

**NOVEL PLATFORMS FOR CO-STIMULATION, NOVEL CAR DESIGNS AND
OTHER ENHANCEMENTS FOR ADOPTIVE CELLULAR THERAPY**

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The application claims priority under 35 U.S.C. §119 to U.S. Provisional Application Serial No. 62/564,249, filed September 27, 2017, the disclosures of which are incorporated herein by reference.

TECHNICAL FIELD

[0002] Provided herein are novel costimulatory module and novel chimeric antigen receptors for adoptive cellular therapies of cancer, infection, allergic, degenerative and immune disorders.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0003] Accompanying this filing is a Sequence Listing entitled "Sequence_ST25.txt", created on September 27, 2018 and having 60,347,260 bytes of data, machine formatted on IBM-PC, MS-Windows operating system. The sequence listing is hereby incorporated herein by reference in its entirety for all purposes.

BACKGROUND

[0004] Adoptive T-cell immunotherapy has risen to the forefront of treatment approaches for cancer. T cells can be engineered to express the genes of chimeric antigen receptors (CARs) that recognize tumor associated antigens. CARs are engineered immune-receptors, which can redirect T cells to selectively kill tumor cells. The general premise for their use in cancer immunotherapy is to rapidly generate tumor-targeted T cells, bypassing the barriers and incremental kinetics of active immunization and thereby act as 'living drugs'. Unlike the physiologic T-cell receptor (TCR), which engages HLA-peptide complexes, CARs engage molecules that do not require peptide processing or HLA expression to be recognized. CARs therefore recognize antigen on any HLA background, in contrast to TCRs, which need to be matched to the haplotype of the patient. Furthermore, CARs can target tumor cells that have down-regulated HLA expression or proteasomal antigen processing, two mechanisms that contribute to tumor escape from TCR-mediated immunity. Another feature of the broad applicability of CARs is their ability to bind not only to proteins but also to carbohydrate and glycolipid structures, again expanding the range of potential targets.

SUMMARY

[0005] The disclosure provides an immune cell or immune cell population thereof expressing (i) at least one non-naturally occurring immune receptor and (ii) at least one non-

naturally occurring agent that selectively activates the NF- κ B signaling pathway. In one embodiment, the at least one non-naturally occurring immune receptor comprises at least one antigen-binding domain and at least one transmembrane domain. In another or a further embodiment, the at least one non-naturally occurring immune receptor is capable of recruiting at least one TCR associated signaling module. In another or a further embodiment, the at least one non-naturally occurring immune receptor is a chimeric antigen receptor (CAR) or a recombinant TCR. In another or a further embodiment, the at least one antigen-binding domain of the at least one non-naturally occurring immune receptor binds to an antigen selected from a group consisting of CD5; CD19; CD123; CD22; CD30; CD171; CS1 (also referred to as CD2 subset 1, CRACC, MPL, SLAMF7, CD319, and 19A24); C-type lectin-like molecule-1 (CLL-1 or CLECL1); CD33; epidermal growth factor receptor variant III (EGFRviii); ganglioside G2 (GD2); ganglioside GD3 (aNeu5Ac(2-8)aNeu5Ac(2-3)bDGalp(1-4)bDGlc(1-1)Cer); TNF receptor family member B cell maturation (BCMA); Tn antigen ((Tn Ag) or (GalNAc α -Ser/Thr)); prostate-specific membrane antigen (PSMA); Receptor tyrosine kinase-like orphan receptor 1 (ROR1); Fms Like Tyrosine Kinase 3 (FLT3); Tumor-associated glycoprotein 72 (TAG72); CD38; CD44v6; a glycosylated CD43 epitope expressed on acute leukemia or lymphoma but not on hematopoietic progenitors, a glycosylated CD43 epitope expressed on non-hematopoietic cancers, Carcinoembryonic antigen (CEA); Epithelial cell adhesion molecule (EPCAM); B7H3 (CD276); KIT (CD117); Interleukin-13 receptor subunit alpha-2 (IL-13Ra2 or CD213A2); Mesothelin; Interleukin 11 receptor alpha (IL-11Ra); prostate stem cell antigen (PSCA); Protease Serine 21 (Testisin or PRSS21); vascular endothelial growth factor receptor 2 (VEGFR2); Lewis(Y) antigen; CD24; Platelet-derived growth factor receptor beta (PDGFR-beta); Stage-specific embryonic antigen-4 (SSEA-4); CD20; Folate receptor alpha (FRa or FR1); Folate receptor beta (FRb); Receptor tyrosine-protein kinase ERBB2 (Her2/neu); Mucin 1, cell surface associated (MUC1); epidermal growth factor receptor (EGFR); neural cell adhesion molecule (NCAM); Prostase; prostatic acid phosphatase (PAP); elongation factor 2 mutated (ELF2M); Ephrin B2; fibroblast activation protein alpha (FAP); insulin-like growth factor 1 receptor (IGF-I receptor), carbonic anhydrase IX (CAIX); Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2); glycoprotein 100 (gp100); oncogene fusion protein consisting of breakpoint cluster region (BCR) and Abelson murine leukemia viral oncogene homolog 1 (Abl) (bcr-abl); tyrosinase; ephrin type-A receptor 2 (EphA2); sialyl Lewis adhesion molecule (sLe); ganglioside GM3 (aNeu5Ac(2-3)bDCIalp(1-4)bDGlc(1-1)Cer); transglutaminase 5 (TGS5); high molecular weight-melanoma associated antigen (HMWMAA); o-acetyl-GD2

ganglioside (OAcGD2); tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related (TEM7R); claudin 6 (CLDN6); thyroid stimulating hormone receptor (TSHR); G protein coupled receptor class C group 5, member D (GPRC5D); chromosome X open reading frame 61 (CXORF61); CD97; CD179a; anaplastic lymphoma kinase (ALK); Polysialic acid; placenta-specific 1 (PLAC1); hexasaccharide portion of globoH glycosphingolipid (GloboH); mammary gland differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3 (ADRB3); pannexin 3 (PANX3); G protein-coupled receptor 20 (GPR20); lymphocyte antigen 6 complex, locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame Protein (TARP); Wilms tumor protein (WT1); Cancer/testis antigen 1 (NY-ESO-1); Cancer/testis antigen 2 (LAGE-1a); Melanoma-associated antigen 1 (MAGE-A1); ETS translocation-variant gene 6, located on chromosome 12p (ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, Member 1A (XAGE1); angiopoietin-binding cell surface receptor 2 (Tie 2); melanoma cancer testis antigen-1 (MAD-CT-1); melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; tumor protein p53 (p53); p53 mutant; protein; survivin; telomerase; prostate carcinoma tumor antigen-1 (PCT A-1 or Galectin 8), melanoma antigen recognized by T cells 1 (MelanA or MART1); Rat sarcoma (Ras) mutant; human Telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoints; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease, serine 2 (TMPRSS2) ETS fusion gene); N-Acetyl glucosaminyl-transferase V (NA17); paired box protein Pax-3 (PAX3); Androgen receptor; Cyclin B1; v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Tyrosinase-related protein 2 (TRP-2); Cytochrome P450 1B 1 (CYP1B 1); CCCTC-Binding Factor (Zinc Finger Protein)-Like (BORIS or Brother of the Regulator of Imprinted Sites), Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3); Paired box protein Pax-5 (PAX5); proacrosin binding protein sp32 (OY-TES1); lymphocyte-specific protein tyrosine kinase (LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint 2 (SSX2); Receptor for Advanced Glycation Endproducts (RAGE-1); renal ubiquitous 1 (RUI); renal ubiquitous 2 (RU2); legumain; human papilloma virus E6 (HPV E6); human papilloma virus E7 (HPV E7); intestinal carboxyl esterase; heat shock protein 70-2 mutated (mut hsp70-2); CD79a; CD79b; CD72; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR or CD89); Leukocyte immunoglobulin-like receptor subfamily A member 2 (LILRA2); CD300 molecule-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone

marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2); lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); and immunoglobulin lambda-like polypeptide 1 (IGLL1), MPL, Biotin, c-MYC epitope Tag, CD34, LAMP1 TROP2, GFRalpha4, CDH17, CDH6, NYBR1, CDH19, CD200R, Slea (CA19.9; Sialyl Lewis Antigen); Fucosyl-GM1, PTK7, gpNMB, CDH1-CD324, DLL3, CD276/B7H3, IL11Ra, IL13Ra2, CD179b-IGL11, TCRgamma-delta, NKG2D, CD32 (FCGR2A), Tn ag, Tim1-/HVCR1, CSF2RA (GM-CSFR-alpha), TGFbetaR2, Lews Ag, TCR-beta1 chain, TCR-beta2 chain, TCR-gamma chain, TCR-delta chain, FITC, Leutenizing hormone receptor (LHR), Follicle stimulating hormone receptor (FSHR), Gonadotropin Hormone receptor (CGHR or GR), CCR4, GD3, SLAMF6, SLAMF4, HIV1 envelope glycoprotein, HTLV1-Tax, CMV pp65, EBV-EBNA3c, KSHV K8.1, KSHV-gH, influenza A hemagglutinin (HA), GAD, PDL1, Guanylyl cyclase C (GCC), auto antibody to desmoglein 3 (Dsg3), auto antibody to desmoglein 1 (Dsg1), HLA, HLA-A, HLA-A2, HLA-B, HLA-C, HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, HLA-DR, HLA-G, IgE, CD99, Ras G12V, Tissue Factor 1 (TF1), AFP, GPRC5D, Claudin18.2 (CLD18A2 or CLDN18A.2), P-glycoprotein, STEAP1, Liv1, Nectin-4, Cripto, gpA33, BST1/CD157, low conductance chloride channel, and an antigen recognized by TNT antibody. In another or a further embodiment, the at least one non-naturally occurring agent capable of selectively activating NF- κ B pathway is selected from the group consisting of vFLIP K13, K13-opt, a NEMO mutant, a NEMO-fusion protein, IKK1-S176E-S180E, IKK2-S177E-S181E, RIP, IKK α , IKK β , Tcl-1, MyD88-L265, any NF- κ B activating protein or protein fragment, any inhibitor of an inhibitor of NF- κ B pathway, any gene editing system capable of selectively activating NF- κ B, any homolog or variant thereof and any combination thereof. In another or a further embodiment, the at least one non-naturally occurring agent capable of selectively activating NF- κ B pathway is of non-viral origin. In another or a further embodiment, the at least one non-naturally occurring agent capable of selectively activating NF- κ B pathway is a gene editing system. In another or a further embodiment, the at least one non-naturally occurring agent capable of selectively activating NF- κ B pathway induces oligomerization of NEMO/IKK γ . In another or a further embodiment, the at least one non-naturally occurring agent capable of selectively activating NF- κ B pathway induces activation of the IKK complex. In another or a further embodiment, at least one the non-naturally occurring agent capable of selectively activating NF- κ B pathway does not activate the AKT pathway. In another or a further embodiment, the at least one non-naturally occurring agent capable of selectively activating NF- κ B pathway is expressed in a constitutive or inducible

manner. In another or a further embodiment, the at least one non-naturally occurring agent capable of selectively activating NF- κ B pathway is expressed transiently. In another or a further embodiment, the at least one non-naturally occurring agent capable of selectively activating NF- κ B pathway is expressed stably. In another or a further embodiment, the activity of the at least one non-naturally occurring agent capable of selectively activating NF- κ B pathway is controlled post-translationally through contacting the cell with a compound. In another or a further embodiment, the at least one non-naturally occurring agent capable of selectively activating NF- κ B pathway is expressed as a fusion construct with one or more copies of a switch domain. In another or a further embodiment, the activity of the at least one non-naturally occurring agent capable of selectively activating NF- κ B pathway is controlled at the post-translational level by administration of therapeutically effective amount of a compound that induces dimerization of the switch domain. In another or a further embodiment, the switch domain comprises one or more copies of a FKBP12 domain. In another or a further embodiment, the compound is AP20187 or Rimiducid or a homolog thereof. In another or a further embodiment, the immune cell is a T-lymphocyte (T-cell), a CAR-T cell, a TCR-expressing T cell, a tumor infiltrating lymphocyte (TIL), a tissue resident lymphocyte, a stem cell, an induced pluripotent stem cell or a Natural Killer (NK) cell. In another or a further embodiment, the immune cell has been engineered to lack a functional native T-Cell Receptor (TCR) signaling complex and/or β 2 microglobulin. In another or a further embodiment, the at least one non-naturally occurring immune receptor and/or the at least one agent capable of selectively activating NF- κ B signaling pathway are cloned into an endogenous TCR gene such that the expression of the at least one non-naturally occurring immune receptor and/or the at least one agent capable of selectively activating NF- κ B signaling pathway are under control of the endogenous regulatory elements/promoter for the TCR gene. The disclosure also provides for the use of an immune cell or immune cell population as described herein that is used for the prevention and treatment of a disease selected from the group of a cancer, infectious disease, immune disease, and allergic disease. In another or a further embodiment, at least one polynucleotide encodes the at least one non-naturally occurring immune receptor and the at least one non-naturally occurring agent capable of selectively activating NF- κ B signaling pathway are expressed from a single promoter. In another or a further embodiment, at least one polynucleotide encoding the at least one non-naturally occurring immune receptor and the at least one non-naturally occurring agent capable of selectively activating NF- κ B signaling pathway are expressed using two or more separate promoters. In another or a further embodiment, the at least one

polynucleotide comprises a first nucleic acid coding sequence encoding the at least one non-naturally occurring immune receptor separated from a second nucleic acid sequence encoding the non-naturally occurring agent capable of selectively activating NF- κ B such that upon expression of the first and second nucleic acid coding sequences that non-naturally occurring immune receptor and non-naturally occurring agent capable of selectively activating NF- κ B are not physically or chemically linked. In another or a further embodiment, the at least one non-naturally occurring immune receptor and/or the at least one non-naturally occurring agent capable of selectively activating NF- κ B coding polynucleotide(s) are cloned into an endogenous TCR gene such that the at least one non-naturally occurring immune receptor and/or at least one non-naturally occurring agent capable of selectively activating NF- κ B are under control of the endogenous regulatory elements/promoter for the TCR gene. In another or a further embodiment, one or more constant chains of the TCR genes are functionally re-expressed.

[0006] The disclosure also provides at least one recombinant polynucleotide encoding at least one non-naturally occurring immune receptor, the at least one recombinant polynucleotide comprising (a) a first nucleic acid domain encoding a partial or entire transmembrane and/or cytoplasmic domain and optionally the extracellular domain of an endogenous protein, wherein the endogenous protein is expressed on the surface of lymphocytes and triggers the activation and/or proliferation of the lymphocyte; (b) optionally a polynucleotide a linker; (c) a second nucleic acid domain operably linked to the first nucleic acid domain, wherein the second nucleic acid domain encodes one or more non-natural TCR antigen binding domain(s); (d) an optional third nucleic acid domain encoding a costimulatory domain; and (e) an optional additional nucleic acid domain encoding an accessory module.

[0007] The disclosure also provides at least one recombinant polynucleotide comprising a first nucleic acid encoding a non-naturally occurring immune receptor; and a second nucleic acid encoding an accessory module comprising a selective NF- κ B activator. In one embodiment, the first nucleic acid and the second nucleic acid are separated by an oligonucleotide linker encoding a cleavable peptide linker. In another embodiment, the at least one comprises two recombinant polynucleotide such that the first nucleic acid and second nucleic acid are expressed from separate vectors. In another or a further embodiment, the selective NF- κ B activator is a non-naturally occurring selective NF- κ B activator. In another or a further embodiment, the non-naturally occurring immune receptor is selected from the group consisting of a CAR, an Ab-TCR, a TFP, a cTCR, a SIR and a recombinant

TCR. In another or a further embodiment, the non-naturally occurring immune receptor comprises an (i) an extracellular antigen specific domain, (ii) a transmembrane domain, and (iii) an optional intracellular signaling domain comprising an immunoreceptor tyrosine-based activation motif (ITAM), wherein (iii) is located at the C-terminus of the non-naturally occurring immune receptor. In another or a further embodiment, upon expression of the first and second nucleic acids sequences the non-naturally occurring immune receptor and selective NF- κ B activator polypeptide are not physically or chemically linked. In another or a further embodiment, the extracellular antigen-specific domain binds to any one or more of CD5; CD19; CD123; CD22; CD30; CD171; CS1 (also referred to as CD2 subset 1, CRACC, MPL, SLAMF7, CD319, and 19A24); C-type lectin-like molecule-1 (CLL-1 or CLECL1); CD33; epidermal growth factor receptor variant III (EGFRviii); ganglioside G2 (GD2); ganglioside GD3 (aNeu5Ac(2-8)aNeu5Ac(2-3)bDGalp(1-4)bDGlc(1-1)Cer); TNF receptor family member B cell maturation (BCMA); Tn antigen ((Tn Ag) or (GalNAc α -Ser/Thr)); prostate-specific membrane antigen (PSMA); Receptor tyrosine kinase-like orphan receptor 1 (ROR1); Fms Like Tyrosine Kinase 3 (FLT3); Tumor-associated glycoprotein 72 (TAG72); CD38; CD44v6; a glycosylated CD43 epitope expressed on acute leukemia or lymphoma but not on hematopoietic progenitors, a glycosylated CD43 epitope expressed on non-hematopoietic cancers, Carcinoembryonic antigen (CEA); Epithelial cell adhesion molecule (EPCAM); B7H3 (CD276); KIT (CD117); Interleukin-13 receptor subunit alpha-2 (IL-13Ra2 or CD213A2); Mesothelin; Interleukin 11 receptor alpha (IL-11Ra); prostate stem cell antigen (PSCA); Protease Serine 21 (Testisin or PRSS21); vascular endothelial growth factor receptor 2 (VEGFR2); Lewis(Y) antigen; CD24; Platelet-derived growth factor receptor beta (PDGFR-beta); Stage-specific embryonic antigen-4 (SSEA-4); CD20; Folate receptor alpha (FRA or FR1); Folate receptor beta (FRb); Receptor tyrosine-protein kinase ERBB2 (Her2/neu); Mucin 1, cell surface associated (MUC1); AFP/MHC complex; epidermal growth factor receptor (EGFR); neural cell adhesion molecule (NCAM); Prostase; prostatic acid phosphatase (PAP); elongation factor 2 mutated (ELF2M); Ephrin B2; fibroblast activation protein alpha (FAP); insulin-like growth factor 1 receptor (IGF-I receptor), carbonic anhydrase IX (CAIX); Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2); glycoprotein 100 (gp100); oncogene fusion protein consisting of breakpoint cluster region (BCR) and Abelson murine leukemia viral oncogene homolog 1 (Abl) (bcr-abl); tyrosinase; ephrin type-A receptor 2 (EphA2); sialyl Lewis adhesion molecule (sLe); ganglioside GM3 (aNeu5Ac(2-3)bDCIalp(1- 4)bDGlc(1-1)Cer); transglutaminase 5 (TGS5); high molecular weight-melanoma associated antigen (HMWMAA); o-acetyl-GD2

ganglioside (OAcGD2); tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related (TEM7R); claudin 6 (CLDN6); thyroid stimulating hormone receptor (TSHR); G protein coupled receptor class C group 5, member D (GPRC5D); chromosome X open reading frame 61 (CXORF61); CD97; CD179a; anaplastic lymphoma kinase (ALK); Polysialic acid; placenta-specific 1 (PLAC1); hexasaccharide portion of globoH glycosphingolipid (GloboH); mammary gland differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3 (ADRB3); pannexin 3 (PANX3); G protein-coupled receptor 20 (GPR20); lymphocyte antigen 6 complex, locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame Protein (TARP); Wilms tumor protein (WT1); WT1/MHC I complex; Cancer/testis antigen 1 (NY-ESO-1); NY-ESO-1/MHC I complex, Cancer/testis antigen 2 (LAGE-1a); Melanoma-associated antigen 1 (MAGE-A1); ETS translocation-variant gene 6, located on chromosome 12p (ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, Member 1A (XAGE1); angiopoietin-binding cell surface receptor 2 (Tie 2); melanoma cancer testis antigen-1 (MAD-CT-1); melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; tumor protein p53 (p53); p53 mutant; prostatein; survivin; telomerase; prostate carcinoma tumor antigen-1 (PCT A-1 or Galectin 8), melanoma antigen recognized by T cells 1 (MelanA or MART1); Rat sarcoma (Ras) mutant; human Telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoints; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease, serine 2 (TMPRSS2) ETS fusion gene); N-Acetyl glucosaminyl-transferase V (NA17); paired box protein Pax-3 (PAX3); Androgen receptor; Cyclin B1; v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Tyrosinase-related protein 2 (TRP-2); Cytochrome P450 1B 1 (CYP1B 1); CCCTC-Binding Factor (Zinc Finger Protein)-Like (BORIS or Brother of the Regulator of Imprinted Sites), Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3); Paired box protein Pax-5 (PAX5); proacrosin binding protein sp32 (OY-TES1); lymphocyte-specific protein tyrosine kinase (LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint 2 (SSX2); Receptor for Advanced Glycation Endproducts (RAGE-1); renal ubiquitous 1 (RUI); renal ubiquitous 2 (RU2); legumain; human papilloma virus E6 (HPV E6); HPV E6/MHC I complex; human papilloma virus E7 (HPV E7); HPV E7/MHC I complex; AFP/MHC I complex; Ras/MHC I complex; intestinal carboxyl esterase; heat shock protein 70-2 mutated (mut hsp70-2); CD79a; CD79b; CD72; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR or CD89); Leukocyte immunoglobulin-like receptor

