017). Especially pg 4 ln 4-13, pg ln 25-29, pg 27 ln 27-32, claim 1.	1-3, 57, 206-208, 235, 290, 294/290
Y	US 2016/0311901 A1 (BLUEBIRD BIO, INC.) 27 October 2016 (27.10.2016). Especially para [0012], [0018], [0028], [0033], [0085], [0154], [0185], sheet 3 fig 1C.	1-3, 57, 206-208, 235, 290, 294/290
Y	WO 2018/127585 A1 (TXCELL) 12 July 2018 (12.07.2018). Especially pg 46 ln 13-23.	57, 235, 290, 294/290

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
9 April 2020	11 MAY 2020
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Lee Young Telephone No. PCT Helpdesk: 571-272-4300

Form PCT/ISA/210 (second sheet) (July 2019)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/66223

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

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

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

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

3. ☒ Claims Nos.: 4-28, 34-56, 62-83, 87-111, 116-137, 143-163, 169-205, 209-233, 237-261, 267-289, 295-309
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:
----Go to Extra Sheet for continuation-----

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

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

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

Box III-4 (cont). an FRB T2098L multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and a TNFR2 costimulatory domain and where the first and second multimerization domains are different

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Nos 1-3, 57, 206-208, 235, 290, 294 (in part), limited to a) a first polypeptide comprising: an FKBP12 multimerization domain polypeptide or variant thereof; a CD8a transmembrane domain; a CD137 costimulatory domain; and a CD3.zeta primary signaling domain; and (b) a second polypeptide comprising: an antibody or antigen binding fragment thereof; [see above]:

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US 19/66223

Box III: Observations where Unity of Invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-3, 29-33, 57-61, 206-208, 234-236, 262-266, 290-294, drawn to a non-natural cell comprising a polypeptide complex comprising a first polypeptide comprising a first multimerization domain, a second polypeptide comprising a second multimerization domain, and a bridging factor that promotes the formation of a polypeptide complex.
The polypeptide complex will be searched to the extent that it is the most detailed species components indicated (claim 57): (a) a first polypeptide comprising: an FKBP12 multimerization domain polypeptide or variant thereof; a CD8a transmembrane domain; a CD137 costimulatory domain; and a CD3.zeta primary signaling domain; and
(b) a second polypeptide comprising: an antibody or antigen binding fragment thereof;
an FRB T2098L multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and a TNFR2 costimulatory domain and where the first and second multimerization domains are different (claim 2). It is believed that claims 1-3, 57, 206-208, 235, 290, 294 (in part) read on this first named invention and thus these claims will be searched without fee to the extent that they encompass a) a first polypeptide comprising: an FKBP12 multimerization domain polypeptide or variant thereof; a CD8a transmembrane domain; a CD137 costimulatory domain; and a CD3.zeta primary signaling domain; and
(b) a second polypeptide comprising: an antibody or antigen binding fragment thereof;
an FRB T2098L multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and a TNFR2 costimulatory domain and where the first and second multimerization domains are different. Additional first polypeptides with specific multimerization domains, transmembrane domains, co-stimulatory domains, and stimulatory domains with second polypeptides with specific multimerization domains, transmembrane domains and costimulatory domains will be searched upon payment of additional fees. Applicant must specify the claims that encompass any additional first and/or second polypeptide components. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be: (claim 58) (a) a first polypeptide comprising: an FKBP12 multimerization domain polypeptide or variant thereof; a CD8a transmembrane domain; a CD137 costimulatory domain; and a CD3~ primary signaling domain; and
(b) a second polypeptide comprising: an antibody or antigen binding fragment thereof;
an FRB T2098L multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and an OX40 costimulatory domain; where the first and second multimerization domains are different (claim 2)(claims 1-3, 58, 206-208, 234, 291, 294 (in part)).