subfamily A member 2 (LILRA2); CD300 molecule-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2); lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); and immunoglobulin lambda-like polypeptide 1 (IGLL1), MPL, Biotin, c-MYC epitope Tag, CD34, LAMP1 TROP2, GFRalpha4, CDH17, CDH6, NYBR1, CDH19, CD200R, Slea (CA19.9; Sialyl Lewis Antigen); Fucosyl-GM1, PTK7, gpNMB, CDH1-CD324, DLL3, CD276/B7H3, IL11Ra, IL13Ra2, CD179b-IGL11, TCRgamma-delta, NKG2D, CD32 (FCGR2A), Tn ag, Tim1-/HVCR1, CSF2RA (GM-CSFR-alpha), TGFbetaR2, Lews Ag, TCR-beta1 chain, TCR-beta2 chain, TCR-gamma chain, TCR-delta chain, FITC, Leutenizing hormone receptor (LHR), Follicle stimulating hormone receptor (FSHR), Gonadotropin Hormone receptor (CGHR or GR), CCR4, GD3, SLAMF6, SLAMF4, HIV1 envelope glycoprotein, HTLV1-Tax, CMV pp65, EBV-EBNA3c, KSHV K8.1, KSHV-gH, influenza A hemagglutinin (HA), GAD, PDL1, Guanylyl cyclase C (GCC), auto antibody to desmoglein 3 (Dsg3), auto antibody to desmoglein 1 (Dsg1), HLA, HLA-A, HLA-A2, HLA-B, HLA-C, HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, HLA-DR, HLA-G, IgE, CD99, Ras G12V, Tissue Factor 1 (TF1), AFP, GPRC5D, Claudin18.2 (CLD18A2 or CLDN18A.2), P-glycoprotein, STEAP1, Liv1, Nectin-4, Cripto, gpA33, BST1/CD157, low conductance chloride channel, and an antigen recognized by TNT antibody. In another or a further embodiment, the selective NF- κ B activator is selected from the group consisting of vFLIP K13, a NEMO mutant, a NEMO-fusion protein, IKK1-S176E-S180E, IKK2-S177E-S181E, RIP, FKBPx2-RIP-ID, IKK1, FKBPx2-IKKa, IKK2, FKBPx2-IKK2, Tcl-1, MyD88-L265, any NF- κ B activating protein or protein fragment, any inhibitor of an inhibitor of NF- κ B pathway, a gene editing system capable of selectively activating NF- κ B, an RNA interference system that selectively activating NF- κ B and any combination thereof. In another or a further embodiment, the selective NF- κ B activator is expressed as a fusion construct with one or more copies of FKBP domain. In another or a further embodiment, the extracellular antigen specific domain is selected from the group consisting of: the variable region of the heavy chain (vH) of an antibody or a fragment thereof specific for a predefined target antigen; the variable region of the light chain (vL) of an antibody or a fragment thereof specific for a predefined target antigen; a single chain variable fragment (scFv) or a fragment thereof specific for a predefined target antigens; an antibody fragment (*e.g.*, Fv, a Fab, a (Fab')₂) specific for a predefined target antigen; a single domain antibody (SDAB) fragments specific for a predefined target antigen; a camelid vHH domain specific for a predefined target

antigen; a non-immunoglobulin antigen binding scaffolds specific for a predefined target antigen; a receptors specific or a fragment thereof for a predefined target antigen; a ligands or a fragment thereof specific for a predefined target antigen; a bispecific-antibody, -antibody fragment, -scFV, -vHH, -SDAB, -non-immunoglobulin antigen binding scaffold, -receptor or -ligand specific for one or more predefined target antigens; and an autoantigen or a fragment thereof.

[0008] The disclosure also provides at least one vector comprising the at least one polynucleotide of any of the foregoing polynucleotides constructs described herein and above. In one embodiment, the vector is selected from the group consisting of a DNA vector, an RNA vector, a plasmid, a lentivirus vector, adenoviral vector, AAV vector, a retrovirus vector, a baculovirus vector, a sleeping beauty transposon vector, and a piggybac transposon vector.

[0009] The disclosure also provides an immune effector cell or stem cell comprising at least one recombinant polynucleotide, construct or vector described herein and above. In one embodiment the immune cell is an antigen presenting cell. In another or a further embodiment, the immune effector cell is a human T cell, a human NKT cell or a synthetic T cell, NK cell, or a stem cell that can give rise to an immune effector cell, optionally, wherein the T cell is diacylglycerol kinase (DGK) and/or Ikaros deficient and/or Brd4 deficient.

[0010] The disclosure also provides a method to (i) extend the life span of an immune cell expressing, (ii) stimulate proliferation of an immune cell, (iii) stimulate cytokine production by an immune cell, (iv) enhance antigen presentation by an immune cell, (v) protect an immune cell from apoptosis, the method comprising transfecting or transforming the immune cells with a polynucleotide encoding a selective NF- κ B activator or a NF- κ B specific stimulatory polypeptide. In one embodiment, the selective NF- κ B activator or a NF- κ B specific stimulatory polypeptide is selected from the group consisting of vFLIP K13, K13-opt, a NEMO mutant, a NEMO-fusion protein, IKK1-S176E-S180E, IKK2-S177E-S181E, RIP, IKK α , IKK β , Tcl-1, MyD88-L265, any NF- κ B activating protein or protein fragment, any inhibitor of an inhibitor of NF- κ B pathway, any homolog or variant thereof and any combination thereof. In another or a further embodiment, the selective NF- κ B activator or a NF- κ B specific stimulatory polypeptide is expressed in a constitutive or inducible manner. In another or a further embodiment, the selective NF- κ B activator or a NF- κ B specific stimulatory polypeptides controlled post-translationally through contacting the T cell with a compound. In another or a further embodiment, the selective NF- κ B activator or a NF- κ B specific stimulatory polypeptide is expressed as a fusion construct with one or more

copies of FKBP domain. In another or a further embodiment, the activity of the selective NF- κ B activator or a NF- κ B specific stimulatory polypeptide is controlled at the post-translational level by administration of therapeutically effective amount of a compound that induces dimerization of the FKBP domain. In another or a further embodiment, the compound is AP20187 or rimiducid.

[0011] The disclosure also provides a method of making a non-naturally occurring immune receptor-expressing immune effector cell, comprising introducing at least one vector or at least one recombinant polynucleotide construct of the disclosure into an immune effector cell or a hematopoietic stem cell or progenitor cell that can give rise to an immune effector cell, under conditions such that a non-naturally occurring immune receptor is expressed and the immune effector cell comprises (i) extended life span, (ii) improved T cell proliferation, and/or (iii) reduced apoptosis compared to a CAR-T cell lacking an NF κ B specific stimulatory polypeptide. In another or a further embodiment, the method further comprises providing a population of immune effector cells; and removing T regulatory cells from the population, thereby providing a population of T regulatory-depleted cells; wherein the steps are performed prior to introducing the vector or recombinant polynucleotide encoding the CAR and/or NF κ B specific stimulatory polypeptide to the population. In another or a further embodiment, the T regulatory cells are removed from the cell population using an anti-CD25 antibody, or an anti-GITR antibody. In another or a further embodiment, the method further comprises a) providing a population of immune effector cells; and b) enriching P-glycoprotein (P-gp or Pgp; MDR1, ABCB1, CD243)-positive cells from the population, thereby providing a population of P-glycoprotein (P-gp or Pgp; MDR1, ABCB1, CD243)-enriched cells; wherein steps a) and b) are performed prior to or after introducing the vector or recombinant polynucleotide encoding the CAR and/or NF κ B specific stimulatory polypeptide. In another or a further embodiment, the P-glycoprotein positive cells are enriched using any one or more of the methods selected from the group consisting of i) immunoselection using one or a cocktail of P-glycoprotein specific antibodies, ii) staining with one or more of fluorescent dyes that are substrates of P-glycoprotein, tetramethylrhodamine methyl ester (TMRM), Adriamycin and actinomycin-D) under conditions at which P-glycoprotein is active as a pump and enriching for cells that stain less with the dye, iii) selection of cells that are resistant to phototoxic compounds that are substrates of P-glycoprotein, such as any one or more of TH9402, 2-(4,5-dibromo-6-amino-3-imino-3H-xanthen-9-yl)-benzoic acid methyl ester hydrochloride, 2-(4,5-dibromo-6-amino-3-imino-3H-xanthen-9-yl)-benzoic acid ethyl ester hydrochloride, 2-(4,5-dibromo-6-amino-3-

imino-3H-xanthen-9-yl)-benzoic acid octyl ester hydrochloride, 2-(4,5-dibromo-6-amino-3-imino-3H-xanthen-9-yl)-benzoic acid n-butyl ester hydrochloride, 2-(6-ethyl amino-3-ethyl imino-3H-xanthen-9-yl)-benzoic acid n-butyl ester hydrochloride, or derivatives thereof or combinations thereof, and iv) selection of cells that are resistant to cytotoxic compounds that are substrates of P-glycoprotein, such as vincristine, vinblastine, taxol, paclitaxel, mitoxantrone, etoposide, adriamycin, daunorubicin and actinomycin-D.

[0012] The disclosure also provide a method of generating a population of RNA-engineered cells comprising introducing *in vitro* transcribed RNA or RNAs or synthetic RNA or RNAs into a cell or population of cells, where the RNA or RNAs comprises a recombinant polynucleotide or polynucleotides of the disclosure.

[0013] The disclosure also provides a method of providing anti-disease immunity in a subject comprising administering to the subject an effective amount of the immune effector cell or a stem cell that can give rise to an immune effector cell of the disclosure, wherein the cell is an autologous T cell or an allogeneic T cell, or an autologous NKT cell or an allogeneic NKT cell or an autologous or an allogeneic hematopoietic stem cell or an autologous or an allogeneic iPSC that can give rise to an immune effector cell. In another or a further embodiment, the allogeneic T cell or allogeneic NKT cell or hematopoietic stem cell or iPSC lacks expression or has low expression of a functional TCR or a functional HLA.

[0014] The disclosure also provides a composition comprising an immune effector cell or a stem cell that can generate immune effector cells comprising a non-naturally occurring immune receptor and a selective NFkB activator, wherein the non-naturally occurring immune receptor comprises an antigen binding domains that bind to a disease-associated antigen associated said disease-associated antigen is selected from a group consisting of: CD5, CD19; CD123; CD22; CD30; CD171; CS-1 (also referred to as CD2 subset 1, CRACC, SLAMF7, CD319, and 19A24); C-type lectin-like molecule-1 (CLL-1 or CLECL1); CD33; epidermal growth factor receptor variant III (EGFRviii); ganglioside G2 (GD2); ganglioside GD3 (aNeu5Ac(2-8)aNeu5Ac(2-3)bDGalp(1-4)bDGlc(1-1)Cer); TNF receptor family member B cell maturation (BCMA); Tn antigen ((Tn Ag) or (GalNAcα-Ser/Thr)); prostate-specific membrane antigen (PSMA); Receptor tyrosine kinase-like orphan receptor 1 (ROR1); FmsLike Tyrosine Kinase 3 (FLT3); Tumor-associated glycoprotein 72 (TAG72); CD38; CD44v6; a glycosylated CD43 epitope expressed on acute leukemia or lymphoma but not on hematopoietic progenitors, a glycosylated CD43 epitope expressed on non-hematopoietic cancers, Carcinoembryonic antigen (CEA); Epithelial cell adhesion molecule (EPCAM); B7H3 (CD276); KIT (CD117); Interleukin-13 receptor subunit alpha-2

(IL-13Ra2 or CD213A2); Mesothelin; Interleukin 11 receptor alpha (IL-11Ra); prostate stem cell antigen (PSCA); Protease Serine 21 (Testisin or PRSS21); vascular endothelial growth factor receptor 2 (VEGFR2); Lewis(Y) antigen; CD24; Platelet-derived growth factor receptor beta (PDGFR-beta); Stage-specific embryonic antigen-4 (SSEA-4); CD20; Folate receptor alpha; Receptor tyrosine-protein kinase ERBB2 (Her2/neu); Mucin 1, cell surface associated (MUC1); epidermal growth factor receptor (EGFR); neural cell adhesion molecule (NCAM); Prostase; prostatic acid phosphatase (PAP); elongation factor 2 mutated (ELF2M); Ephrin B2; fibroblast activation protein alpha (FAP); insulin-like growth factor 1 receptor (IGF-I receptor); carbonic anhydrase IX (CAIX); Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2); glycoprotein 100 (gpl00); oncogene fusion protein consisting of breakpoint cluster region (BCR) and Abelson murine leukemia viral oncogene homolog 1 (Abl) (bcr-abl); tyrosinase; ephrin type-A receptor 2 (EphA2); Fucosyl GM1; sialyl Lewis adhesion molecule (sLe); ganglioside GM3 (aNeu5Ac(2-3)bDClalp(1-4)bDGlcp(1-1)Cer); transglutaminase 5 (TGS5); high molecular weight-melanoma-associated antigen (HMWMAA); o-acetyl-GD2 ganglioside (OAcGD2); tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related (TEM7R); claudin 6 (CLDN6); thyroid stimulating hormone receptor (TSHR); G protein coupled receptor class C group 5, member D (GPRC5D); chromosome X open reading frame 61 (CXORF61); CD97; CD179a; anaplastic lymphoma kinase (ALK); Polysialic acid; placenta-specific 1 (PLAC1); hexasaccharide portion of globoH glycosphingolipid (GloboH); mammary gland differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3 (ADRB3); pannexin 3 (PANX3); G protein-coupled receptor 20 (GPR20); lymphocyte antigen 6 complex, locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame Protein (TARP); Wilms tumor protein (WT1); Cancer/testis antigen 1 (NY-ESO-1); Cancer/testis antigen 2 (LAGE-1a); Melanoma-associated antigen 1 (MAGE-A1); ETS translocation-variant gene 6, located on chromosome 12p (ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, Member 1A (XAGE1); angiopoietin-binding cell surface receptor 2 (Tie 2); melanoma cancer testis antigen-1 (MAD-CT-1); melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; tumor protein p53 (p53); p53 mutant; prostatein; surviving; telomerase; prostate carcinoma tumor antigen-1 (PCT A-1 or Galectin 8), melanoma antigen recognized by T cells 1 (MelanA or MART1); Rat sarcoma (Ras) mutant; human Telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoints; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease, serine 2 (TMPRSS2) ETS fusion gene); N-Acetyl glucosaminyl-

transferase V (NA17); paired box protein Pax-3 (PAX3); Androgen receptor; Cyclin B1; v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Tyrosinase-related protein 2 (TRP-2); Cytochrome P450 1B 1 (CYPIB 1); CCCTC-Binding Factor (Zinc Finger Protein)-Like (BORIS or Brother of the Regulator of Imprinted Sites), Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3); Paired box protein Pax-5 (PAX5); proacrosin binding protein sp32 (OY-TES1); lymphocyte-specific protein tyrosine kinase (LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint 2 (SSX2); Receptor for Advanced Glycation End products (RAGE-1); renal ubiquitous 1 (RUI); renal ubiquitous 2 (RU2); legumain; human papilloma virus E6 (HPV E6); human papilloma virus E7 (HPV E7); intestinal carboxyl esterase; heat shock protein 70-2 mutated (mut hsp70-2); CD79a; CD79b; CD72; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR or CD89); Leukocyte immunoglobulin-like receptor subfamily A member 2 (LILRA2); CD300 molecule-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2); lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); and immunoglobulin lambda-like polypeptide 1 (IGLL1), MPL, Biotin, c-MYC epitope Tag, CD34, LAMP1 TROP2, GFRalpha4, CDH17, CDH6, NYBR1, CDH19, CD200R, Slea (CA19.9; Sialyl Lewis Antigen) Fucosyl-GM1, PTK7, gpNMB, CDH1-CD324, DLL3, CD276/B7H3, IL11Ra, IL13Ra2, CD179b-IGL11, ALK TCRgamma-delta, NKG2D, CD32 (FCGR2A), CSPG4-HMW-MAA, Tim1-/HVCR1, CSF2RA (GM-CSFR-alpha), TGFbetaR2, VEGFR2/KDR, Lews Ag, TCR-beta1 chain, TCR-beta2 chain, TCR-gamma chain, TCR-delta chain, FITC, Leutenizing hormone receptor (LHR), Follicle stimulating hormone receptor (FSHR), Chorionic Gonadotropin Hormone receptor (CGHR), CCR4, SLAMF6, SLAMF4, HIV1 envelope glycoprotein, HTLV1-Tax, CMV pp65, EBV-EBNA3c, influenza A hemagglutinin (HA), GAD, PDL1, Guanylyl cyclase C (GCC), KSHV-K8.1 protein, KSHV-gH protein, auto-antibody to desmoglein 3 (Dsg3), autoantibody to desmoglein 1 (Dsg1), HLA, HLA-A, HLA-A2, HLA-B, HLA-C, HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, HLA-DR, HLA-G, IGE, CD99, RAS G12V, Tissue Factor 1 (TF1), AFP, GPRC5D, claudin18.2 (CLD18A2 OR CLDN18A.2)), P-glycoprotein, STEAP1, LIV1, NECTIN-4, CRIPTO, GPA33, BST1/CD157, low conductance chloride channel, and antigen recognized by TNT antibody.

[0015] The disclosure also provides a method of treating or preventing a disease associated with expression of a disease-associated antigen in a subject, comprising

administering to the subject an effective amount of an immune effector cell comprising a non-naturally occurring immune receptor and a selective NFkB activator, wherein the non-naturally occurring immune receptor comprises an antigen binding domains that bind to a disease-associated antigen associated said disease-associated antigen is selected from a group consisting of: CD5; CD19; CD123; CD22; CD30; CD171; CS-1 (also referred to as CD2 subset 1, CRACC, SLAMF7, CD319, and 19A24); C-type lectin-like molecule-1 (CLL-1 or CLECL1); CD33; epidermal growth factor receptor variant III (EGFRviii); ganglioside G2 (GD2); ganglioside GD3 (aNeu5Ac(2-8)aNeu5Ac(2-3)bDGalp(1-4)bDGlc(1-1)Cer); TNF receptor family member B cell maturation (BCMA); Tn antigen ((Tn Ag) or (GalNAc α -Ser/Thr)); prostate-specific membrane antigen (PSMA); Receptor tyrosine kinase-like orphan receptor 1 (ROR1); FmsLike Tyrosine Kinase 3 (FLT3); Tumor-associated glycoprotein 72 (TAG72); CD38; CD44v6; a glycosylated CD43 epitope expressed on acute leukemia or lymphoma but not on hematopoietic progenitors, a glycosylated CD43 epitope expressed on non-hematopoietic cancers, Carcinoembryonic antigen (CEA); Epithelial cell adhesion molecule (EPCAM); B7H3 (CD276); KIT (CD117); Interleukin-13 receptor subunit alpha-2 (IL-13Ra2 or CD213A2); Mesothelin; Interleukin 11 receptor alpha (IL-11Ra); prostate stem cell antigen (PSCA); Protease Serine 21 (Testisin or PRSS21); vascular endothelial growth factor receptor 2 (VEGFR2); Lewis(Y) antigen; CD24; Platelet-derived growth factor receptor beta (PDGFR-beta); Stage-specific embryonic antigen-4 (SSEA-4); CD20; Folate receptor alpha; Receptor tyrosine-protein kinase ERBB2 (Her2/neu); Mucin 1, cell surface associated (MUC1); epidermal growth factor receptor (EGFR); neural cell adhesion molecule (NCAM); Prostase; prostatic acid phosphatase (PAP); elongation factor 2 mutated (ELF2M); Ephrin B2; fibroblast activation protein alpha (FAP); insulin-like growth factor 1 receptor (IGF-I receptor), carbonic anhydrase IX (CAIX); Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2); glycoprotein 100 (gp100); oncogene fusion protein consisting of breakpoint cluster region (BCR) and Abelson murine leukemia viral oncogene homolog 1 (Abl) (bcr-abl); tyrosinase; ephrin type-A receptor 2 (EphA2); Fucosyl GM1; sialyl Lewis adhesion molecule (sLe); ganglioside GM3 (aNeu5Ac(2-3)bDGalp(1-4)bDGlc(1-1)Cer); transglutaminase 5 (TGS5); high molecular weight-melanoma-associated antigen (HMWMAA); o-acetyl-GD2 ganglioside (OAcGD2); tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related (TEM7R); claudin 6 (CLDN6); thyroid stimulating hormone receptor (TSHR); G protein coupled receptor class C group 5, member D (GPRC5D); chromosome X open reading frame 61 (CXORF61); CD97; CD179a; anaplastic lymphoma kinase (ALK); Polysialic acid; placenta-specific 1 (PLAC1);

hexasaccharide portion of globoH glycosphingolipid (GloboH); mammary gland differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3 (ADRB3); pannexin 3 (PANX3); G protein-coupled receptor 20 (GPR20); lymphocyte antigen 6 complex, locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame Protein (TARP); Wilms tumor protein (WT1); Cancer/testis antigen 1 (NY-ESO-1); Cancer/testis antigen 2 (LAGE-1a); Melanoma-associated antigen 1 (MAGE-A1); ETS translocation-variant gene 6, located on chromosome 12p (ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, Member 1A (XAGE1); angiopoietin-binding cell surface receptor 2 (Tie 2); melanoma cancer testis antigen-1 (MAD-CT-1); melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; tumor protein p53 (p53); p53 mutant; prostein; surviving; telomerase; prostate carcinoma tumor antigen-1 (PCT A-1 or Galectin 8), melanoma antigen recognized by T cells 1 (MelanA or MART1); Rat sarcoma (Ras) mutant; human Telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoints; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease, serine 2 (TMPRSS2) ETS fusion gene); N-Acetyl glucosaminyl-transferase V (NA17); paired box protein Pax-3 (PAX3); Androgen receptor; Cyclin B1; v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Tyrosinase-related protein 2 (TRP-2); Cytochrome P450 1B 1 (CYP1B 1); CCCTC-Binding Factor (Zinc Finger Protein)-Like (BORIS or Brother of the Regulator of Imprinted Sites), Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3); Paired box protein Pax-5 (PAX5); proacrosin binding protein sp32 (OY-TES1); lymphocyte-specific protein tyrosine kinase (LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint 2 (SSX2); Receptor for Advanced Glycation End products (RAGE-1); renal ubiquitous 1 (RUI); renal ubiquitous 2 (RU2); legumain; human papilloma virus E6 (HPV E6); human papilloma virus E7 (HPV E7); intestinal carboxyl esterase; heat shock protein 70-2 mutated (mut hsp70-2); CD79a; CD79b; CD72; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR or CD89); Leukocyte immunoglobulin-like receptor subfamily A member 2 (LILRA2); CD300 molecule-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2); lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); and immunoglobulin lambda-like polypeptide 1 (IGLL1), MPL, Biotin, c-MYC epitope Tag, CD34, LAMP1 TROP2, GFRalpha4, CDH17, CDH6, NYBR1, CDH19, CD200R, SleA (CA19.9; Sialyl Lewis Antigen) Fucosyl-GM1,

PTK7, gpNMB, CDH1-CD324, DLL3, CD276/B7H3, IL11Ra, IL13Ra2, CD179b-IGL11, ALK TCRgamma-delta, NKG2D, CD32 (FCGR2A), CSPG4-HMW-MAA, Tim1-/HVCR1, CSF2RA (GM-CSFR-alpha), TGFbetaR2, VEGFR2/KDR, Lews Ag, TCR-beta1 chain, TCR-beta2 chain, TCR-gamma chain, TCR-delta chain, FITC, Leutenizing hormone receptor (LHR), Follicle stimulating hormone receptor (FSHR), Chorionic Gonadotropin Hormone receptor (CGHR), CCR4, SLAMF6, SLAMF4, HIV1 envelope glycoprotein, HTLV1-Tax, CMV pp65, EBV-EBNA3c, influenza A hemagglutinin (HA), GAD, PDL1, Guanylyl cyclase C (GCC), KSHV-K8.1 protein, KSHV-gH protein, auto-antibody to desmoglein 3 (Dsg3), autoantibody to desmoglein 1 (Dsg1), HLA, HLA-A, HLA-A2, HLA-B, HLA-C, HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, HLA-DR, HLA-G, IGE, CD99, RAS G12V, Tissue Factor 1 (TF1), AFP, GPRC5D, claudin18.2 (CLD18A2 OR CLDN18A.2)), P-glycoprotein, STEAP1, LIV1, NECTIN-4, CRIPTO, GPA33, BST1/CD157, low conductance chloride channel, and antigen recognized by TNT antibody, thereby treating the subject or preventing a disease in the subject. In another or a further embodiment, the disease associated with expression of the disease associated antigen is selected from the group consisting of a proliferative disease, a precancerous condition, a cancer, and a non-cancer related indication associated with expression of the disease-associated antigen. In another or a further embodiment, the cancer is a hematologic cancer chosen from one or more of chronic lymphocytic leukemia (CLL), acute leukemias, acute lymphoid leukemia (ALL), B-cell acute lymphoid leukemia (B-ALL), T-cell acute lymphoid leukemia (T-ALL), chronic myelogenous leukemia (CML), B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma, primary effusion lymphoma, follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin's lymphoma, Hodgkin's lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, or pre-leukemia. In another or a further embodiment, the cancer is selected from the group consisting of colon cancer, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, cancer of the small intestine, cancer of the esophagus, melanoma, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina,

carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, solid tumors of childhood, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, Merkel cell cancer, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers, combinations of said cancers, and metastatic lesions of said cancers. In another or a further embodiment, the disease is associated with infection by a virus including but not limited to HIV1, HIV2, HTLV1, Epstein Barr virus (EBV), cytomegalovirus (CMV), adenovirus, adeno-associated virus, BK virus, Human Herpesvirus 6, Human Herpesvirus 8 influenza virus, parainfluenza virus, avian flu virus, MERS and SARS coronaviruses, Crimean Congo Hemorrhagic fever virus, rhino virus, enterovirus, Dengue virus, West Nile virus, Ebola virus, Marburg virus, Lassa fever virus, zika virus, RSV, measles virus, mumps virus, rhino virus, varicella virus, herpes simplex virus 1 and 2, varicella zoster virus, HIV-1, HTLV1, Hepatitis virus, enterovirus, hepatitis B virus, Hepatitis C virus, Nipah and Rift valley fever viruses, Japanese encephalitis virus, Merkel cell polyomavirus, or is associated with infection with mycobacterium tuberculosis, atypical mycobacteria species, Pneumocystis jirovecii, toxoplasmosis, rickettsia, nocardia, aspergillus, mucor, or candida. In another or a further embodiment, the disease is an immune or degenerative disease including but not limited to diabetes mellitus, multiple sclerosis, rheumatoid arthritis, pemphigus vulgaris, ankylosing spondylitis, Hoshimoto's thyroiditis, SLE, sarcoidosis, scleroderma, mixed connective tissue disease, graft versus host disease or Alzheimer's disease.

[0016] The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] **Figure 1** depicts a cartoon of current an antibody, T-cell receptor (TCR), CAR and next generation CARs and SIRs.

[0018] **Figure 2** depicts a cartoon comparing second generation CAR biological activity and structure to an embodiment of the present disclosure depicting a CAR lacking CD28 or 41BB but expressing a NF- κ B stimulatory molecule (NEMO and/or K13, or mutants thereof).

[0019] **Figure 3** shows strong activation of NF- κ B by mNEMO-K270A, hNEMO-K277A and weak activation by hNEMO-K277I and hNEMO-K277G mutant.

[0020] **Figure 4** shows activity of a Bispecific T cell engager targeting MPL and using a 161-scFv targeting domain. HEL-pLenti-hGluc and T cells were pre-incubated separately with the following supernatants at 4° C for 2h Medium alone and pLenti-161-StreptagII-CD3-Myc-His-P02 (042517-P02-SC). Post-incubation, cells were co-cultured in U-bottom 96-well plate at an E:T ratio of 1:1 or 5:1 for 4h at 37 C. 50 μ l of cells+sup/well were transferred to 384 well plate in triplicate. hGLuc assay was performed using 15 μ l of CTZ assay buffer (1:100).

[0021] **Figure 5A-C** shows CRISPR/Cas9-mediated TFP gene targeting into the TRAC locus and strategies to rescue TRAC expression. a, Top, TRAC locus with the 5' end (grey) of the TRAC first exon, the TRAC gRNA (blue) and the corresponding PAM sequence (red). The two blue arrows indicate the predicted Cas9 double strand break. Bottom, CRISPR/Cas9-targeted integration into the TRAC locus. The targeting construct (AAV) contains a splice acceptor (SA), followed by a F2A coding sequence, the TFP gene and a polyA sequence, flanked by sequences homologous to the TRAC locus (LHA and RHA, left and right homology arm). Once integrated, the endogenous TCR α promoter drives TFP expression, while the TRAC locus is disrupted. B) The targeting construct expresses TFP and coexpresses TRAC (TCR α constant chain) through a 2A sequence. C) The targeting construct expresses TFP and coexpresses via a 2A sequence a signal peptide which is in frame with the first exon present in the RHA so that TCR α promoter drives TFP expression as well as that of TRAC which is lacking the TCR α variable region (TRAV); TRAJ, TCR α joining region; 2A, the self-cleaving 2A sequence. pA: SV40/ β -globin polyA sequence.

[0022] **Figure 6A-E** shows various construct designs for targeting cassette to direct an Ab-TCR to the TRAC locus.

[0023] **Figure 7A-F** shows various construct designs for targeting cassette to direct a cTCR (SIR) to TRAC locus.

[0024] **Figure 8A-D** shows various construct designs for targeting cassette to direct a cTCR (SIR) and a TCR to TRAC locus.

[0025] **Figure 9A-D** shows various construct designs for targeting cassette to direct a single chain cTCR (SIR) to TRAC locus.

DETAILED DESCRIPTION

[0026] Initial first-generation CARs were constructed through the fusion of a scFv (single chain fragment variable)-based antigen binding domain to an inert CD8

transmembrane domain, linked to a cytoplasmic signaling domain derived from the CD3- ζ or Fc receptor γ chains (**Fig. 1**).

[0027] Although CD3- ζ chain aggregation is sufficient to enable lytic activity of T-cells, they failed to elicit a robust cytokine response, including interleukin-2 (IL-2), and support T-cell expansion upon repeated exposure to antigen. For optimal activation and proliferation, T cells require both T-cell receptor engagement and signaling, as well as costimulatory signaling through costimulatory receptors (*i.e.*, CD28, 4-1BB, OX-40) on T cells binding to cognate ligands (*i.e.*, CD80/86, 4-1BBL, OX-40L) expressed either by the targeted tumor cell or the antigen-presenting cells. To overcome the lack of T-cell co-stimulation, first generation CARs were further modified by incorporating the cytoplasmic signaling domains of T-cell costimulatory receptors. These second-generation CARs enhanced signaling strength and persistence of the modified T cells, leading to superior antitumor activity. Signaling through the costimulatory domains present in the 2nd generation CAR constructs results in activation of several signaling pathways, such as NF- κ B, AKT and ERK. In particular, AKT activation promotes T cell activation but has been also shown to results in terminal differentiation, exhaustion and lack of persistence.

[0028] **Fig. 2** depicts a cartoon of a 2nd generation CAR as described above next to a first generation CAR plus a specific NF- κ B stimulatory molecule depicting the biological activity associated with each.

[0029] The CAR constructs in current clinical use are artificial in design as they represent fusion of several different proteins. In particular, inclusion of co-stimulatory domain in the 2nd generation CAR construct results in non-physiological signaling through the receptor, which in turn could contribute to their toxicity. Some CARs show tonic antigen-independent signaling, which leads to unrestrained cellular activation, eventually resulting in apoptosis, excessive cytokine release independent of cognate antigens, and immunologic exhaustion. Tonic signaling through co-stimulatory domains (*e.g.*, 41BB and CD28 domain) has been shown to impede T cell survival. Thus, there is a need for improving the CAR design to achieve long term persistence of CAR modified T cells without the risk of excessive toxicity, such as cytokine release syndrome (CRS).

[0030] To overcome some of the design limitation of conventional 2nd generation CARs, several alternative designs, collectively termed next generation CARs, have been described, including Ab-TCR (WO 2017/070608 A1 incorporated herein by reference), TCR receptor fusion proteins or TFP (WO 2016/187349 A1 incorporated herein by reference), Synthetic Immune Receptors (SIRs) (see, WO 2018/102795 A1, incorporated herein by

reference), Tri-functional T cell antigen coupler (Tri-TAC) (see, WO 2015/117229 A1, incorporated herein by reference). These alternative CAR designs, in general, lack a co-stimulatory domain.

[0031] To overcome the limitations of AKT activation and tonic signaling, this disclosure demonstrates the use of selective NF- κ B activators, such as NEMO-mutants (*e.g.*, hNEMO-K277A, hNEMO-K277A-DeltaV249-K255, mouse NEMO-K270A), K13-opt, IKK2-S177E-S181E, or IKK1-S176E-S180E, to provide costimulatory function. In contrast to 41BB- and CD28-derived costimulatory domains that activate a multitude of signaling pathways (see, 2nd and 3rd generation CARs in **Fig. 1**), selective NF- κ B activators, such as, for example, hNEMO-K277A, hNEMO-K277A-DeltaV249-K255, mouse NEMO-K270A, K13-opt, IKK2-S177E-S181E, or IKK1-S176E-S180E, selectively activate the NF- κ B pathway by activating the I-kappaB kinase (IKK) complex. The disclosure further describes an alternative non-naturally occurring immune receptor, *e.g.*, CAR, design in which the costimulation is provided by an accessory module comprising a selective NF- κ B activator that is co-expressed with the non-naturally occurring immune receptor (*e.g.*, a CAR). However, in contrast to the 2nd generation CAR constructs in which the co-stimulatory domain is a component of the mature CAR polypeptide, the accessory module comprising the selective NF- κ B activator is not necessarily an integral part of the mature immune receptor *e.g.*, CAR, polypeptide. Such a design has advantage as it overcomes the problems of tonic signaling, excessive cytokine production and early exhaustion of T cells caused by the aggregation and non-physiological signaling through the costimulatory domains. The disclosure further provides a method to regulate the activity of the NF- κ B activators by expressing them in fusion with switch domains, such as in fusion with tandem copies of a FKBP12v36 domain.

[0032] The disclosure demonstrates that expression of selective NF- κ B activators, such, for example, as hNEMO-K277A, hNEMO-K277A-DeltaV249-K255, mouse NEMO-K270A, IKK2-S177E-S181E, IKK1-S176-S180E and K13-opt, in T cells extends their ability to proliferate long term in culture without undergoing senescence, thereby demonstrating for the first time that activation of a single pathway (*i.e.*, NF- κ B) is sufficient for postponing senescence of T cells. For example, CD19-CAR constructs co-expressing hNEMO-K277A or hNEMO-K277A-DeltaV249-K255 but lacking any costimulatory domain demonstrate superior *in vivo* efficacy as compared to 2nd generation CAR construct containing the 41BB costimulatory domain. The disclosure further demonstrates that selective activation of NF- κ B is sufficient to promote the proliferation of T cells, delay senescence and improve the

performance of T cells for adoptive cell therapy, including CAR-T cell therapy. Thus, the disclosure provides composition and methods to enhance the survival, proliferation, cytokine secretion, delay exhaustion and senescence and improve the *in vivo* expansion, persistence and anti-tumor activity of an immune cell, *e.g.*, T cell, *e.g.*, CAR-T or TCR-T or SIR-T cell, and/or an immune cell expressing a non-naturally occurring immune receptor, via selective or preferential (*i.e.*, without AKT activation) activation of the NF- κ B pathway in the immune cell. Moreover, the disclosure demonstrates that the use of selective NF- κ B activators, such as, for example, hNEMO-K277A or hNEMO-K277A-DeltaV249-K255, is not limited to its use in CAR-T cells as they can be used in any T cell for adoptive cellular therapy, including T cells expressing endogenous TCR (*e.g.*, tumor infiltrating lymphocytes), exogenous TCR, SIR and the like.

[0033] The disclosure further demonstrates that selective NF- κ B activators, such as, for example, hNEMO-K277A, hNEMO-K277A-DeltaV249-K255, mouse NEMO-K270A, K13-opt, IKK2-S177E-S181E, or IKK1-S176E-S180E, can be used to improve the performance of vaccines by promoting cytokine secretion and antigen presentation by immune cells, *e.g.*, antigen presenting cells, *e.g.*, dendritic cells. For example, bone marrow derived dendritic cells (DC) expressing selective NF- κ B activators, such as hNEMO-K277A, hNEMO-K277A-DeltaV249-K255, mouse NEMO-K270A, K13-opt, IKK2-S177E-S181E, or IKK1-S176E-S180E, show superior cytokine production, antigen presentation, and immune response (*e.g.*, anti-tumor response or anti-infectious agent response) as compared to control DC.

[0034] The disclosure further provides NF- κ B activators, including selective NF- κ B activators that are of human origin and therefore are less immunogenic.

[0035] The disclosure further provides NF- κ B activators, including selective NF- κ B activators that can be expressed in the cytosol. The disclosure further provides NF- κ B activators, including selective NF- κ B activators, that are constitutively active and do not require a stimulus, *e.g.*, treatment with a ligand, for their ability to activate NF- κ B.

[0036] The disclosure further provides several antigen binding domains that can be used in the generation of conventional CARs (*e.g.*, 2nd generation CAR containing 41BB costimulatory domain) as well next generation CARs such as SIRs, zSIRs, Ab-TCR, and TFPs, for applications in adoptive cellular therapy. In some embodiments, these antigen binding domains are derived from antibodies and target antigens expressed in both hematologic malignancies and solid tumors. The SEQ ID Nos. of vL, vH and scFv fragments of these antigen binding domains are shown in **Tables 6A-C**. The SEQ ID Nos of the

complementary determining regions (CDRs) of the light (vL) and heavy (vH) chains are shown in **Tables 6A-B**. The nucleic acid and amino acid SEQ IDs of exemplary 2nd generation CARs containing 41BB costimulatory domains and next generation CARs (*e.g.*, zCAR-K13, zCAR-NEMO-K277A, SIRs, Ab-TCRs and TFP) based on these antigen binding domains are provided in **Tables 10-14**. The CARs containing these antigen binding domains show diverse *in vitro* and *in vivo* properties, such as binding affinity to the target antigens, cytokine secretion, proliferation, cytotoxicity, exhaustion, and long term persistence. As such, the non-naturally occurring immune receptors, *e.g.*, CARs, containing these target antigens can be used to generate a diverse immune response. The polynucleotide, polypeptides, expression constructs, recombinantly engineered cells expressing CARs comprising the antigen binding domains of the disclosure, as well as method of making and using such polypeptides, polynucleotides and cells are described in methods known in the art and methods described in PCT/US2017/024843, WO 2014/160030 A2, WO 2016/187349 A1, WO 2017/070608 A1 and WO 2018/102795 A1, which are incorporated herein by reference in their entirety. The immune cells expressing the CARs comprising these antigen binding domains can be generated and used for adoptive cellular therapy of cancer, infectious and immune disorders using methods known in the art and methods described in WO 2017/070608 A1, WO 2016/187349 A1, WO 2018/102795 A1, WO 2015/117229 A1, which are incorporated herein by reference in their entirety.

[0037] The disclosure further provides novel methods for generating allogeneic T cells expressing TCR and CARs, including next generation CARs (*e.g.*, TFP, SIR, Ab-TCR, cTCR), for the purpose of off-the-shelf adoptive cellular therapy.

[0038] The disclosure further provides novel methods of combination therapies using autologous and allogeneic T cells expressing TCR and CARs, including next generation CARs (*e.g.*, TFP, SIR, Ab-TCR and cTCR). The disclosure provides methods of restoring the expression and/or activity of TFPs based on CD3 ϵ , CD3 γ and CD δ chains in T cells lacking the expression of native TCR α , TCR β , TCR γ or TCR δ chains by coexpressing in the cells expressing the TFPs the constant chains of TCR α , TCR β , TCR γ or TCR δ . The disclosure further provides methods of restoring the expression and/or activity of TFPs based on CD3 ϵ , CD3 γ and CD δ chains in T cells lacking the expression of native TCR α , TCR β , TCR γ or TCR δ chains by coexpressing in the cells expressing the TFPs either SIRs or Ab-TCR that encode the full length or fragments of constant chains of TCR α , TCR β , TCR γ or TCR δ . The disclosure provides that TFPs based on CD3 ϵ , CD3 γ and CD δ chains can be combined with SIRs or Ab-TCR encoding the constant chains of TCR α , TCR β , TCR γ or TCR δ constant

chains in T cells lacking the native TCR α , TCR β , TCR γ or TCR δ chains for the purpose of allogeneic and off-the-shelf therapy.

[0039] As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells and reference to "the polynucleotide" includes reference to one or more polynucleotides and so forth.

[0040] Also, the use of "or" means "and/or" unless stated otherwise. Similarly, "comprise," "comprises," "comprising" "include," "includes," and "including" are interchangeable and not intended to be limiting.

[0041] It is to be further understood that where descriptions of various embodiments use the term "comprising," those skilled in the art would understand that in some specific instances, an embodiment can be alternatively described using language "consisting essentially of" or "consisting of."

[0042] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Allen *et al.*, *Remington: The Science and Practice of Pharmacy* 22nd ed., Pharmaceutical Press (September 15, 2012); Hornyak *et al.*, *Introduction to Nanoscience and Nanotechnology*, CRC Press (2008); Singleton and Sainsbury, *Dictionary of Microbiology and Molecular Biology* 3rd ed., revised ed., J. Wiley & Sons (New York, NY 2006); Smith, *March's Advanced Organic Chemistry Reactions, Mechanisms and Structure* 7th ed., J. Wiley & Sons (New York, NY 2013); Singleton, *Dictionary of DNA and Genome Technology* 3rd ed., Wiley-Blackwell (November 28, 2012); and Green and Sambrook, *Molecular Cloning: A Laboratory Manual* 4th ed., Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY 2012), provide one skilled in the art with a general guide to many of the terms used in the present application. For references on how to prepare antibodies, see Greenfield, *Antibodies A Laboratory Manual* 2nd ed., Cold Spring Harbor Press (Cold Spring Harbor NY, 2013); Köhler and Milstein, *Derivation of specific antibody-producing tissue culture and tumor lines by cell fusion*, Eur. J. Immunol. 1976 Jul, 6(7):511-9; Queen and Selick, *Humanized immunoglobulins*, U. S. Patent No. 5,585,089 (1996 Dec); and Riechmann *et al.*, *Reshaping human antibodies for therapy*, Nature 1988 Mar 24, 332(6162):323-7 All headings and subheading provided herein are solely for ease of reading and should not be construed to limit the invention. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references

mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and specific examples are illustrative only and not intended to be limiting.