Group II+: 84-86, 112-115, 138-142, 164-168, drawn to a fusion polypeptide comprising a first polypeptide comprising a first multimerization domain, a polypeptide cleavage signal, and a second polypeptide comprising a second multimerization domain.
Group II+ will be searched upon payment of additional fee(s). The fusion polypeptide composition may be searched, for example, to the extent that its' species are defined by (a) a first polypeptide comprising: an FKBP multimerization domain polypeptide or variant thereof; a first transmembrane domain; a costimulatory domain; and/or a primary signaling domain;
(b) a polypeptide cleavage signal; and
(c) a second polypeptide comprising: an antibody or antigen binding fragment thereof;
an FRB multimerization domain polypeptide or variant thereof; a second transmembrane domain; and an OX40 costimulatory domain (claim 138), where the multimerization domains are different (claim 85) for an additional fee and election as such. It is believed that claims 84-86, 112, 115 (in part), 138, 142 (in part) read on this exemplary invention. Additional fusion polypeptides will be searched upon the payment of additional fees. Applicants must indicate, if applicable, which claims read on this named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the '+' group(s) will result in only the first named invention to be searched/examined. An exemplary election would be: a) a first polypeptide comprising: an FRB T2098L multimerization domain polypeptide or variant thereof; a CD8a transmembrane domain; a CD137 costimulatory domain; and a CD3.zeta primary signaling domain;
(b) a polypeptide cleavage signal; and (c) a second polypeptide comprising: an antibody or antigen binding fragment thereof; an FKBP12 multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and an OX40 costimulatory domain (claim 167) where the multimerization domains are different (claim 85) (claims 84-86, 112, 115 (in part), 167, 168 (in part)).

The inventions listed as Groups I+ and II+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I+ have the special technical feature of a bridging factor that promotes the formation of a polypeptide complex, not required by Group II+.

Group II+ inventions have the special technical feature of a fusion protein comprising a polypeptide cleavage signal, not required by Group I+.

-----continued on next sheet------

----continued from previous sheet------

Among the inventions listed as Groups I+ are the first polypeptide different multimerization domains, transmembrane domains, costimulatory domains, second polypeptide multimerization domains, transmembrane domains, costimulatory domains recited therein. Each invention requires a specific first polypeptide multimerization domain, transmembrane domain costimulatory domain, second polypeptide multimerization domain, transmembrane domain, costimulatory domain not required by any other inventions.

Among the inventions listed as Groups II+ are the first polypeptide different multimerization domains, transmembrane domains, costimulatory domains, second polypeptide multimerization domains, transmembrane domains, costimulatory domains recited therein. Each invention requires a specific first polypeptide multimerization domain, transmembrane domain costimulatory domain, second polypeptide multimerization domain, transmembrane domain, costimulatory domain not required by any other inventions.

Common Technical Feature:

Group I+ and Group II+ inventions share the common technical features of:

1. a first polypeptide comprising: a first multimerization domain polypeptide or variant thereof; a first transmembrane domain; a first costimulatory domain; and/or a primary signaling domain.

2. a second polypeptide comprising: an extracellular binding domain; a second multimerization domain polypeptide or variant thereof; a second transmembrane domain; and a second costimulatory domain.

3. multimerization domain pairs FKBP and FKBP-rapamycin binding (FRB), FKBP12 and FRB T2098L

4. transmembrane domains CD8a, CD4

5. costimulatory domains CD137, OX40 and TNFR2

6. primary signaling domain CD3
Group I+ inventions share the common technical feature of:

7. a bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides.
Group II+ inventions share the common technical feature of:

8. a polypeptide cleavage signal

However, said common technical features do not represent a contribution over the prior art, and is disclosed by of WO 2017/180993 A1 to Bluebird Bio, Inc. (hereinafter "Bluebird '993"), in view of US 2016/0311901 A1 to Bluebird Bio, Inc (hereinafter "Bluebird '901"), in view of WO 2018/127585 A1 to TxCell (hereinafter "TxCell")

As to common technical feature #1, Bluebird '993 discloses a first polypeptide comprising: a first multimerization domain polypeptide or variant thereof; a first transmembrane domain; a first costimulatory domain; and/or a primary signaling domain (claim 1; "A salvage chimeric antigen receptor (CAR) comprising: a) an extracellular antigen binding domain; b) a multimerization domain; b) a transmembrane domain; c) one or more intracellular co-stimulatory signaling domains; and/or d) a primary signaling domain").

As to common technical feature #2, Bluebird '993 discloses a polypeptide comprising: an extracellular binding domain; a second multimerization domain polypeptide or variant thereof; a second transmembrane domain; and a second costimulatory domain. (claim 1; "A salvage chimeric antigen receptor (CAR) comprising: a) an extracellular antigen binding domain; b) a multimerization domain; b) a transmembrane domain; c) one or more intracellular co-stimulatory signaling domains). In addition, Bluebird '901 discloses there is a first fusion polypeptide and a second fusion polypeptide which interact by means of multimerization domains (para [0012]; "In various embodiments, a non-natural cell is provided, comprising: a first nucleic acid molecule encoding a first fusion protein comprising a first multimerization domain, a hydrophobic domain, and an actuator domain, wherein the first multimerization domain localizes extracellularly when the first fusion protein is expressed; and a second nucleic acid molecule encoding a second fusion protein comprising a binding domain and a second multimerization domain, wherein the second fusion protein localizes extracellularly when expressed; wherein a first bridging factor promotes the formation of a polypeptide complex on the non natural cell surface with the bridging factor associated with and disposed between the multimerization domains of the first and second fusion proteins").