[0043] All publications herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. Any references cited are not an admission that any of the information provided therein is prior art or relevant to the presently claimed invention, or that any publication specifically or implicitly referenced is prior art.

[0044] The term "about" when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of $\pm 20\%$ or in some instances $\pm 10\%$, or in some instances $\pm 5\%$, or in some instances $\pm 1\%$, or in some instances $\pm 0.1\%$ from the specified value, as such variations are appropriate to perform the disclosed methods or describe the compositions herein. Moreover, any value or range (*e.g.*, less than 20 or similar terminology) explicitly includes any integer between such values or up to the value. Thus, for example, "one to five mutations" explicitly includes 1, 2, 3, 4, and/or 5 mutations.

[0045] The term "Ab-TCR" or "AbTCR" refers to a next generation CAR platform as described in WO 2017/070608 A1 which is incorporated herein by reference. In an embodiment, an Ab-TCR comprises an antibody moiety that specifically binds to a target antigen fused to a TCR module capable of recruiting at least one TCR signaling module. Exemplary TCR modules that can be used in the construction of Ab-TCR are provided in SEQ ID NO: 959-964 (**Table 6D**) and in WO 2017/070608 A1 which is incorporated herein by reference. In the TCR module TCRb-IAH-6MD three amino acid residues (F133, E136 and Q139) found in human TCRb chain (SEQ ID NO: 15053) (see **Tables 4, 5 & 6D**) are mutated to the residues Isoleucine, Alanine, and Histidine found in the murine TCRb chain, respectively, so as to enhance the expression of this module. Similarly, in the TCR module IgG1-CH1-TCRa-SDVP-6MD four amino acid residues (P91, E92, S93, S94) found in human TCRa chain (SEQ ID NO: 15041) are mutated to the residues S, D, V, P found in the murine TCRa chain so as to enhance the expression of this module (see **Tables 3 & 6D**). Exemplary Ab-TCRs co-expressing an accessory module encoding NEMO-K277A are provided in SEQ ID NO: 3124-3523 (**Table 14**). However, the accessory module encoding NEMO-K277A is optional. Ab-TCR with the antigen binding domains (*i.e.*, vL and vH fragments, ligands and receptors *etc.*) described in this disclosure can be constructed without NEMO-K277A. As such this accessory module along with the upstream Furine-SGSG-F2A

sequence can be deleted from the Ab-TCR. Alternatively, the accessory module encoding NEMO-K277A can be replaced by accessory modules encoding other proteins, such as hNEMO-K277A-deltaV249-K555, mNEMO-K270A, K13-opt, IKK2-S177E-S181E, or IKK1-S176E-S180E, and MyD88-L265P, FKBPx2-NEMO, NEMO-L600-FKBPx2 *etc.* Furthermore, the TCR modules present in the Ab-TCR can be substituted by other TCR modules described in WO 2017/070608 A1. For example, the Ab-TCR represented by SEQ ID NO: 3124-3323 contain TCR modules IgCL-TCRb-IAH-6MD (SEQ ID NO: 960) and IgG1-CH1-TCRa-SDVP-6MD (SEQ ID NO: 963) which can be substituted by TCR modules IgCL-TCRb-wt2-opt-6MD (SEQ ID NO: 961) and IgG1-CH1-TCRa-wt2-opt-6MD (SEQ ID NO: 964), respectively. Exemplary Ab-TCRs co-expressing an accessory module encoding NEMO-K277A and containing the TCR modules IgCL-TCRg-6MD (SEQ ID NO: 959) and IgG1-CH1-TCRd-6MD (SEQ ID NO: 962) are provided in SEQ ID NO: 3324-3523. The order of the antigen binding domains in these constructs is the same as the order of the constructs shown in Table 14 and therefore a Ab-TCR based on IgCL-TCRg-6MD (SEQ ID NO: 959) and IgG1-CH1-TCRd-6MD (SEQ ID NO: 962) targeting a particular antigen and containing a specific antigen binding domain can be identified by referring to **Table 14**.

[0046] The term “accessory module” refers to any one or more of hNEMO-K277A (or NEMO-K277A), hNEMO-K277A-delta-V249-K555, mNEMO-K270A, K13-opt, IKK2-S177E-S181E (or IKK2-SS/EE), IKK1-S176E-S180E (or IKK1-SS/EE), MyD88-L265P, TCL-1a, MTCP-1, CMV-141, 41BBL, CD40L, vFLIP-K13, MC159, cFLIP-L/MRIT α , cFLIP-p22, HTLV1 Tax, HTLV2 Tax, HTLV2 Tax-RS mutant, FKBPx2-K13, FKBPx2-HTLV2-Tax, FKBPx2-HTLV2-Tax-RS, IL6R-304-vHH-Alb8-vHH, IL12f, PD1-4H1 scFV, PD1-5C4 scFV, PD1-4H1-Alb8-vHH, PD1-5C4-Alb8-vHH, CTLA4-Ipilimumab-scFv, CTLA4-Ipilimumab-Alb8-vHH, IL6-19A-scFV, IL6-19A-scFV-Alb8-vHH, sHVEM, sHVEM-Alb8-vHH, hTERT, Fx06, shRNA targeting Brd4, IgSP-[hTRAC-opt2], IgSP-[hTRBC-opt2] and combination thereof that is expressed in an immune cell (*e.g.*, T cell, *e.g.*, CAR-T cell or TCR-T cell) to decrease, regulate or modify the activity of the immune cell. In some embodiments, the accessory module is co-expressed with an immune receptor such as a CAR or a TCR to increase, decrease, regulate or modify the expression or activity of a CAR or a TCR or a CAR-expressing or a TCR-expressing cell. The accessory module can be co-expressed with a CAR or a TCR using a single vector or using two or more different vectors. In a further embodiment, the accessory module comprises an FKBP (FK506 binding protein)-fusion protein, such as FKBPx2-NEMO, whose activity can be controlled by the

administration of a dimerizer molecule. In some embodiments, the accessory module is expressed in an antigen presenting cell, *e.g.*, a dendritic cell.

[0047] As used herein "affinity" is meant to describe a measure of binding strength. Affinity, in some instances, depends on the closeness of stereochemical fit between a binding agent and its target (*e.g.*, between an antibody and antigen including epitopes specific for the binding domain), on the size of the area of contact between them, and on the distribution of charged and hydrophobic groups. Affinity generally refers to the "ability" of the binding agent to bind its target. There are numerous ways used in the art to measure "affinity". For example, methods for calculating the affinity of an antibody for an antigen are known in the art, including use of binding experiments to calculate affinity. Binding affinity may be determined using various techniques known in the art, for example, surface plasmon resonance, bio-layer interferometry, dual polarization interferometry, static light scattering, dynamic light scattering, isothermal titration calorimetry, ELISA, analytical ultracentrifugation, and flow cytometry. An exemplary method for determining binding affinity employs surface plasmon resonance. Surface plasmon resonance is an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.). As used herein, the term "specific binding" means the contact between an antibody and an antigen with a binding affinity of at least 10^{-6} M. In certain aspects, antibodies bind with affinities of at least about 10^{-7} M, and preferably 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, or 10^{-12} M.

[0048] The "AKT Pathway" or "PI3K-AKT Pathway" as used herein is a signal transduction pathway that promotes survival and growth in response to extracellular signals. Key proteins involved are PI3K (phosphatidylinositol 3-kinase) and Akt (Protein Kinase B).

[0049] The term "antibody," as used herein, refers to a protein, or polypeptide sequence derived from an immunoglobulin molecule which specifically binds with an antigen. Antibodies can be monoclonal, or polyclonal, multiple or single chain, or intact immunoglobulins, and may be derived from natural sources or from recombinant sources. Antibodies can be tetramers of immunoglobulin molecules. The antibody may be 'humanized', 'chimeric' or non-human.

[0050] The term "antibody fragment" refers to at least one portion of an antibody, that retains the ability to specifically interact with (*e.g.*, by binding, steric hindrance, stabilizing/destabilizing, spatial distribution) an epitope of an antigen. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab'h, Fv fragments, scFv antibody

fragments, disulfide-linked Fvs (sdFv), a Fd fragment consisting of the VH and CH1 domains, linear antibodies, single domain antibodies (sdAb) such as either vL or vH, camelid vHH domains, multi-specific antibodies formed from antibody fragments such as a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region, and an isolated CDR or other epitope binding fragments of an antibody. An antigen binding fragment can also be incorporated into single domain antibodies, maxibodies, minibodies, nanobodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (see, *e.g.*, Hollinger and Hudson, *Nature Biotechnology* 23:1126-1136, 2005). Antigen binding fragments can also be grafted into scaffolds based on polypeptides such as a fibronectin type III (Fn3) (see U.S. Patent No.: 6,703,199, which describes fibronectin polypeptide minibodies).

[0051] The term "antibody heavy chain," refers to the larger of the two types of polypeptide chains present in antibody molecules in their naturally occurring conformations, and which normally determines the class to which the antibody belongs.

[0052] The term "antibody light chain," refers to the smaller of the two types of polypeptide chains present in antibody molecules in their naturally occurring conformations. Kappa (κ) and lambda (λ) light chains refer to the two major antibody light chain isotypes.

[0053] "Anticancer agent" refers to agents that inhibit aberrant cellular division and growth, inhibit migration of neoplastic cells, inhibit invasiveness or prevent cancer growth and metastasis. The term includes chemotherapeutic agents, biological agent (*e.g.*, siRNA, viral vectors such as engineered MLV, adenoviruses, herpes virus that deliver cytotoxic genes), antibodies and the like.

[0054] The term "anticancer effect" refers to a biological effect which can be manifested by various means, including but not limited to, a decrease in tumor volume, a decrease in the number of cancer cells, a decrease in the number of metastases, an increase in life expectancy, decrease in cancer cell proliferation, decrease in cancer cell survival, or amelioration of various physiological symptoms associated with the cancerous condition. An "anticancer effect" can also be manifested by the ability of the CARs in prevention of the occurrence of cancer in the first place.

[0055] The term "antigen" or "Ag" refers to a molecule that provokes an immune response. This immune response may involve either antibody production, or the activation of specific immunologically-competent cells, or both. The skilled artisan will understand that any macromolecule, including virtually all proteins or peptides, can serve as an antigen. Furthermore, antigens can be derived from recombinant or genomic DNA. A skilled artisan

will understand that any DNA, which comprises a nucleotide sequences or a partial nucleotide sequence encoding a protein that elicits an immune response therefore encodes an "antigen" as that term is used herein. Furthermore, one skilled in the art will understand that an antigen need not be encoded solely by a full length nucleotide sequence of a gene. The disclosure includes, but is not limited to, the use of partial nucleotide sequences of more than one gene and that these nucleotide sequences are arranged in various combinations to encode polypeptides that elicit the desired immune response. Moreover, a skilled artisan will understand that an antigen need not be encoded by a "gene" at all. It is readily apparent that an antigen can be generated synthesized or can be derived from a biological sample, or might be macromolecule besides a polypeptide. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a fluid with other biological components.

[0056] Non-limiting examples of target antigens include: CD5; CD19; CD123; CD22; CD30; CD171; CS1 (also referred to as CD2 subset 1, CRACC, MPL, SLAMF7, CD319, and 19A24); C-type lectin-like molecule-1 (CLL-1 or CLECL1); CD33; epidermal growth factor receptor variant III (EGFRviii); ganglioside G2 (GD2); ganglioside GD3 (aNeu5Ac(2-8)aNeu5Ac(2-3)bDGalp(1-4)bDGlc(1-1)Cer); TNF receptor family member B cell maturation (BCMA); Tn antigen ((Tn Ag) or (GalNAc α -Ser/Thr)); prostate-specific membrane antigen (PSMA); Receptor tyrosine kinase-like orphan receptor 1 (ROR1); Fms Like Tyrosine Kinase 3 (FLT3); Tumor-associated glycoprotein 72 (TAG72); CD38; CD44v6; a glycosylated CD43 epitope expressed on acute leukemia or lymphoma but not on hematopoietic progenitors, a glycosylated CD43 epitope expressed on non-hematopoietic cancers, Carcinoembryonic antigen (CEA); Epithelial cell adhesion molecule (EPCAM); B7H3 (CD276); KIT (CD117); Interleukin-13 receptor subunit alpha-2 (IL-13Ra2 or CD213A2); Mesothelin; Interleukin 11 receptor alpha (IL-11Ra); prostate stem cell antigen (PSCA); Protease Serine 21 (Testisin or PRSS21); vascular endothelial growth factor receptor 2 (VEGFR2); Lewis(Y) antigen; CD24; Platelet-derived growth factor receptor beta (PDGFR-beta); Stage-specific embryonic antigen-4 (SSEA-4); CD20; Folate receptor alpha (FRa or FR1); Folate receptor beta (FRb); Receptor tyrosine-protein kinase ERBB2 (Her2/neu); Mucin 1, cell surface associated (MUC1); epidermal growth factor receptor (EGFR); neural cell adhesion molecule (NCAM); Prostase; prostatic acid phosphatase (PAP); elongation factor 2 mutated (ELF2M); Ephrin B2; fibroblast activation protein alpha (FAP); insulin-like growth factor 1 receptor (IGF-I receptor), carbonic anhydrase IX (CAIX); Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2); glycoprotein 100 (gp100); oncogene fusion protein consisting of breakpoint cluster region (BCR) and Abelson murine

leukemia viral oncogene homolog 1 (Abl) (bcr-abl); tyrosinase; ephrin type-A receptor 2 (EphA2); sialyl Lewis adhesion molecule (sLe); ganglioside GM3 (aNeu5Ac(2-3)bDCIalp(1-4)bDGlc(1-1)Cer); transglutaminase 5 (TGS5); high molecular weight-melanoma associated antigen (HMWMAA); o-acetyl-GD2 ganglioside (OAcGD2); tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related (TEM7R); claudin 6 (CLDN6); thyroid stimulating hormone receptor (TSHR); G protein coupled receptor class C group 5, member D (GPRC5D); chromosome X open reading frame 61 (CXORF61); CD97; CD179a; anaplastic lymphoma kinase (ALK); Polysialic acid; placenta-specific 1 (PLAC1); hexasaccharide portion of globoH glycosphingolipid (GloboH); mammary gland differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3 (ADRB3); pannexin 3 (PANX3); G protein-coupled receptor 20 (GPR20); lymphocyte antigen 6 complex, locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame Protein (TARP); Wilms tumor protein (WT1); Cancer/testis antigen 1 (NY-ESO-1); Cancer/testis antigen 2 (LAGE-1a); Melanoma-associated antigen 1 (MAGE-A1); ETS translocation-variant gene 6, located on chromosome 12p (ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, Member 1A (XAGE1); angiopoietin-binding cell surface receptor 2 (Tie 2); melanoma cancer testis antigen-1 (MAD-CT-1); melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; tumor protein p53 (p53); p53 mutant; prostein; survivin; telomerase; prostate carcinoma tumor antigen-1 (PCT A-1 or Galectin 8), melanoma antigen recognized by T cells 1 (MelanA or MART1); Rat sarcoma (Ras) mutant; human Telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoints; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease, serine 2 (TMPRSS2) ETS fusion gene); N-Acetyl glucosaminyl-transferase V (NA17); paired box protein Pax-3 (PAX3); Androgen receptor; Cyclin B1; v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Tyrosinase-related protein 2 (TRP-2); Cytochrome P450 1B 1 (CYP1B 1); CCCTC-Binding Factor (Zinc Finger Protein)-Like (BORIS or Brother of the Regulator of Imprinted Sites), Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3); Paired box protein Pax-5 (PAX5); proacrosin binding protein sp32 (OY-TESI); lymphocyte-specific protein tyrosine kinase (LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint 2 (SSX2); Receptor for Advanced Glycation Endproducts (RAGE-1); renal ubiquitous 1 (RUI); renal ubiquitous 2 (RU2); legumain; human papilloma virus E6 (HPV E6); human papilloma virus E7 (HPV E7); intestinal carboxyl esterase; heat shock protein 70-2 mutated (mut hsp70-2); CD79a; CD79b; CD72;

Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR or CD89); Leukocyte immunoglobulin-like receptor subfamily A member 2 (LILRA2); CD300 molecule-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2); lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); and immunoglobulin lambda-like polypeptide 1 (IGLL1), MPL, Biotin, c-MYC epitope Tag, CD34, LAMP1 TROP2, GFRalpha4, CDH17, CDH6, NYBR1, CDH19, CD200R, Slea (CA19.9; Sialyl Lewis Antigen); Fucosyl-GM1, PTK7, gpNMB, CDH1-CD324, DLL3, CD276/B7H3, IL11Ra, IL13Ra2, CD179b-IGL1, TCRgamma-delta, NKG2D, CD32 (FCGR2A), Tn ag, Tim1-/HVCR1, CSF2RA (GM-CSFR-alpha), TGFbetaR2, , Lews Ag, TCR-beta1 chain, TCR-beta2 chain, TCR-gamma chain, TCR-delta chain, FITC, Leutenizing hormone receptor (LHR), Follicle stimulating hormone receptor (FSHR), Gonadotropin Hormone receptor (CGHR or GR), CCR4, GD3, SLAMF6, SLAMF4, HIV1 envelope glycoprotein, HTLV1-Tax, CMV pp65, EBV-EBNA3c, KSHV K8.1, KSHV-gH, influenza A hemagglutinin (HA), GAD, PDL1, Guanylyl cyclase C (GCC), auto antibody to desmoglein 3 (Dsg3), auto antibody to desmoglein 1 (Dsg1), HLA, HLA-A, HLA-A2, HLA-B, HLA-C, HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, HLA-DR, HLA-G, IgE, CD99, Ras G12V, Tissue Factor 1 (TF1), AFP, GPRC5D, Claudin18.2 (CLD18A2 or CLDN18A.2), P-glycoprotein, STEAP1, Liv1, Nectin-4, Cripto, gpA33, BST1/CD157, low conductance chloride channel, and the antigen recognized by TNT antibody.

[0057] The term "antigen presenting cell" or "APC" refers to an immune system cell such as an accessory cell (*e.g.*, a B-cell, a dendritic cell, and the like) that displays a foreign antigen complexed with major histocompatibility complexes (MHC's) on its surface. T-cells may recognize these complexes using their T-cell receptors (TCRs). APCs process antigens and present them to T-cells.

[0058] The term "anti-infection effect" refers to a biological effect which can be manifested by various means, including but not limited to, *e.g.*, decrease in the titer of the infectious agent, a decrease in colony counts of the infectious agent, amelioration of various physiological symptoms associated with the infectious condition. An "anti-infectious effect" can also be manifested by the ability of the peptides, polynucleotides, cells and antibodies in prevention of the occurrence of infection in the first place.

[0059] The term "antitumor effect" or "anti-cancer effect" refers to a biological effect which can be manifested by various means, including but not limited to, *e.g.*, a decrease in

tumor volume, a decrease in the number of tumor cells, a decrease in tumor cell proliferation, or a decrease in tumor cell survival.

[0060] An “antigen binding domain” or “antigen binding module” or “antigen binding segment” or “antigen specific domain” (ASD) refers to a polypeptide or peptide that due to its primary, secondary or tertiary sequence, post-translational modifications and/or charge binds to an antigen with a high degree of specificity. The antigen binding domain may be derived from different sources, for example, an antibody (full length heavy chain, Fab fragments, single chain Fv (scFv) fragments, divalent single chain antibodies or diabodies), a non-immunoglobulin binding protein, a ligand or a receptor. There are, however, numerous alternatives, such as linked cytokines (which leads to recognition of cells bearing the cytokine receptor), affibodies, ligand binding domains from naturally occurring receptors, soluble protein/peptide ligand for a receptor (for example on a tumor cell), peptides, and vaccines to prompt an immune response, which may each be used in various embodiments of the invention. In some embodiments, almost any molecule that binds a given antigen with high affinity can be used as an ASD, as will be appreciated by those of skill in the art. In some embodiments, the antigen binding domain comprises T cell receptors (TCRs) or portions thereof. In exemplary embodiments, nucleic acids encoding antigen binding domains comprising scFVs are set forth herein in SEQ ID NOs: 642-902 and in **Table 6C**. In exemplary embodiments, amino acids encoding antigen binding domains comprising scFVs are set forth herein in SEQ ID NOs: 4555-4815 in **Table 6C**.

[0061] The term “Association constant (K_a)” is defined as the equilibrium constant of the association of a receptor and ligand.

[0062] “Autoantibody” refers to an antibody that is produced by a B-cell specific for an autoantigen.

[0063] The term “autoantigen” refers to an endogenous antigen that stimulates production of an autoimmune response, such as production of autoantibodies. Autoantigen also includes a self-antigen or antigen from a normal tissue that is the target of a cell mediated or an antibody-mediated immune response that may result in the development of an autoimmune disease. Examples of autoantigens include, but are not limited to, desmoglein 1, desmoglein 3, and fragments thereof.

[0064] “Avidity” refers to the strength of the interaction between a binding agent and its target (*e.g.*, the strength of the interaction between an antibody and its antigen target, a receptor and its cognate and the like). The avidity can be weak or strong. Methods for calculating the affinity of an antibody for an antigen are known in the art, including use of

binding experiments to calculate affinity. Antibody activity in functional assays (*e.g.*, flow cytometry assay) is also reflective of antibody affinity. Antibodies and affinities can be phenotypically characterized and compared using functional assays (*e.g.*, flow cytometry assay).

[0065] As used herein, the term “backbone” refers to the specific combination of CARs (**Table 1**) and accessory modules as described in **Table 2**. In exemplary embodiments, specific combinations of CARs and accessory modules which comprise various backbones are described in **Table 2**. In one embodiment, the CAR and the accessory module are encoded by a single nucleic acid molecule. In another embodiment, the CAR is encoded by the first nucleic acid molecule and the accessory module is encoded by a second nucleic acid molecule. In some embodiments, the accessory module is encoded by more than one nucleic acid molecule, depending on the number of components in the accessory modules.

[0066] As used herein “beneficial results” may include, but are in no way limited to, lessening or alleviating the severity of the disease condition, preventing the disease condition from worsening, curing the disease condition, preventing the disease condition from developing, lowering the chances of a patient developing the disease condition and prolonging a patient’s life or life expectancy. As non-limiting examples, “beneficial results” or “desired results” may be alleviation of one or more symptom(s), diminishment of extent of the deficit, stabilized (*i.e.*, not worsening) state of cancer progression, delay or slowing of metastasis or invasiveness, and amelioration or palliation of symptoms associated with the cancer.

[0067] As used herein, the term “binding domain” or “antibody molecule” refers to a protein, *e.g.*, an immunoglobulin chain or fragment thereof, ligand domain or fragment thereof (as the case may be), comprising at least one domain, *e.g.*, immunoglobulin variable domain sequence that can bind to a target with affinity higher than a non-specific domain. The term encompasses antibodies and antibody fragments, or ligands and ligand fragments. In another embodiment, an antibody molecule is a multispecific antibody molecule, *e.g.*, it comprises a plurality of immunoglobulin variable domain sequences, wherein a first immunoglobulin variable domain sequence of the plurality has binding specificity for a first epitope and a second immunoglobulin variable domain sequence of the plurality has binding specificity for a second epitope. In another embodiment, a multispecific antibody molecule is a bispecific antibody molecule. A bispecific antibody has specificity for two antigens. A bispecific antibody molecule is characterized by a first immunoglobulin variable domain sequence which has binding specificity for a first epitope and a second immunoglobulin

variable domain sequence that has binding specificity for a second epitope. A bispecific molecule may be a bispecific T cell engaging antibody in which first antigen binding domain binds to an antigen (*e.g.*, CD3e) expressed on T cells and the second antigen binding domain binds to an antigen expressed on a disease causing or disease associated cell (*e.g.*, a cancer cell). The bispecific antibodies can be used for inducing T cell mediated cytotoxicity against cells expressing the target antigen recognized by their second antigen binding domain. The antigen binding domains described in this disclosure can be used to construct bispecific T cell engagers. The nucleic acid sequences of exemplary bispecific T cell engagers comprising the antigen binding domains (*e.g.* scFv) described in this disclosure are presented in SEQ ID NO: 3545-3830 (**Table 13**). The corresponding amino acid sequences are presented in SEQ ID NO: 7458-7721.

[0068] "Binds the same epitope as" means the ability of an antibody, scFv, or other antigen binding domain to bind to a target antigen and having the same epitope as an exemplified antibody, scFv, or other antigen binding domain. As an example, the epitopes of the exemplified antibody, scFv, or other binding agent and other antibodies can be determined using standard epitope mapping techniques. Epitope mapping techniques, well known in the art include Epitope Mapping Protocols in Methods in Molecular Biology, Vol. 66 (Glenn E. Morris, Ed., 1996) Humana Press, Totowa, New Jersey. For example, linear epitopes may be determined by, *e.g.*, concurrently synthesizing large numbers of peptides on solid supports, the peptides corresponding to portions of the protein molecule, and reacting the peptides with antibodies while the peptides are still attached to the supports. Such techniques are known in the art and described in, *e.g.*, U.S. Patent No. 4,708,871; Geysen et al, (1984) Proc. Natl. Acad. Sci. USA 81:3998-4002; Geysen et al, (1985) Proc. Natl. Acad. Sci. USA 82:78-182; Geysen et al, (1986) Mol. Immunol. 23: 709-715. The epitope bound by the antigen binding domain of a CAR can be also determined by the Epitope Binning assay. Epitope binning is a competitive immunoassay used to characterize and then sort a library of monoclonal antibodies against a target protein. Antibodies against a similar target are tested against all other antibodies in the library in a pairwise fashion to see if antibodies block one another's binding to the epitope of an antigen. After each antibody has a profile created against all of the other antibodies in the library, a competitive blocking profile is created for each antibody relative to the others in the library. Closely related binning profiles indicate that the antibodies have the same or a closely related epitope and are "binned" together. Similarly, conformational epitopes are readily identified by determining spatial conformation of amino acids such as by, *e.g.*, hydrogen/deuterium exchange, x-ray crystallography and

two-dimensional nuclear magnetic resonance. See, *e.g.*, Epitope Mapping Protocols, *supra*. Antigenic regions of proteins can also be identified using standard antigenicity and hydropathy plots, such as those calculated using, *e.g.*, the Omega version 1.0 software program available from the Oxford Molecular Group. This computer program employs the Hopp/Woods method, Hopp et al, (1981) Proc. Natl. Acad. Sci USA 78:3824-3828; for determining antigenicity profiles, and the Kyte-Doolittle technique, Kyte et al, (1982) J.Mol. Biol. 157: 105-132; for hydropathy plots. To determine if selected monoclonal antibodies against a target (*e.g.*, CD19) bind to unique epitopes, each antibody can be biotinylated using commercially available reagents (Pierce, Rockford, Ill.). Competition studies using unlabeled monoclonal antibodies and biotinylated monoclonal antibodies can be performed using CD19-extracellular domain coated-ELISA plates. Biotinylated mAb binding can be detected with a strep-avidin-alkaline phosphatase probe. Exemplary epitopes of human CD20 antigen bound by scFv and CARs of the current disclosure are provided in SEQ ID NO: 15149-15154. Exemplary epitopes of human BCMA bound by scFv and CARs of the current disclosure are provided in SEQ ID NO: 15155-15159. An exemplary epitope of human MPL antigen bound by scFv and CARs of the current disclosure is provided in SEQ ID NO: 15160.

[0069] As used herein, the term "biological equivalent thereof" is intended to be synonymous with "equivalent thereof" when referring to a reference protein, antibody or fragment thereof, polypeptide or nucleic acid, intends those having minimal homology while still maintaining desired structure or functionality. Unless specifically recited herein, it is contemplated that any of the above also includes equivalents thereof. For example, an equivalent intends at least about 70% homology or identity, or at least 80% homology or identity and alternatively, or at least about 85%, or alternatively at least about 90%, or alternatively at least about 95%, or alternatively at least 98% percent homology or identity and exhibits substantially equivalent biological activity to the reference protein, polypeptide, antibody or fragment thereof or nucleic acid. Alternatively, when referring to polynucleotides, an equivalent thereof is a polynucleotide that hybridizes under stringent conditions to the reference polynucleotide or its complement. Alternatively, when referring to polypeptides or proteins, an equivalent thereof is an expressed polypeptide or protein from a polynucleotide that hybridizes under stringent conditions to the polynucleotide or its complement that encodes the reference polypeptide or protein.

[0070] As used herein, the term "CDR" or "complementarity determining region" is intended to mean the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. These particular regions have been

described by Kabat *et al.*, J. Bioi. Chem. 252:6609-6616 (1977); Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequences of proteins of immunological interest" (1991); Chothia *et al.*, J. Mol. Bioi. 196:901-917 (1987); and MacCallum *et al.*, J. Mol. Bioi. 252:732-745 (1996), where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or grafted antibodies or variants thereof is intended to be within the scope of the term as defined and used herein. As used herein, the different CDRs of an antibody could be also defined by a combination of the different definitions. For example, vHCDR1 could be defined based on Kabat and VHCDR2 could be defined based on Chothia. The amino acid residues which encompass the CDRs as defined by each of the above cited references are as follows:

	CDR DEFINITIONS		
	Kabat	Chothia	MacCallum
VHCDR1	31-35	26-32	30-35
VHCDR2	50-65	53-55	47-58
VHCDR3	95-102	96-10	193-101
VLCDR1	24-34	26-32	30-36
VLCDR2	50-56	50-52	46-55
VLCDR3	89-97	91-96	89-96

(Residue Numbers correspond to the identified reference).

[0071] The SEQ IDs of the CDRs of the different vL and vH segments that can make up antigen binding domains of CARs of the disclosure are provided in SEQ ID NO: 13204-14121 and SEQ ID NO: 14122-15039, respectively (**Tables 6A, B**) and in Tables 5-6 in PCT/US2017/064379, which are incorporated herein by reference.

[0072] In some embodiments, reference to an antigen-binding module (such as a Fab-like or Fv-like antigen-binding module) that specifically binds to a target antigen means that the antigen-binding module binds to the target antigen with (a) an affinity that is at least about 10 (*e.g.*, about 10, 20, 30, 40, 50, 75, 100, 200, 300, 400, 500, 750, 1000 or more) times its binding affinity for other molecules; or (b) a K_d no more than about 1/10 (*e.g.*, 1/10, 1/20, 1/30, 1/40, 1/50, 1/75, 1/100, 1/200, 1/300, 1/400, 1/500, 1/750, 1/1000 or less) times its K_d for binding to other molecules. Binding affinity can be determined by methods known in the art, such as ELISA, fluorescence activated cell sorting (FACS) analysis, or radioimmunoassay (RIA). K_d can be determined by methods known in the art, such as surface plasmon resonance (SPR) assay utilizing, for example, Biacore instruments, or kinetic exclusion assay (KinExA) utilizing, for example, Sapidyne instruments.

[0073] “Cancer” and “cancerous” refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to B-cell lymphomas (Hodgkin’s lymphomas and/or non-Hodgkins lymphomas), T cell lymphomas, myeloma, myelodysplastic syndrome, skin cancer, brain tumor, breast cancer, colon cancer, rectal cancer, esophageal cancer, anal cancer, cancer of unknown primary site, endocrine cancer, testicular cancer, lung cancer, hepatocellular cancer, gastric cancer, pancreatic cancer, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, cancer of reproductive organs thyroid cancer, renal cancer, carcinoma, melanoma, head and neck cancer, brain cancer (*e.g.*, glioblastoma multiforme), prostate cancer, including but not limited to androgen-dependent prostate cancer and androgen-independent prostate cancer, and leukemia. Other cancer and cell proliferative disorders will be readily recognized in the art. The terms “tumor” and “cancer” are used interchangeably herein, *e.g.*, both terms encompass solid and liquid, *e.g.*, diffuse or circulating, tumors. As used herein, the term “cancer” or “tumor” includes premalignant, as well as malignant cancers and tumors. The term “cancer” is meant to include all types of cancerous growths or oncogenic processes, metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. Exemplary solid tumors include malignancies, *e.g.*, adenocarcinomas, sarcomas, and carcinomas, of the various organ systems, such as those affecting breast, liver, lung, brain, lymphoid, gastrointestinal (*e.g.*, colon), genitourinary tract (*e.g.*, renal, urothelial cells), prostate and pharynx. Adenocarcinomas include cancers such as most colon cancers, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus. In one embodiment, the cancer is a melanoma, *e.g.*, an advanced stage melanoma. Metastatic lesions of the aforementioned cancers can also be treated or prevented using the methods and compositions of the disclosure. Examples of other cancers that can be treated or prevented include pancreatic cancer, bone cancer, skin cancer, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the head or neck, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin Disease, non-Hodgkin lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic

myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, and combinations of said cancers. Treatment of metastatic cancers, *e.g.*, metastatic cancers that express PD-L1 (Iwai *et al.* (2005) *Int. Immunol.* 17:133-144) can be effected using the antibody molecules described herein. Exemplary cancers whose growth can be inhibited include cancers typically responsive to immunotherapy. Additionally, recurrent or are refractory malignancies can be treated using the molecules described herein.

[0074] “Chemotherapeutic agents” are compounds that are known to be of use in chemotherapy for cancer. Non-limiting examples of chemotherapeutic agents can include alkylating agents such as thiotepa and CYTOXAN® cyclophosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (*e.g.*, calicheamicin, especially calicheamicin gammaII and calicheamicin omegaII (see, *e.g.*, Agnew, *Chem. Intl. Ed. Engl.*, 33: 183-186 (1994)); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabycin, caminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® doxorubicin (including morpholino-doxorubicin, cyanomorpholino-

doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitio stanol, mepitio stanol, testolactone; anti-adrenals such as aminogluthethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, *e.g.*, TAXOL® paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE® Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumburg, Ill.), and TAXOTERE® doxetaxel (Rhone-Poulenc Rorer, Antony, France); chloranbucil; GEMZAR® gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as *cis*platin, oxaliplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE; vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (Camptosar, CPT-11) (including the treatment regimen of irinotecan with 5-FU and leucovorin); topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine; combretastatin; leucovorin (LV); oxaliplatin, including the oxaliplatin treatment regimen (FOLFOX); lapatinib (Tykerb); inhibitors of PKC- α , Raf, H-Ras, EGFR (*e.g.*, erlotinib (Tarceva®)) and VEGF-A that reduce cell proliferation and pharmaceutically acceptable salts, acids or derivatives of any of the above or combinations thereof.

[0075] “Chimeric antigen receptors” (CARs) are artificial (non-naturally occurring) immune cell (*e.g.*, T cell) receptors contemplated for use as a therapy for cancer, using a technique called adoptive cell transfer. CARs are also known as artificial T-cell receptors, chimeric T-cell receptors or chimeric immunoreceptors. The antigen-binding, signaling, and stimulatory functions of the complex have been manipulated by genetic recombination methods to a single polypeptide chain, generally referred to as a Chimeric Antigen Receptor (CAR). See, *e.g.*, Eshhar, U.S. Pat. No. 7,741,465; Eshhar, U.S. Patent Application Publication No. 2012/0093842. CARs are constructed specifically to stimulate T cell activation and proliferation in response to a specific antigen to which the CAR binds. Generally, a CAR refers to a set of polypeptides, typically two in the simplest embodiments, which when expressed in an immune effector cell, provides the cell with specificity for a target cell, typically a cancer cell, and with intracellular signal generation. In some embodiments, a CAR comprises at least an extracellular antigen binding domain, a transmembrane domain and a cytoplasmic signaling domain (also referred to herein as “an intracellular signaling domain”) comprising a functional signaling domain derived from a stimulatory molecule and/or costimulatory molecule. In some aspects, the set of polypeptides are contiguous with each other. In one aspect, the stimulatory molecule is the zeta chain associated with the T cell receptor complex. In one aspect, the cytoplasmic signaling domain further comprises one or more functional signaling domains derived from at least one costimulatory molecule as defined below. In one embodiment, the costimulatory molecule is chosen from the costimulatory molecules described herein, *e.g.*, 4-1BB (*i.e.*, CD137), CD27 and/or CD28. In one embodiment, the CAR comprises an optional leader sequence at the amino-terminus (N-ter) of the CAR fusion protein. In one embodiment, the CAR further comprises a leader sequence at the N-terminus of the extracellular antigen binding domain, wherein the leader sequence is optionally cleaved from the antigen binding domain (*e.g.*, a scFv) during cellular processing and localization of the CAR to the cellular membrane. In various embodiments, CARs are recombinant polypeptides comprising an antigen-specific domain (ASD), a hinge region (HR), a transmembrane domain (TMD), an optional co-stimulatory domain (CSD) and an intracellular signaling domain (ISD). The optional costimulatory domain is generally absent in the 1st generation CAR constructs. The target antigen, antigen binding domain name and nucleic acid sequences of several exemplary 1st generation CARs comprising the different antigen binding domains (*e.g.*, vL and vH fragments, vHH, ligands and receptors *etc.*) described in this disclosure and coexpressing the accessory modules encoding NEMO-K277A and PAC are presented in SEQ ID NO: 1594-

1899 (**Tables 12**). These CAR constructs carry a human CD8 signal peptide, a CD8 hinge and transmembrane region and human CD3 ζ intracellular signaling domain. These constructs also carry a MYC linker between the antigen binding domain and the CD8 hinge region, which is optional. The nucleic acid sequences of several exemplary 1st generation CARs comprising the different antigen binding domains (*e.g.*, vL and vH fragments, vHH, ligands and receptors *etc.*) described in this disclosure and coexpressing the accessory modules encoding vFLIP K13 and PAC are presented in SEQ ID NO: 1016-1317 (**Table 13**). The nucleic acid sequences of several exemplary 2nd generation CARs comprising the different antigen binding domains (*e.g.*, vL and vH fragments, vHH, ligands and receptors *etc.*) described in this disclosure and incorporating the 41BB costimulatory domain are presented in SEQ ID NO: 1318-1593 (**Table 13**). These CAR constructs also carry a MYC linker between the antigen binding domain and the transmembrane domain and an accessory module encoding puromycin resistance gene (PAC) that is separated from the CAR cassette by a Furine-SGSG-T2A sequence. The accessory module encoding vFLIP-K13, NEMO-K277A and PAC are optional in the above described 1st and 2nd generation CARs. Thus, CARs with the antigen binding domains (*i.e.*, vL and vH fragments, vHH, ligands and receptors *etc.*) described in this disclosure can be constructed without vFLIP-K13, NEMO-K277A and/or PAC. As such, these accessory modules along with the upstream cleavage linker sequences (*e.g.*, F2A, P2A, or T2A) can be deleted from the CARs represented by SEQ ID NO: 1016-1899. Alternatively, the accessory module encoding vFLIP-K13, NEMO-K277A and/or PAC can be replaced by accessory modules encoding other proteins, such as hNEMO-K277A-deltaV249-K555, mNEMO-K270A, K13-opt, IKK2-S177E-S181E, or IKK1-S176E-S180E, and MyD88-L265P, FKBPx2-NEMO, NEMO-L600-FKBPx2, TCL-1A, MTCP-1, and CMV-141 *etc.* As used herein, the term “CAR” or “CARs” also encompasses newer approaches to conferring antigen specificity onto cells, such as Antibody-TCR chimeric molecules or Ab-TCR or Ab-TCR (WO 2017/070608 A1 incorporated herein by reference), TCR receptor fusion proteins or TFP (WO 2016/187349 A1 incorporated herein by reference), Synthetic Immune Receptors (SIRs) (see, WO 2018/102795 A1, incorporated herein by reference), Tri-functional T cell antigen coupler (Tri-TAC) (see, WO 2015/117229 A1, incorporated herein by reference). The nucleic acid sequences of several exemplary TFPs comprising the different antigen binding domains (*e.g.*, vL and vH fragments, vHH, ligands and receptors *etc.*) described in this disclosure and based on CD3 ϵ , CD3 δ , CD3 γ and CD3 ζ chains and co-expressing the optional accessory module NEMO-K277A are presented in SEQ ID NO: 1900-2205, 2206-2511, 2512-2817, 2818-3123,

respectively (**Table 13**). The order of the antigen binding domains contained in the construct of different CAR architectures and BiTE listed in **Table 13** is the same as the order of the constructs on the zCAR-K277A architecture presented in **Table 12**. Thus, the amino acid and nucleic acid SEQ ID NO of a CAR belonging to a given architecture (e.g., zCAR-K13) and containing a specific antigen binding domain can be determined by examination of **Tables 12** and **Table 13**. Thus, **Table 12** shows that a CAR on the zCAR-NEMO-K277A architecture and containing the huFMC63-11-(vL-vH) antigen binding domain is the 2nd construct in the **Table 12** and is represented by nucleic acid and amino acid SEQ ID NOs: 1595 and 5508, respectively. The nucleic acid and amino acid SEQ ID Nos of a corresponding CAR on the zCAR-K13 architecture can be determine by examination of **Table 13** which shows that the 2nd construct on this architecture has the nucleic acid and amino acid SEQ ID NOs: 1017 and 4930, respectively. A similar approach can be used to determine the nucleic acid and amino acid SEQ ID Nos of other CAR constructs belonging to different architectures and BiTEs. **Table 10** provides the nucleic acid and amino acid SEQ ID Nos of several exemplary CARs belonging to different backbones and targeting HIV-1 Envelop Glycoprotein based on HIV1-N49P6 vL and vH antigen binding domains. **Table 11** provides the nucleic acid and amino acid SEQ ID Nos of several exemplary CARs belonging to the backbones shown in **Table 10** but containing different antigen binding domains. Thus, the nucleic acid and amino acid SEQ ID Nos of a CAR on a particular backbone containing the antigen binding domain shown in **Table 11** can be determined by first determining its rank order in the **Table 10**. Thus, since the 1st generation CAR containing the vFLIP-K13 backbone is the third CAR on the list in **Table 10**, the nucleic acid SEQ ID NO of a 1st generation CAR co-expressing vFLIP-K13 and containing the HIV1-N49P7 antigen binding domain can be easily determined from **Table 11** to be the SEQ ID NO: 8740 (i.e., the 3rd construct in the series starting at 8738). Using a similar approach, the amino acid SEQ ID NO of this CAR construct is determined to be SEQ ID NO: 11438. As the CARs are modular in design, the nucleic acid and amino acid sequence of a CAR/BiTE containing different antigen binding domains or accessory modules can be easily determined by person with ordinary skill in the art by using the sequence of the different modules and exemplary CAR and BiTE constructs disclosed in this disclosure. Typically, “CAR-T cells” are used, which refer to T-cells that have been engineered to express a chimeric antigen receptor. Thus, T lymphocytes bearing such CARs are generally referred to as CAR-T lymphocytes. CARs can be also expressed in cells other than T cells, such as hematopoietic stem cells, induced pluripotent stem cells (iPSC), NK cells and macrophage.