----continued on next sheet------

----continued from previous sheet-----

As to common technical feature #3, Bluebird '993 discloses a multimerization domain pairs FKBP and FKBP-rapamycin binding (FRB) (pg 4 ln 1-2; "the multimerization domain is selected from the group consisting of: an FKBP polypeptide an FRB polypeptide"), FKBP12 and FRB T2098L (pg 4 ln 4-5; "In additional embodiments, the multimerization domain is selected from the group consisting of: an FKBP 12 polypeptide and an FRB T2098L polypeptide").

As to common technical feature #4, Bluebird '993 discloses transmembrane domains CD8a, CD4 (pg 4 ln 10-11; "the transmembrane domain is isolated from a polypeptide selected from the group consisting of: CD8a; CD4").

As to common technical feature #5, Bluebird '993 discloses costimulatory domains CD137, OX40 (pg 4 ln 16-20; "a co-stimulatory molecule selected from the group?.. CD134 (OX40), CD137 (4-1BB)").

As to common technical feature #5, TxCell discloses co-stimulatory domain TNFR2 (pg 46 ln 13-23; "Other examples of intracellular domains of a T cell costimulatory molecule include, but
are not limited to, the signaling domains of proteins selected in the group?.TNFR2").

As to common technical feature #6, Bluebird '993 discloses a primary signaling domain CD3 (pg 4 ln 29; "In particular embodiments, the primary signaling domain isolated from a CD3.zeta").

As to common technical feature #7, Bluebird '901 discloses bridging factor associated with and disposed between the multimerization domains of the first and second polypeptides (para [0012]; "a first bridging factor promotes the formation of a polypeptide complex on the non natural cell surface with the bridging factor associated with and disposed between the multimerization domains of the first and second fusion proteins"; para [0018]; " In one embodiment, the bridging factor is sirolimus, everolimus, novolimus, pimecrolimus, ridaforolimus, tacrolimus, temsirolimus, umirolimus, or zotarolimus").

As to common technical feature #8, Bluebird '901 discloses a polypeptide cleavage signal (para [0198-0199]; "a single polycistronic nucleic acid molecule that encodes a first fusion protein and second fusion protein, or single nucleic acid molecule that encodes a first fusion protein, a self-cleaving amino acid sequence and a second fusion protein.[0199] Suitable protease cleavages sites and self-cleaving peptides are known to the skilled person").

As the common technical features were known in the art at the time of the invention, they cannot be considered common special technical features that would otherwise unify the groups. The inventions lack unity with one another.

Therefore, Groups I+ and II+ lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Note concerning claim 140: Claim 140 is indicated as a fusion polypeptide comprising a first polypeptide and a second polypeptide, but unlike all other independent claims comprising a fusion polypeptide (claims 84, 112, 113, 138, 139, 141, 164-167), fails to include "(b) a polypeptide cleavage signal". For the purposes of the International Search & Opinion, this will be considered an erroneous omission, and claim 140 will therefore be interpreted to include the limitation "(b) a polypeptide cleavage signal".

Item 1(iii) (cont): Will establish the international search report on those parts of the international application which relate to the invention fi rst mentioned in claims Nos 1-3, 57, 206-208, 235, 290, 294 (in part), limited to a) a first polypeptide comprising: an FKBP12 multimerization domain polypeptide or variant thereof; a CD8a transmembrane domain; a CD137 costimulatory domain; and a CD3.zeta primary signaling domain; and
(b) a second polypeptide comprising: an antibody or antigen binding fragment thereof;
an FRB T2098L multimerization domain polypeptide or variant thereof; a CD4 transmembrane domain; and a TNFR2 costimulatory domain and where the first and second multimerization domains are different.

Item 4 (cont.) Claims 4-28, 34-56, 62-83, 87-111, 116-137, 143-163, 169-205, 209-233, 237-261, 267-289, 295-309 are multiple dependent claims and are not drafted according to the second and third sentences of PCT Rule 6.4(a).