[0076] “Codon optimization” or “controlling for species codon bias” refers to the preferred codon usage of a particular host cell. As will be understood by those of skill in the art, it can be advantageous to modify a coding sequence to enhance its expression in a particular host. The genetic code is redundant with 64 possible codons, but most organisms typically use a subset of these codons. The codons that are utilized most often in a species are called optimal codons, and those not utilized very often are classified as rare or low-usage codons.

[0077] Optimized coding sequences containing codons preferred by a particular prokaryotic or eukaryotic host (see also, Murray *et al.* (1989) Nucl. Acids Res. 17:477-508) can be prepared, for example, to increase the rate of translation or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, as compared with transcripts produced from a non-optimized sequence. Translation stop codons can also be modified to reflect host preference. Those of skill in the art will recognize that, due to the degenerate nature of the genetic code, a variety of DNA compounds differing in their nucleotide sequences can be used to encode a given polypeptide of the disclosure.

[0078] As used herein, “co-express” refers to expression of two or more polynucleotides or genes. Genes may be nucleic acids encoding, for example, a single protein or a chimeric protein as a single polypeptide chain. A CAR or a TCR described herein may be encoded by a single polynucleotide chain and expressed as single polypeptide chain, which is subsequently cleaved into different polypeptides, each representing a distinct functional unit. In some embodiments, where the CAR or a TCR consists of two or more functional polypeptide units, the different functional units are coexpressed using one or more polynucleotide chains. In one embodiment, costimulation is provided by an accessory module that is co-expressed with the CAR or a TCR but is not an integral part of the CAR or TCR polypeptide. Such an accessory module that provides costimulation to a CAR- or TCR-expressing cell or any cell but is not an integral part of the CAR or the TCR polypeptide is termed a CAR independent costimulatory module or CICM. In another embodiment, the different polynucleotide chains are linked by nucleic acid sequences that encode for cleavable linkers (*e.g.* T2A, F2A, P2A, E2A *etc.*) (**Table 6D**). In another embodiment, a Ser-Gly-Ser-Gly (SGSG) motif (SEQ ID NO: 4844) is also added upstream of the cleavable linker sequences to enhance the efficiency of cleavage. The polynucleotides encoding the different units of a CAR or a TCR may be linked by IRES (Internal Ribosomal Entry Site) sequences. Alternately, the different functional units of a CAR or TCR are encoded by two different polynucleotides that are not linked via a linker but are instead encoded by, for example, two

different vectors. The nucleic acid and amino acid sequences of exemplary cleavable linkers and Furine cleavage sites are provided in **Table 6D**.

[0079] A “conservative substitution” or “conservative sequence modifications” refers to amino acid modifications that do not significantly affect or alter the binding characteristics or function of the encoded protein. For example, “conservative sequence modifications” refers to amino acid modifications that do not significantly affect or alter the binding characteristics or function of a CAR construct of the disclosure (*e.g.*, a conservative change in the constant chain, antibody, antibody fragment, or non-immunoglobulin binding domains). Such conservative modifications include amino acid substitutions, additions and deletions. Modifications can be introduced by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within a CAR of the disclosure can be replaced with other amino acid residues from the same side chain family and the altered CAR can be tested using the binding and/or functional assays described herein.

[0080] The term “constant region of T cell receptor-alpha” or “constant chain of T cell receptor-alpha” or “TCR α ” or “C α ” is defined as the protein provided as SEQ ID NO: 15041 or the equivalent residues (*i.e.*, a homolog) from a non-human species, *e.g.*, mouse, rodent, monkey, ape and the like. The disclosure also provides certain mutations to TCR α polypeptides which can be used in the construction of SIRs and Ab-TCR (**Tables 3 and 6D**). For example, sites of mutation in C α that demonstrate increased expression and decreased mispairing are located at positions 91, 92, 93, and 94 of SEQ ID NO 15041. A TCR polypeptide with a Thr 48 Cys (T48C) mutation in C α and a Ser-57-Cys (S57C) mutation in C β 1 or C β 2 chain (described more fully elsewhere herein) results in an additional disulfide bond between the two TCR constant chains (α and β). This, in turn, results in reduced mispairing with endogenous TCR chains in an immune cell and enhanced functionality. Similarly, a CAR with a Ser 61 Arg (S61R) mutation in C α (SEQ ID NO:15048) and an Arg

79 Gly (R79G) mutation in C β 1 or C β 2 chain (described more fully elsewhere herein) results in reduced mispairing with the endogenous TCR chains and enhanced functionality due to a “knob and hole” design for pairing. The disclosure provides C α polypeptides having one or more or all of the mutations according to **Table 3** below which can be used in the construction of SIRs and Ab-TCR.

Table 3 : Mutations according to the disclosure in the human constant TCR-alpha region (Cα)			
Position (SEQ ID NO: 15041)	Amino acid in wild-type	Mutation	TYPE
10	Y	C	disulfide bond
15	S	C	disulfide bond
45	T	C	disulfide bond
48	T	C	disulfide bond
61	S	R	Knob into Hole
91	P	S	Murinization
92	E	D	Murinization
93	S	V	Murinization
94	S	P	Murinization

[0081] The human genome encodes for two highly homologous TCR beta constant chains; TCR beta1 (TCR β 1 or TCRb1 or c β 1) and TCR beta 2 (TCR β 2 or TCRb2 or c β 2). The CARs of the disclosure can comprise either of these two chains. Similarly, either TCR beta1 or TCR beta2 chains of other mammalian species can be used in the methods of the disclosure.

[0082] The term “constant chain of T cell receptor-beta 1” or “constant region of T cell receptor-beta 1” (TCR-beta1 or TCR β 1 or TCRb1 or hTCR-beta1 or C β 1) is defined as a protein provided as SEQ ID NO: 15051 or the equivalent residues (*i.e.*, a homolog) from a non-human species, *e.g.*, mouse, rodent, monkey, ape and the like.

[0083] The term “constant chain of T cell receptor-beta 2” or “constant region of T cell receptor-beta 2” (TCR-beta2 or TCR β 2 or TCRb2 or C β 2) is defined as the protein provided as SEQ ID NO: 15052 or the equivalent residues (*i.e.*, a homolog) from a non-human species, *e.g.*, mouse, rodent, monkey, ape and the like.

[0084] The term “constant chain of T cell receptor-beta” or “constant region of T cell receptor-beta” (TCR-beta or TCR β or TCRb or C β) is defined as the protein provided as SEQ ID NO: 15051-15053 or the equivalent residues (*i.e.*, a homolog) from a non-human species, *e.g.*, mouse, rodent, monkey, ape and the like.

[0085] The protein sequences for both C β 2 (SEQ ID NO: 15052) and C β 1 (SEQ ID NO: 15051) are known (**Table 6D**). Differences between the sequences of C β 2 and β 1 are easily identified by alignment of the sequences using typical and ordinary skill in the art. The

disclosure also provides certain mutations to TCR β 's that can be used in the construction of SIRs and Ab-TCRs. For example, sites of mutation in C β s that demonstrate increased expression and decreased mispairing with the endogenous TCR α chains are provided herein. These mutation sites in C β 1 and C β 2 are located at positions 18, 22, 57, 79, 133, 136, and 139 of SEQ ID NOs: 15051 and 15052 and are summarized in the **Tables 4** and **5** below. The mutation sites in C β 1 and C β 2 are identical in their positions. The only difference between the two sequences is that a mutation at position 136. At this position, a glutamic acid (E) is present in C β 2, whereas a valine is present in C β 1.

Table 4: Mutations according to the disclosure in the human constant TCR-beta region1 (Cβ1)			
Position (SEQ ID NO: 15051)	Amino acid in wild-type	Mutation	TYPE
15	E	C	disulfide bond
17	S	C	disulfide bond
18	E	K or R	Murinization
22	S	A	Murinization
57	S	C	disulfide bond
59	D	C	disulfide bond
77	S	C	disulfide bond
79	R	G	Knob into Hole
133	F	I	Murinization
136	V	A	Murinization
139	Q	H	Murinization
Table 5: Mutations according to the disclosure in the human constant TCR-beta region2 (Cβ2)			
Position (SEQ ID NO: 15052)	Amino acid in wild-type	Mutation	TYPE
15	E	C	disulfide bond
17	S	C	disulfide bond
18	E	K or R	Murinization
22	S	A	Murinization
57	S	C	disulfide bond
59	D	C	disulfide bond
77	S	C	disulfide bond
79	R	G	Knob into Hole
133	F	I	Murinization
136	E	A	Murinization
139	Q	H	Murinization

[0086] The term “constant chain of TCR-gamma” or “constant region of TCR-gamma” (TCR-gamma or TCR γ or TCRg or TCR-gamma1 or TCR γ 1 or TCRg1 or C γ) is defined as the protein provided as SEQ ID NO: 15068 or the equivalent residues (*i.e.*, a homolog) from a non-human species, *e.g.*, mouse, rodent, monkey, ape and the like.

[0087] The term “constant chain of TCR-delta” or “constant region of TCR-delta” (TCR-delta or TCR δ or TCRd or C δ) is defined as the proteins provided as SEQ ID NO: 15069 or the equivalent residues (*i.e.*, a homolog) from a non-human species, *e.g.*, mouse, rodent, monkey, ape and the like.

[0088] It will be recognized that proteins can have identity or homology to one another and retain similar or identical functions. The disclosure includes TCR constant regions that have 85%, 90%, 95%, 97%, 98%, 98.5%, 99% or 99.9% identity to any of the sequences described herein while retaining the biological activity.

[0089] Accordingly, the disclosure provides a T-cell receptor constant chain having a sequence selected from the group consisting of: (a) an amino acid sequence that is at least 98% identical to SEQ ID NO:15041 and which can have one or more mutations at positions 61, 91, 92, 93, and/or 94; (b) an amino acid sequence that is at least 98% identical to SEQ ID NO:15051 and can have one or more mutations at positions 18, 22, 57, 79, 133, 136 and/or 139; (c) an amino acid sequence that is at least 98% identical to SEQ ID NO:15052 and can have one or more mutations at position 18, 22, 57, 79, 133, 136 and/or 139; (d) an amino acid sequence that is at least 98% identical to SEQ ID NO:15068; and (e) an amino acid sequence that is at least 98% identical to SEQ ID NO:15069. The T-cell receptor constant chains of any of (a)-(e) retain at least one biological activity of the wild-type T-cell receptor constant chain to which it has identity or homology.

[0090] The term “constitutively active” refers to a molecule, *e.g.*, a protein, that has signaling activity without the need of a stimulus. Exemplary constitutive active proteins are NEMO-K277A and vFLIP K13 as they can activate NF- κ B signaling when expressed in a suitable cell without the need of an additional stimulus.

[0091] The term a “costimulatory molecule” or a “costimulatory receptor” refers to a cognate binding partner on a T cell that specifically binds with a costimulatory ligand, thereby mediating a costimulatory response by the T cell, such as, but not limited to, proliferation. Costimulatory extracellular molecules are cell surface molecules other than antigen receptors or their ligands that contribute to an efficient immune response. Costimulatory molecules include, but are not limited to an MHC class I molecule, BTLA and a Toll ligand receptor, as well as OX40, Dap10, CD27, CD28, CD2, CD5, CD8, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), Lck, TNFR-I, TNFR-II, Fas, CD30, CD40 and 4-1BB (CD137). Further examples of such costimulatory molecules include CD8, ICAM-1, GITR, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD160, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4,

VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IP0-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand that specifically binds with CD83. A co-stimulatory receptor may be expressed on cells other T cells, such as NK cells or macrophages.

[0092] A “costimulatory intracellular signaling domain” or “costimulatory domain” (CSD) can be the intracellular portion of a costimulatory receptor. A costimulatory molecule can be represented in the following protein families: TNF receptor proteins, Immunoglobulin-like proteins, cytokine receptors, integrins, signaling lymphocytic activation molecules (SLAM proteins), and activating NK cell receptors. Examples of such molecules include CD27, CD28, 4-1BB (CD137), OX40, GITR, CD30, CD40, ICOS, BAFFR, HVEM, ICAM-1, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD8, CD7, CD287, LIGHT, NKG2C, NKG2D, SLAMF7, NKp80, NKp30, NKp44, NKp46, CD160, B7-H3, and a ligand that specifically binds with CD83, and the like. The intracellular signaling domain can comprise the entire intracellular portion, or the entire native intracellular signaling domain, of the molecule from which it is derived, or a functional fragment or derivative thereof. The CARs of the disclosure may comprise one or more co-stimulatory domains.

[0093] The term “cTCR” refers to a wild-type TCR nucleic acid coding sequence and the corresponding wild-type TCR protein linked to an antigen binding domain. cTCRs are used in some embodiments and reference controls. For example, a cTCR having a CD19 binding domain and a CD19-CAR (comprising a mutant TCR chain and CD19 binding domain) will have different expression and/or difference binding affinities to the target antigen.

[0094] The term “cytosolic” or “cytoplasmic” refers to an agent, e.g., a protein, that is situated in the cytoplasm of a cell in its mature form. A cytosolic protein can translocate into the nucleus but is not a transmembrane protein and is not secreted outside the cell. An exemplary cytosolic protein is vFLIP K13.

[0095] The term “degenerative disorders” refers to a disease that is the result of a continuous process based on degenerative cell changes, affecting tissues or organs, which will increasingly deteriorate over time, whether due to normal bodily wear or lifestyle choices such as exercise or eating habits. Exemplary degenerative diseases include Alzheimer's

disease, Charcot–Marie–Tooth disease, Creutzfeldt–Jakob disease, Friedreich's ataxia, Diabetes mellitus (type II), and Atherosclerosis.

[0096] "Derived from" as that term is used herein, indicates a relationship between a first and a second molecule. It generally refers to structural similarity between the first molecule and a second molecule and does not connote or include a process or source limitation on a first molecule that is derived from a second molecule. For example, in the case of an antigen binding domain that is derived from an antibody molecule, the antigen binding domain retains sufficient antibody structure such that it has the required function, namely, the ability to bind to an antigen. It does not connote or include a limitation to a particular process of producing the antibody, *e.g.*, it does not mean that, to provide the antigen binding domain, one must start with an antibody sequence and delete unwanted sequence, or impose mutations, to arrive at the antigen binding domain.

[0097] "Dimerization molecule," as that term is used herein refers to a molecule that promotes the association of a first switch domain with a second switch domain. In embodiments, the dimerization molecule does not naturally occur in the subject, or does not occur in concentrations that would result in significant dimerization. In embodiments, the dimerization molecule is a small molecule, *e.g.*, rapamycin or a rapalogue, *e.g.*, RAD001, Rimiducid or AP20187. Rimiducid (AP1903) is a lipid-permeable tacrolimus analogue with homodimerizing activity. Rimiducid homodimerizes an analogue of human protein FKBP12 (Fv) which contains a single acid substitution (Phe36Val). Rimiducid is used to homodimerize the Fv-containing drug-binding domains of non-naturally occurring immune receptor resulting in downstream signaling activation during cell therapy. Rimiducid can be at about 0.01-1 mg/kg and has an EC₅₀ in cell culture of about 0.1nM. AP20187 can be administered from about 2-10 mg/kg/day in single or multi-doses.

[0098] The phrase "disease associated with expression of a target antigen" or "disease associated antigen as described herein" includes, but is not limited to, a disease associated with expression of a target antigen as described herein or condition associated with cells which express a target antigen as described herein including, *e.g.*, proliferative diseases such as a cancer or malignancy or a precancerous condition such as a myelodysplasia, a myelodysplastic syndrome or a pre leukemia; or a noncancer related indication associated with cells which express a target antigen as described herein. In one aspect, a cancer associated with expression of a tumor antigen as described herein is a hematological cancer. In one aspect, a cancer associated with expression of a tumor antigen as described herein is a solid cancer. Further diseases associated with expression of a tumor antigen described herein

include, but are not limited to, atypical and/or non-classical cancers, malignancies, precancerous conditions or proliferative diseases associated with expression of a tumor antigen as described herein. Non-cancer related indications associated with expression of a target antigen as described herein include, but are not limited to, *e.g.*, autoimmune disease, (*e.g.*, lupus), inflammatory disorders (allergy and asthma) and transplantation. In some embodiments, the target antigen-expressing cells express, or at any time expressed, mRNA encoding the target antigen. In another embodiment, the target antigen -expressing cells produce the target antigen protein (*e.g.*, wild-type or mutant), and the target antigen protein may be present at normal levels or reduced levels. In another embodiment, the target antigen -expressing cells produced detectable levels of a target antigen protein at one point, and subsequently produced substantially no detectable target antigen protein.

[0099] “Disease targeted by genetically modified cells” as used herein encompasses the targeting of any cell involved in any manner in any disease by the genetically modified cells of the invention, irrespective of whether the genetically modified cells target diseased cells or healthy cells to effectuate a therapeutically beneficial result. The genetically modified cells include but are not limited to genetically modified T-cells, NK cells, hematopoietic stem cells, pluripotent embryonic stem cells or embryonic stem cells. The genetically modified cells express the conventional CARs and novel backbones containing conventional CARs with accessory modules of the invention, which CARs may target any of the antigens expressed on the surface of target cells. Examples of antigens which may be targeted include but are not limited to antigens expressed on B-cells; antigens expressed on carcinomas, sarcomas, lymphomas, leukemia, germ cell tumors, and blastomas; antigens expressed on various immune cells; and antigens expressed on cells associated with various hematologic diseases, autoimmune diseases, and/or inflammatory diseases. Other antigens that may be targeted will be apparent to those of skill in the art and may be targeted by the CARs of the invention in connection with alternate embodiments thereof.

[00100] The term “Dissociation constant (K_d)” is defined as the equilibrium constant of the dissociation of a receptor–ligand interaction.

[00101] As used herein a “diverse set of non-naturally occurring immune receptors” or “diverse set of SIRs” or “diverse set of CARs” refers to a plurality of non-naturally occurring immune receptors having the same binding domain linked to a diverse set of T cell receptor constant chains or “backbones” wherein each construct comprising a binding domain and a different T cell constant chain or backbone provide a diverse range of binding to a target antigen and/or varied expression levels. For example, depending upon the mutation

composition of the constant domain (*e.g.*, mutant TCRA+TCRB), the binding affinity of the binding domain to its target varies. In some embodiments, a SIR of the disclosure (single strand or heterodimer) comprises a binding affinity that is greater than a wild-type TCR (*e.g.*, cTCR) with the same binding domain. In one embodiment a SIR has a higher expression level than a cTCR by at least 1.25 fold to about 10,000 fold higher (and any number in between), wherein the SIR and cTCR differ only in the mutation in the TCR domain. In another embodiment, a SIR has a binding affinity for a target that is at least 1.5 fold higher to about 10,000 fold higher than a cTCR having a binding domain to the same antigen. In yet another embodiment, the SIR has a higher binding affinity than a cTCR to the same antigen, but less than a chimeric antigen receptor (CAR) having the same binding domain. In some embodiments, the binding of a SIR expressing effector cell to the target antigen is at least 1.25-fold more than the binding of a corresponding cTCR-expressing effector cell but less than 100,000 fold more than the corresponding cTCR. In some embodiment, the antigen binding domain has a disassociation constant (K_D , reflecting its binding affinity) from between about 10^{-4} M to 10^{-8} M. In some embodiments, the antigen binding domain binds to one or more of the antigens recited above. In some embodiment, the antigen binding domain has a K_D of between about 10^{-4} M to 10^{-8} M, *e.g.*, between about 10^{-5} M to 10^{-7} M, *e.g.*, between about 10^{-5} M to 10^{-6} M, for the target antigen. In one embodiment, the binding affinity of the antigen binding domain is at least five-fold, 10-fold, 20-fold, 30-fold, 50-fold, 100-fold or 1,000-fold less than a reference antibody. In one embodiment, the encoded antigen binding domain has a binding affinity at least 5-fold less than a reference antibody. In some embodiments, the reference antibody is an antibody from which the antigen binding domain is derived. For example, the disclosure contemplates a diverse population of SIRs against a particular antigen target that can be designed and screened based upon the nucleic acid sequence codon optimization and/or the mutation in the TCR chain to promote pairing or expression and/or the use of a linker between the binding domain and the TCR domain.

[00102] As used herein, an "epitope" is defined to be the portion of an antigen capable of eliciting an immune response, or the portion of an antigen that binds to an antibody or antibody fragment. Epitopes can be a protein sequence or subsequence.

[00103] The term "expression vector" refers to a vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in an *in vitro* expression system. Expression vectors include all those known in the art, including cosmids,

plasmids (*e.g.*, naked or contained in liposomes) and viruses (*e.g.*, lentiviruses, retroviruses, adenoviruses, and adena-associated viruses) that incorporate the recombinant polynucleotide.

[00104] The term "functional portion" when used in reference to a CAR refers to any part or fragment of the CAR, which part or fragment retains the biological activity of the CAR of which it is a part (the parent CAR). Functional portions encompass, for example, those parts of a CAR that retain the ability to recognize target cells, or detect, treat, or prevent a disease, to a similar extent, the same extent, or to a higher extent, as the parent CAR. In reference to the parent CAR, the functional portion can comprise, for instance, about 10%, 25%, 30%, 50%, 68%, 80%, 90%, 95%, or more, of the parent CAR.

[00105] "Genetically modified cells", "redirected cells", "genetically engineered cells" or "modified cells" as used herein refer to cells that express a CAR of the disclosure. In some embodiments, the genetically modified cells comprise vectors that encode a CAR. In some embodiments, the genetically modified cells comprise vectors that encode a CAR and one or more accessory molecules in the same vector. In some embodiments, the genetically modified cells comprise a first vector that encodes a CAR and a second vector that encodes the accessory molecule. In some embodiments, the genetically modified cells comprise a first vector that encodes a CAR and a second vector that encodes more than one accessory molecule. In some embodiments, the genetically modified cells comprise a first vector that encodes a CAR and a second vector that encodes the first accessory molecule and a third vector that encodes a second accessory molecule.

[00106] "Hinge region" (HR) as used herein refers to the hydrophilic region which is between the antigen binding domain and the transmembrane domain. The hinge regions include but are not limited to Fc fragments of antibodies or fragments or derivatives thereof, hinge regions of antibodies or fragments or derivatives thereof, CH2 regions of antibodies, CH3 regions of antibodies, artificial spacer sequences or combinations thereof. Examples of hinge regions include but are not limited to CD8a hinge, and artificial spacers made of polypeptides which may be as small as, for example, Gly3 or CH1 and CH3 domains of IgGs (such as human IgG4). In some embodiments, the hinge region is any one or more of (i) a hinge, CH2 and CH3 regions of IgG4, (ii) a hinge region of IgG4, (iii) a hinge and CH2 of IgG4, (iv) a hinge region of CD8a, (v) a hinge, CH2 and CH3 regions of IgG1, (vi) a hinge region of IgG1 or (vi) a hinge and CH2 region of IgG1. Other hinge regions will be apparent to those of skill in the art and may be used in connection with alternate embodiments of the disclosure.

[00107] The term "immune disorder" refers to a disease characterized by dysfunction of immune system. An autoimmune disease is a condition arising from an abnormal immune response to a normal body part. There are at least 80 types of autoimmune diseases.

[00108] "Immune cell" as used herein refers to the cells of the mammalian immune system including but not limited to antigen presenting cells, B-cells, basophils, cytotoxic T-cells, dendritic cells, eosinophils, granulocytes, helper T-cells, leukocytes, lymphocytes, macrophages, mast cells, memory cells, monocytes, natural killer cells, neutrophils, phagocytes, plasma cells and T-cells.

[00109] "Immune effector cell," as that term is used herein, refers to a cell that is involved in an immune response, *e.g.*, in the promotion of an immune effector response. Examples of immune effector cells include T cells, *e.g.*, alpha/beta T cells and gamma/delta T cells, B cells, natural killer (NK) cells, natural killer T (NKT) cells, mast cells, and myeloid-derived phagocytes.

[00110] "Immune effector function" or "immune effector response," "effector function" refers to the specialized function of a differentiated cell. Effector function of a T-cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines. For example, an immune effector function or response refers a property of a T or NK cell that promotes killing or the inhibition of growth or proliferation, of a target cell. In the case of a T cell, primary stimulation and co-stimulation are examples of immune effector function or response. In case of antigen presenting cells (*e.g.*, dendritic cells) antigen presentation and cytokine secretion are examples of effector functions.

[00111] "Immune response" as used herein refers to immunities including but not limited to innate immunity, humoral immunity, cellular immunity, immunity, inflammatory response, acquired (adaptive) immunity, autoimmunity and/or overactive immunity.

[00112] An "intracellular signaling domain," (ISD) or "cytoplasmic domain" as the term is used herein, refers to an intracellular signaling portion of a molecule. The intracellular signaling domain generates a signal that promotes an immune effector function of the cell. Examples of immune effector function include cytolytic activity and helper activity, including the secretion of cytokines. Examples of domains that transduce the effector function signal include but are not limited to the z chain of the T-cell receptor complex or any of its homologs (*e.g.*, h chain, FcεR1g and b chains, MB1 (Iga) chain, B29 (Igb) chain, *etc.*), human CD3 zeta chain, CD3 polypeptides (D, d and e), syk family tyrosine kinases (Syk, ZAP 70, *etc.*), src family tyrosine kinases (Lck, Fyn, Lyn, *etc.*) and other molecules involved in T-cell transduction, such as CD2, CD5 and CD28. Other intracellular signaling domains

will be apparent to those of skill in the art and may be used in connection with alternate embodiments of the disclosure.

[00113] In another embodiment, the intracellular signaling domain can comprise a primary intracellular signaling domain. Exemplary primary intracellular signaling domains include those derived from the molecules responsible for primary stimulation, or antigen dependent stimulation. In another embodiment, the intracellular signaling domain can comprise a costimulatory intracellular domain. Exemplary costimulatory intracellular signaling domains include those derived from molecules responsible for costimulatory signals, or antigen independent stimulation. For example, a primary intracellular signaling domain can comprise a cytoplasmic sequence of CD3z, and a costimulatory intracellular signaling domain can comprise cytoplasmic sequence from co-receptor or costimulatory molecule, such as CD28 or 41BB.

[00114] A primary intracellular signaling domain can comprise a signaling motif which is known as an immunoreceptor tyrosine-based activation motif or ITAM. Examples of ITAM containing primary cytoplasmic signaling sequences include, but are not limited to, those derived from CD3 zeta, common FeR gamma (FCER1G), Fe gamma RIIa, FeR beta (Fe Epsilon R1b), CD3 gamma, CD3 delta, CD3 epsilon, CD79a, CD79b, DAPIO, and DAP12.

[00115] The term “isolated” as used herein refers to molecules or biologicals or cellular materials being substantially free from other materials. In one aspect, the term “isolated” refers to nucleic acid, such as DNA or RNA, or protein or polypeptide (*e.g.*, an antibody or derivative thereof), or cell or cellular organelle, or tissue or organ, separated from other DNAs or RNAs, or proteins or polypeptides, or cells or cellular organelles, or tissues or organs, respectively, that are present in the natural source. The term “isolated” also refers to a nucleic acid or peptide that is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Moreover, an “isolated nucleic acid” is meant to include nucleic acid fragments which are not naturally occurring as fragments and would not be found in the natural state. The term “isolated” is also used herein to refer to polypeptides which are isolated from other cellular proteins and is meant to encompass both purified and recombinant polypeptides. The term “isolated” is also used herein to refer to cells or tissues that are isolated from other cells or tissues and is meant to encompass both, cultured and engineered cells or tissues.

[00116] As used herein, the term “linker” (also “linker domain” or “linker region”) refers to an oligo or a polypeptide (or an oligo encoding the polypeptide) that joins together two or more domains or regions of a CAR polynucleotide or polypeptide, respectively, disclosed herein. The linker can be anywhere from 1 to 500 amino acids in length or 3 to 1500 nucleotide in length. In some embodiments the “linker” is cleavable or non-cleavable. Unless specified otherwise, the term “linker” used herein means a non-cleavable linker. Said non-cleavable linkers may be composed of flexible residues which allow freedom of motion of adjacent protein domains relative to one another. Non-limiting examples of such residues include glycine and serine. In some embodiments, linkers include non-flexible residues. Examples of cleavable linkers include 2A linkers (for example T2A), 2A-like linkers or functional equivalents thereof and combinations thereof. In some embodiments, the linkers include the picornaviral 2A-like linker, CHYSEL sequences of porcine teschovirus (P2A), *Thosea asigna* virus (T2A) or combinations, variants and functional equivalents thereof. In some embodiments, the linker sequences may comprise a motif that results in cleavage between the 2A glycine and the 2B proline (see, e.g., T2A sequence, SEQ ID NO: 4839 and 4840, C-terminal Gly-Pro). The nucleic sequences of several exemplary cleavable linkers are provided in SEQ ID NO: 925 to SEQ ID NO: 930 and amino acid sequences of several exemplary linkers are provided in SEQ ID NO: 4838 to SEQ ID NO: 4843. Other cleavable linkers that may be used herein are readily appreciated by those of skill in the art.

[00117] In an embodiment, a Ser-Gly-Ser-Gly (SGSG) motif (SEQ ID NOs: 931-32) is also added upstream of the cleavable linker sequences to enhance the efficiency of cleavage. A potential drawback of the cleavable linkers is the possibility that the small 2A tag left at the end of the N-terminal protein may affect protein function or contribute to the antigenicity of the proteins. To overcome this limitation, in some embodiments, a furine cleavage site (RAKR) (SEQ ID NO: 933-935) is added upstream of the SGSG motifs to facilitate cleavage of the residual 2A peptide following translation.

[00118] The term “flexible polypeptide linker” as used herein refers to a peptide linker that consists of amino acids such as glycine and/or serine residues used alone or in combination, to link polypeptide chains together (e.g., variable heavy and variable light chain regions together). In one embodiment, the flexible polypeptide linker is a Gly/Ser linker and comprises the amino acid sequence (Gly-Gly-Gly-Ser)_n, (SEQ ID NO: 4191-4192) where n is a positive integer equal to or greater than 1. For example, n=1, n=2, n=3, n=4, n=5 and n=6, n=7, n=8, n=9 and n=10. In one embodiment, the flexible polypeptide linkers include, but are not limited to, (Gly₄Ser)₄ or (Gly₄Ser)₃ (SEQ ID NO: 4193 or 4194). Also included within the

scope of the disclosure are linkers described in W02012/138475, incorporated herein by reference).

[00119] The term "lentivirus" refers to a genus of the Retroviridae family. Lentiviruses are unique among the retroviruses in being able to infect non-dividing cells; they can deliver a significant amount of genetic information into the DNA of the host cell, so they are one of the most efficient methods of a gene delivery vector. HIV, SIV, and FIV are all examples of lenti viruses.

[00120] The term "lentiviral vector" refers to a vector derived from at least a portion of a lentivirus genome, including especially a self-inactivating lentiviral vector as provided in Milone *et al.*, Mol. Ther. 17(8): 1453-1464 (2009). Other examples of lentivirus vectors that may be used in the clinic, include but are not limited to, *e.g.*, the LENTIVECTOR® gene delivery technology from Oxford BioMedica, the LENTIMAX™ vector system from Lentigen and the like. Nonclinical types of lentiviral vectors are also available and would be known to one skilled in the art. Other examples of lentivirus vectors are pLENTI-EF1 α (SEQ ID NO: 3837), pLENTI-EF1 α -DWPRE (SEQ ID NO: 3838), pCCLc-MNDU3-WPRE (SEQ ID NO: 7779) and pCCLc-MNDU3-Eco-Nhe-Sal-WPRE (SEQ ID NO: 7780). pLenti-EF1 α -DWPRE was derived from the pLENTI-EF1 α vector by deletion of WPRE sequence. An internal Sac II fragment was deleted from the EF1 α promoter to generate EF1 α -D-SACII-Promoter (SEQ ID NO: 3842). In an exemplary embodiment, the nucleic acid fragment encoding a CAR, CAR plus accessory module(s), or the accessory module(s) can be cloned between the *Nhe* I and *Sal* I sites present in the pLENTI-EF1 α and the pCCLc-MNDU3-Eco-Nhe-Sal-WPRE vectors using methods known in the art.

[00121] "Mammal" as used herein refers to any member of the class *Mammalia*, including, without limitation, humans and nonhuman primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats and guinea pigs, and the like. The term does not denote a particular age or sex. Thus, adult and newborn subjects, as well as fetuses, whether male or female, are intended to be included within the scope of this term.

[00122] "Naked DNA" as used herein refers to DNA encoding a CAR cloned in a suitable expression vector in proper orientation for expression. Viral vectors which may be used include but are not limited SIN lentiviral vectors, retroviral vectors, foamy virus vectors, adeno-associated virus (AAV) vectors, hybrid vectors and/or plasmid transposons (for example sleeping beauty transposon system) or integrase based vector systems. Other

vectors that may be used in connection with alternate embodiments of the invention will be apparent to those of skill in the art.

[00123] “Native” or “Naturally occurring” or “endogenous” as used herein refers to a gene, protein, nucleic acid (*e.g.*, DNA, RNA *etc.*) or fragment thereof that is native to a cell or is naturally expressed in a cell. Thus, a native or endogenous TCR α chain polypeptide of a T cell consists of a variable domain (V α) joined to a TCR α constant chain. The native or endogenous TCR α chain precursor polypeptide also consists of an amino-terminal signal peptide that is cleaved from the mature polypeptide.

[00124] NF-Kappa-B Essential Modulator (NEMO) refers to a scaffolding protein component of I κ B kinase complex required for NF- κ B activation. NF- κ B is a transcription factor that controls inflammation, cell proliferation and apoptosis.

[00125] “NF- κ B pathway” or “NF- κ B signaling pathway” refers to a signal transduction pathway that results in the nuclear translocation of NF- κ B subunits and transcriptional activation of NF- κ B subunit responsive genes. NF- κ B refers to family of transcription factors that are involved in the regulated expression of several genes involved in the inflammatory and immune response. Five known members of this family have been characterized to date and include c-Rel, NF- κ B1 (p50 and its precursor p105), NF- κ B2 (p52 and its precursor p105), p65(RelA) and RelB. Although many dimeric forms of NF- κ B have been described, the classical NF- κ B complex is a heterodimer of the p65/RelA and p50 subunits and is found in most cells in association with a family of inhibitory proteins, called I κ Bs, of which the most common is I κ B α . In the classical NF- κ B pathway, stimulation by a number of cytokines, such as TNF α and IL-1, results in the activation of a multi-subunit I κ B kinase (IKK) complex, which contains two catalytic subunits, IKK1/IKK α and IKK2/IKK β , and a regulatory subunit, NEMO/IKK γ . The activated IKK complex leads to the inducible phosphorylation of I κ B proteins and their subsequent degradation, thereby releasing NF- κ B from their inhibitory influence. Once released, NF- κ B is free to migrate to the nucleus and bind to the promoter of specific genes possessing its cognate binding site. The transcriptional activity of the NF- κ B dimers in the nucleus is further modified by their phosphorylation. An alternative (or noncanonical) pathway of NF- κ B activation, that involves proteasome-mediated processing of p100/NF- κ B2 into p52 subunit, has been described.

[00126] “NF- κ B stimulatory molecule” or “NF- κ B stimulator” or “NF- κ B activator” refers to a subset of accessory molecules that promote the activity of the NF- κ B signaling pathway or the activity/expression of the downstream target genes of the NF- κ B signaling

pathway. In some embodiments, a NF- κ B activator is a non-naturally occurring NF- κ B activating agent. An exemplary non-naturally occurring NF- κ B activating agent is hNEMO-K277A. In one embodiment, the NF- κ B stimulatory molecule or NF- κ B stimulator is a selective NF- κ B stimulator or a selective NF- κ B activator. A “selective NF- κ B activator” or a “selective NF- κ B stimulator” as described herein, refers to an agent that activates the NF- κ B signaling pathway selectively with no or minimal activation of the other signaling pathways. In one embodiment, a selective NF- κ B activator activates NF- κ B signaling pathway with no or minimal activation of one or more of signaling pathways selected from the group of AKT, PI3K, JNK, p38 kinase, ERK, JAK/STAT and interferon signaling pathways. In one embodiment, a selective NF- κ B activator activates NF- κ B signaling pathway with no or minimal activation of AKT signaling pathway. In one embodiment, a selective NF- κ B activator activates NF- κ B signaling pathway with no or minimal activation of AKT signaling pathway. In one embodiment, a selective NF- κ B activator activates NF- κ B signaling pathway with no or minimal activation of PI3K signaling pathway. In one embodiment, a selective NF- κ B activator activates NF- κ B signaling pathway with no or minimal activation of ERK signaling pathway. In one embodiment, a selective NF- κ B activator activates NF- κ B signaling pathway with no or minimal activation of JNK signaling pathway. In one embodiment, a selective NF- κ B activator activates NF- κ B signaling pathway with no or minimal activation of p38 kinase signaling pathway. In one embodiment, a selective NF- κ B activator activates NF- κ B signaling pathway with no or minimal activation of JAK/STAT signaling pathway. In one embodiment, a selective NF- κ B activator activates NF- κ B signaling pathway with no or minimal activation of interferon signaling pathway. A number of methods to measure the activation of the NF- κ B signaling pathways are known in the art, including but not limited to measurement of phosphorylated I κ B α , phosphorylated p65/RelA, total I κ B α , p65 nuclear translocation, upregulation of NF- κ B responsive genes, electrophoretic mobility-shift assay (EMSA) and NF- κ B-based reporter assay *etc.* These assays can be used in the methods of the disclosure either singly or in combinations to identify selective activators of NF- κ B pathway. A number of methods to measure the activation of the signaling pathways (*e.g.*, AKT, PI3K, JNK, p38 kinase, ERK, JAK/STAT and interferon signaling pathways) are known in the art, including but not limited to measurement of phosphorylation of the different kinases and downstream substrates belonging to the different pathways, nuclear translocation of downstream transcription factors, upregulation of the downstream responsive genes, electrophoretic mobility shift assay (EMSA) and luciferase based reporter assay *etc.* These assays can be used in the methods of

the disclosure either singly or in combinations to select selective activators of NF- κ B signaling pathway. A selective NF- κ B stimulator specifically activates NF- κ B compared to other accessory molecules such as 41BB. A NF- κ B stimulatory molecule, including a selective NF- κ B activator, has one or more of the following effects: (i) extend the life span of T cells, *e.g.*, CAR-T cells or TCR-T cells, (ii) stimulate T cell proliferation, (iii) protect T cells, *e.g.*, CAR-T cells, from apoptosis, (iv) delay senescence of T cells, *e.g.*, CAR-T cells or TCR-T cells (v) delay exhaustion of T cells, *e.g.*, CAR-T cells or TCR-T cells, (vi) delay terminal differentiation of T cells, (vii) promote production of cytokines, such as IL2, by T cells, (viii) promote *in vivo* expansion of T cells, including CAR-T cells and TCR-T cells, (ix) promote *in vivo* persistence of T cells, including CAR-T cells and TCR-T cells, (x) improve the *in vivo* activity (*e.g.*, anti-tumor activity) of the T cells, including CAR-T and TCR-T cells. A NF- κ B stimulatory molecule, including a selective NF- κ B activator, may be expressed in cells other than T cells, such as antigen presenting cells, *e.g.*, dendritic cells. A NF- κ B stimulatory molecule, including a selective NF- κ B activator, may be used to enhance the antigen presentation, cytokine production and immune response generated by antigen presenting cells. An NF- κ B stimulatory molecule, including a selective NF- κ B activator, may be of viral or non-viral (*e.g.*, human) origin. An NF- κ B stimulatory molecule, including a selective NF- κ B activator, may be expressed in a cell transiently or stably. An NF- κ B stimulatory molecule, including a selective NF- κ B activator, may be expressed in a cell in a constitutive or inducible manner. An NF- κ B stimulatory molecule, including a selective NF- κ B activator, may be expressed in a cell in fusion with a switch domain, *e.g.*, tandem copies of a FKBP12v36 domain. Exemplary switch domain containing NF- κ B stimulatory molecules are provided in SEQ ID NO: 973-977, 1006-1009, 7763-7767 and 7781-7782 (**Table 7**). An NF- κ B stimulatory molecule, including a selective NF- κ B activator, can be expressed from a vector containing a coding sequence for a CAR/TCR or may be present on a different vector. For example, in some embodiments, vectors comprising polynucleotides encoding CARs/TCRs further comprise polynucleotides encoding viral and cellular signaling proteins which are NF- κ B stimulatory molecule or selective NF- κ B activator that (i) extend the life span of T cells, *e.g.*, CAR-T cells or TCR-T cells, (ii) stimulate T cell proliferation, (iii) protect T cells, *e.g.*, CAR-T cells, from apoptosis, (iv) delay senescence of T cells, *e.g.*, CAR-T cells or TCR-T cells (v) delay exhaustion of T cells, *e.g.*, CAR-T cells or TCR-T cells, (vi) delay terminal differentiation of T cells, (vii) promote production of cytokines, such as IL2, by T cells, (viii) promote *in vivo* expansion of T cells, including CAR-T cells and TCR-T cells, (ix) promote *in vivo* persistence of T cells, including CAR-T cells and

TCR-T cells, and/or (x) improve the *in vivo* activity (*e.g.*, anti-tumor activity) of the T cells, including CAR-T and TCR-T cells.. In some embodiments, the coding sequence for a NF- κ B stimulatory molecule is linked to a CAR backbone coding sequence by an oligonucleotide encoding a cleavable linker. In exemplary embodiments, such NF- κ B stimulatory molecules include but are not limited to vFLIP-K13 from Kaposi's sarcoma associated herpes virus, a codon optimized K13 (K13-opt), NEMO mutant ((*e.g.*, hNEMO-K277A, hNEMO-K277L, hNEMO-K277A-deltaV249-K255, mNEMO-K270A *etc*), IKK2-S177E-S181E, IKK1-S176E-S180E, MyD88-L265P, TCL-1A, MTCP-1, IKK α , and IKK β (**Table 7**). In one embodiment, vectors encoding CARs further encode vFLIP-K13. In another embodiment, vectors encoding CARs further encode hNEMO-K277A. In some embodiments, the NF- κ B stimulatory molecule is encoded by a vector that is distinct from the vector encoding the CAR described herein. In some embodiments, effector cells comprising vectors encoding CARs also comprise vectors encoding NF- κ B stimulatory molecule. In some embodiments, the NF- κ B stimulatory molecules are encoded by modifying the genomic locus encoding the corresponding endogenous protein. For example, one or more copies of hNEMO gene can be modified by homologous recombination to mutate it to K277A mutant form. An exemplary targeting constructs that can be used to create K277A mutation in the endogenous human NEMO gene is presented by SEQ ID NO: 7771. An exemplary targeting constructs that can be used to create K277A-Delta-V249-K255 mutation in the endogenous human NEMO gene is presented by SEQ ID NO: 7772. These targeting constructs can be introduced into human T cells with a gene editing system targeting NEMO, *e.g.*, CRISP/Cas9 or TALON, using techniques known in the art. Exemplary NEMO gRNA target sequences for *Streptococcus Pyogenes* Cas9 are provided in SEQ ID NO: 7759-7762. In one embodiment, the CAR and the NF- κ B stimulatory molecule are encoded by a single polynucleotide. In another embodiment, the CAR is encoded by the first nucleic acid molecule and the NF- κ B stimulatory molecule is encoded by a second nucleic acid molecule. In some embodiments, the NF- κ B stimulatory molecule is encoded by more than one nucleic acid molecule, depending on the number of NF- κ B stimulatory molecules. In certain portions of the disclosure the abbreviation "CAR/NF κ B" is used to indicate, for example, a cell that expresses both a CAR of the disclosure and an NF- κ B stimulatory molecule (*e.g.*, a NF- κ B specific stimulatory molecule). For example, the term "CAR/NF κ B-expressing T cell" refers to a CAR-T cells having any number of possible different antigen binding domains that also expresses, for example, an NF- κ B specific stimulatory molecule selected from the group consisting of vFLIP-K13 from Kaposi's sarcoma associated herpes virus, a codon optimized

K13 (K13-opt), hNEMO-K277A, hNEMO-K277A-deltaV249-K555, mNEMO-K270A, IKK2-S177E-S181E, IKK1-S176E-S180E, MyD88-L265P, TCL-1A, MTCP-1, IKK1/IKK α , and IKK2/IKK β , or any combination thereof. The NF- κ B stimulatory molecule may be directly linked to the cytoplasmic domain of the CAR or may be independently expressed in the cell. The NF- κ B stimulatory molecule may be a molecule that blocks the expression and or activity of an inhibitor of NF- κ B signaling pathway. For example, a NF- κ B stimulatory molecule that blocks the expression and or activity of an inhibitor of NF- κ B signaling pathway is a genetic (*e.g.*, siRNA, shRNA, gRNA, TALON, or Zn finger nuclease), chemical or biological inhibitor of A20. Other embodiments include NEMO-fusion constructs as NF- κ B stimulatory molecules (*e.g.*, hNEMO-FKBP $\times$ 2, FKBP $\times$ 2-hNEMO-L600 *etc.*).

[00127] As used herein a “non-naturally occurring agent” or “non-native” or “exogenous” refers to an agent that is not naturally expressed in a cell. Stated another way, the non-naturally occurring agent is “engineered” to be expressed in a cell. A non-naturally occurring agent may be a cloned version of a naturally occurring agent. Exemplary non-naturally occurring agents include CARs, SIRs, Ab-TCRs, TFPs, recombinant TCR, NEMO-K277A, vFLIP-K13 and K13-opt. A non-naturally occurring agent may be expressed into a cell using techniques of gene transfer known in the art, such as lentiviral or retroviral mediated gene transfer. A non-naturally occurring agent may be expressed in an immune cell using an exogenous promoter (*e.g.*, EF1 α promoter) or an endogenous promoter (*e.g.*, TCR α promoter). When an endogenous gene (*e.g.*, IKK1, IKK2, IKK γ /NEMO) is cloned and ectopically expressed in a cell, it represents another example of a non-naturally occurring agent.

[00128] As used herein a “non-naturally occurring immune receptor” or “exogenous immune receptor” refers to an immune receptor that is not naturally expressed in an immune cell. Stated another way, the non-naturally occurring immune receptor is “engineered” to be expressed in an immune cell. A non-naturally occurring immune receptor may be a cloned version of a naturally occurring immune receptor. Alternatively, a non-naturally occurring immune receptor may be a chimeric receptor that is produced using recombinant molecular biology techniques. Exemplary non-naturally occurring immune receptors include CARs, SIR, Ab-TCRs, TFPs and recombinant TCR. A non-naturally occurring immune receptor may be introduced into an immune cell using techniques of gene transfer known in the art, such as lentiviral or retroviral mediated gene transfer. A non-naturally occurring immune receptor may be expressed in an immune cell using an exogenous promoter (*e.g.*, EF1 α promoter) or an endogenous promoter (*e.g.*, TCR α promoter).

[00129] As used herein a “non-naturally occurring TCR antigen binding domain” or “exogenous TCR antigen binding domain” refers to a binding domain operably linked to a TCR constant region that is chimeric and non-naturally occurring with respect to a TCR present in nature. Stated another way, the non-naturally occurring TCR antigen binding domain is “engineered” using recombinant molecular biology techniques to be operably linked to a TCR and moreover, that the antigen binding domain is obtain or derived from a molecule that is distinct from a TCR found in nature. An antigen binding domain that is distinct from a TCR in nature includes antibody vH and vL fragments, humanized antibody fragments, chimeric antibody fragments, receptor ligands, and the like.

[00130] As used herein a “non-viral origin” refers to an agent (*e.g.*, a protein) that is not wholly or in part encoded by a virus or has any domain or region of more than 10 amino acids (*e.g.*, more than 15 amino acids, 20 amino acids, 25 amino acids or 50 amino acids) with greater than 80% (*e.g.*, more than 85%, 90%, 95%, or 99%) sequence homology to a virally encoded protein. In an embodiment, an agent of non-viral origin is of human origin. In an embodiment, an agent of non-viral origin is a selective NF- κ B activator. An exemplary agent of non-viral origin that is a selective NF- κ B activator is human NEMO-K277A (SEQ ID NO: 4892).

[00131] The term “operably linked” or “functionally linked” refers to functional linkage or association between a first component and a second component such that each component can be functional. For example, operably linked includes the association between a regulatory sequence and a heterologous nucleic acid sequence resulting in expression of the latter. For example, a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. In the context of two polypeptides that are operably linked a first polypeptide functions in the manner it would independent of any linkage and the second polypeptide functions as it would absent a linkage between the two.

[00132] “Percent identity” in the context of two or more nucleic acids or polypeptide sequences, refers to two or more sequences that are the same. Two sequences are “substantially identical” if two sequences have a specified percentage of amino acid residues or nucleotides that are the same (*e.g.*, 60% identity, optionally 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% identity over a specified region, or, when not specified, over the entire sequence), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of

the following sequence comparison algorithms or by manual alignment and visual inspection. Optionally, the identity exists over a region that is at least about 50 nucleotides (or 10 amino acids) in length, or more preferably over a region that is 100 to 500 or 1000 or more nucleotides (or 20, 50, 200 or more amino acids) in length.

[00133] For sequence comparison, generally one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters. Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith and Waterman, (1970) *Adv. Appl. Math.* 2:482c, by the homology alignment algorithm of Needleman and Wunsch, (1970) *J. Mol. Biol.* 48:443, by the search for similarity method of Pearson and Lipman, (1988) *Proc. Nat'l. Acad. Sci. USA* 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (see, *e.g.*, Brent *et al.*, (2003) *Current Protocols in Molecular Biology*).

[00134] Two examples of algorithms that can be used for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.*, (1977) *Nuc. Acids Res.* 25:3389-3402; and Altschul *et al.*, (1990) *J. Mol. Biol.* 215:403-410, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information.

[00135] The percent identity between two amino acid sequences can also be determined using the algorithm of E. Meyers and W. Miller, (1988) *Comput. Appl. Biosci.* 4:11-17) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (1970) *J. Mol. Biol.* 48:444-453) algorithm which has been incorporated into the GAP program in the GCG software package (available at www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

[00136] The term "polynucleotide", "nucleic acid", or "recombinant nucleic acid" refers to polymers of nucleotides such as deoxyribonucleic acid (DNA), and, where appropriate, ribonucleic acid (RNA).

[00137] A "protein" or "polypeptide", which terms are used interchangeably herein, comprises one or more chains of chemical building blocks called amino acids that are linked together by chemical bonds called peptide bonds.

[00138] The term "retrovirus vector" refers to a vector derived from at least a portion of a retrovirus genome. Examples of retrovirus vector include MSCVneo, MSCV-pac (or MSCV-puro), MSCV-hygro as available from Addgene or Clontech.

[00139] The term "Sleeping Beauty Transposon" or "Sleeping Beauty Transposon Vector" refers to a vector derived from at least a portion of a Sleeping Beauty Transposon genome.

[00140] The term "single chain variable region" or "scFv" refers to a fusion protein comprising at least one antibody fragment comprising a variable region of a light chain and at least one antibody fragment comprising a variable region of a heavy chain, wherein the light and heavy chain variable regions are contiguously linked, *e.g.*, via a synthetic linker, *e.g.*, a short flexible polypeptide linker, and capable of being expressed as a single chain polypeptide, and wherein the scFv retains the specificity of the intact antibody from which it is derived. Unless specified, as used herein an scFv may have the vL and vH variable regions in either order, *e.g.*, with respect to the N-terminal and C-terminal ends of the polypeptide, the scFv may comprise vL-linker-vH or may comprise vH-linker-vL. In this invention, a scFv is also described as vL-Gly-Ser-Linker-vH. Alternatively, a scFv is also described as (vL+vH) or (vH+vL).

[00141] The term "signaling domain" refers to the functional region of a protein which transmits information within the cell to regulate cellular activity via defined signaling pathways by generating second messengers or functioning as effectors by responding to such messengers.

[00142] The term "Synthetic Immune Receptor" or alternatively a "SIR" refers to a set of polypeptides, typically two in some embodiments, which when expressed in an effector cell, provides the cell with specificity for a target cell, typically a cancer cell, and with intracellular signal generation. SIRs represent next generation CAR platforms that are described in WO 2018/102795 A1 which is incorporated herein by reference. In a typical embodiment, a SIR comprises one or more antigen binding domains (*e.g.*, antibody or antibody fragment, a ligand or a receptor) that bind to antigens as described herein, and are

joined to one or more T cell receptor constant chains or regions via an optional linker. In some embodiments, the set of polypeptides are contiguous with each other. In some embodiments, a SIR comprises two or more sets of two or more polypeptides. The polypeptides of each set of SIR are contiguous with each other (functional polypeptide unit 1) but are not contiguous with the polypeptides of the other set (functional polypeptide unit 2). In some aspects, the T cell receptor constant chains (or regions) of the SIR is chosen from the constant chain of human T cell receptor-alpha (TCR-alpha or TCR α or TCR α or hTCR-alpha or hTCR α or hTCR α or C α), human T cell receptor-beta1 (TCR-beta1 or TCR β 1 or TCR β 1 or hTCR-beta1 or hTCR β 1 or hTCR β 1 or C β 1), human T cell receptor-beta 2 (TCR-beta2 or TCR β 2 or TCR β 2 or hTCR-beta2 or hTCR β 2 or hTCR β 2 or C β 2 also designated TCR-beta, TCR β or TCR β or C β), human Pre-T cell receptor alpha ((preTCR-alpha or preTCR α or preTCR α or preC α), human T cell receptor-gamma (TCR-gamma or TCR γ or TCR γ or hTCR-gamma or hTCR γ or hTCR γ or hTCR γ 1 or hTCRgamma1, or C γ), or human T cell receptor-delta (TCR-delta or TCR δ or TCR δ or hTCR-delta or hTCR δ or hTCR δ or C δ). In some embodiments, the TCR constant chains of SIR are encoded by their wild-type nucleotide sequences while in other aspects the TCR constant chains of SIR are encoded by the nucleotide sequences that are not wild-type. In some embodiments, the TCR constant chains of SIR are encoded by their codon optimized sequences. In some embodiments, the TCR constant chains of SIR encode for the wild-type polypeptide sequences while in other embodiments the TCR constant chains of SIR encoded for polypeptides that carry one or more mutations. In some embodiments, the TCR constant chains of SIR are encoded by their codon optimized sequences that carry one or more mutations. A SIR that comprises an antigen binding domain (*e.g.*, a scFv, or vHH) that targets a specific tumor maker "X", such as those described herein, is also referred to as X-SIR or XSIR. For example, a SIR that comprises an antigen binding domain that targets CD19 is referred to as CD19-SIR or CD19SIR. The TCR constant chain/domain of a SIR can be derived from the same species in which the SIR will ultimately be used. For example, for use in humans, it may be beneficial for the TCR constant chain of the SIR to be derived from or comprised of human TCR constant chains. However, in some instances, it is beneficial for the TCR constant chain to be derived from the same species in which the SIR will ultimately be used in, but modified to carry amino acid substitutions that enhance the expression of the TCR constant chains. For example, for use in humans, it may be beneficial for the TCR constant chain of the SIR to be derived from or comprised of human TCR constant chains but in which certain amino acids are replaced by the corresponding amino acids from the murine TCR constant chains. Such

murinized TCR constant chains provide increased expression of the SIR. The SIR or functional portion thereof, can include additional amino acids at the amino or carboxy terminus, or at both termini, which additional amino acids are not found in the amino acid sequence of the TCR or antigen binding domain which make up the SIR. Desirably, the additional amino acids do not interfere with the biological function of the SIR or functional portion, *e.g.*, recognize target cells, detect cancer, treat or prevent cancer, *etc.* More desirably, the additional amino acids enhance the biological activity, as compared to the biological activity of the parent SIR. The nucleic acid and amino acid sequences of exemplary SIRs are provided in SEQ ID NO: 3878-3879 and in **Tables 10-11**.

[00143] The term "stimulation," refers to a primary response induced by binding of a stimulatory molecule (*e.g.*, a TCR/CD3 complex) with its cognate ligand (or target antigen) thereby mediating a signal transduction event, such as, but not limited to, signal transduction via the TCR/CD3. Stimulation can mediate altered expression of certain molecules.

[00144] The term "stimulatory molecule," refers to a molecule expressed by an immune cell (*e.g.*, T cell, NK cell, B cell) that provides the cytoplasmic signaling sequence(s) that regulate activation of the immune cell in a stimulatory way for at least some aspect of the immune cell signaling pathway. In one aspect, the signal is a primary signal that is initiated by, for instance, binding of a TCR/CD3 complex with an MHC molecule loaded with peptide, and which leads to mediation of a T cell response, including, but not limited to, proliferation, activation, differentiation, and the like. A primary cytoplasmic signaling sequence (also referred to as a "primary signaling domain") that acts in a stimulatory manner may contain a signaling motif which is known as immunoreceptor tyrosine-based activation motif or ITAM. Examples of an ITAM containing cytoplasmic signaling sequence includes, but is not limited to, those derived from CD3 zeta, common γ (FCER1G), γ IIIa, γ (Fe Epsilon Rib), CD3 γ , CD3 δ , CD3 ϵ , CD79a, CD79b, DAP10, and DAP12.

[00145] The term "subject" is intended to include living organisms in which an immune response can be elicited (*e.g.*, any domesticated mammals or a human).

[00146] "Switch domain," or a "dimerization domain" as used herein, typically refers to a polypeptide-based entity that, in the presence of a dimerization molecule, associates with another switch domain. The association results in a functional coupling of a first entity linked to, *e.g.*, fused to, a first switch domain, and a second entity linked to, *e.g.*, fused to, a second switch domain. A first and second switch domain are collectively referred to as a dimerization switch. In embodiments, the first and second switch domains are the same as

one another, *e.g.*, they are polypeptides having the same primary amino acid sequence, and are referred to collectively as a homodimerization switch. In embodiments, the switch is intracellular. In embodiments, the switch domain is a polypeptide-based entity, *e.g.*, FKBP (FK506 binding protein), and the dimerization molecule is small molecule, *e.g.*, AP20187.

[00147] The terms “T-cell” and “T-lymphocyte” are interchangeable and used synonymously herein. Examples include but are not limited to naïve T cells (“lymphocyte progenitors”), central memory T cells, effector memory T cells, stem memory T cells (T_{scm}), iPSC-derived T cells, synthetic T cells or combinations thereof.

[00148] The term “TCR-associated signaling module” refers to a molecule having a cytoplasmic immunoreceptor tyrosine-based activation motif (ITAM) that is part of the TCR-CD3 complex. TCR-associated signaling modules include $CD\gamma\epsilon$, $CD\delta\epsilon$ and $CD3\zeta\zeta$.

[00149] “Therapeutic agents” as used herein refers to agents that are used to, for example, treat, inhibit, prevent, mitigate the effects of, reduce the severity of, reduce the likelihood of developing, slow the progression of and/or cure, a disease. Diseases targeted by the therapeutic agents include but are not limited to infectious diseases, carcinomas, sarcomas, lymphomas, leukemia, germ cell tumors, blastomas, antigens expressed on various immune cells, and antigens expressed on cells associated with various hematologic diseases, and/or inflammatory diseases.

[00150] “Therapeutic Controls” as used herein refers to an element used for controlling the activity of a CAR expressing cell. In some embodiments, therapeutic controls for controlling the activity of the CAR expressing cells of the invention comprise any one or more of truncated epidermal growth factor receptor (tEGFR), truncated epidermal growth factor receptor viii (tEGFRviii), truncated CD30 (tCD30), truncated BCMA (tBCMA), truncated CD19 (tCD19), thymidine kinase, cytosine deaminase, nitroreductase, xanthine-guanine phosphoribosyl transferase, human caspase 8, human caspase 9, inducible caspase 9, purine nucleoside phosphorylase, linamarase/linamarin/glucose oxidase, deoxyribonucleoside kinase, horseradish peroxidase (HRP)/indole-3-acetic (IAA), Gamma-glutamylcysteine synthetase, CD20/alphaCD20, CD34/thymidine kinase chimera, dox-depedent caspase-2, mutant thymidine kinase (HSV-TKSR39), AP1903/Fas system, a chimeric cytokine receptor (CCR), a selection marker, and combinations thereof.

[00151] The term “therapeutic effect” refers to a biological effect which can be manifested by various means, including but not limited to, *e.g.*, decrease in tumor volume, a decrease in the number of cancer cells, a decrease in the number of metastases, an increase in life expectancy, decrease in cancer cell proliferation, decrease in cancer cell survival,

decrease in the titer of the infectious agent, a decrease in colony counts of the infectious agent, amelioration of various physiological symptoms associated with a disease condition. A "therapeutic effect" can also be manifested by the ability of the peptides, polynucleotides, cells and antibodies in prevention of the occurrence of disease in the first place or in the prevention of relapse of the disease.

[00152] The term "therapeutically effective amount" as used herein refers to the amount of a pharmaceutical composition comprising one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof, to decrease at least one or more symptom of the disease or disorder, and relates to a sufficient amount of pharmacological composition to provide the desired effect. The phrase "therapeutically effective amount" as used herein means a sufficient amount of the composition to treat a disorder, at a reasonable benefit/risk ratio applicable to any medical treatment.

[00153] A therapeutically or prophylactically significant reduction in a symptom is, *e.g.* at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 125%, at least about 150% or more in a measured parameter as compared to a control or non-treated subject or the state of the subject prior to administering the oligopeptides described herein. Measured or measurable parameters include clinically detectable markers of disease, for example, elevated or depressed levels of a biological marker, as well as parameters related to a clinically accepted scale of symptoms or markers for diabetes. It will be understood, however, that the total daily usage of the compositions and formulations as disclosed herein will be decided by the attending physician within the scope of sound medical judgment. The exact amount required will vary depending on factors such as the type of disease being treated, gender, age, and weight of the subject.

[00154] The term "TCR receptor fusion proteins or TFP" refers to a next generation CAR platform as described in WO 2016/187349 A1 which is incorporated herein by reference. In an embodiment, a TFP comprises an antibody moiety that specifically binds to a target antigen fused to a TCR chain such as CD3 ϵ , CD3 γ , CD3 δ , TCR α or TCR β . Exemplary TCR chains that can be used in the construction of TFP are represented by SEQ ID NOs: 944-945, 948, 949-950 and 958 and are provided in WO 2017/070608 A1 which is incorporated herein by reference. A TFP incorporating CD3 ϵ chain is referred to as a CD3 ϵ TFP. A TFP incorporating CD3 γ chain is referred to as a CD3 γ TFP. A TFP incorporating CD3 δ chain is referred to as a CD3 δ TFP. The TFP incorporating CD3 ϵ , CD3 γ or CD3 δ chains are collectively referred to as CD3 $\epsilon/\gamma/\delta$ TFP. Exemplary TFPs incorporating different

antigen binding domains (*e.g.*, vL and vH fragments, ligands, receptors *etc.*) described in this disclosure and co-expressing an accessory module encoding NEMO-K277A are provided in SEQ ID NO: 1900-3123 (**Table 13**). The SEQ ID Nos, antigen binding domains and target antigens of these TFPs can be determined by referring to **Table 12** as these TFP constructs have identical antigen binding domains to the first generation CAR constructs coexpressing NEMO-K277A shown in **Table 12** and are numbered in identical order. However, the accessory module encoding NEMO-K277A is optional. TFP with the antigen binding domains (*i.e.*, vL and vH fragments, ligands and receptors *etc.*) described in this disclosure can be constructed without NEMO-K277A. As such, this accessory module along with the upstream Furine-SGSG-F2A sequence can be deleted from the TFPs represented by SEQ ID NO: 1900-3123. Alternatively, the accessory module encoding NEMO-K277A can be replaced by accessory modules encoding other signaling proteins, such as hNEMO-K277A-deltaV249-K555, mNEMO-K270A, K13-opt, IKK2-S177E-S181E, or IKK1-S176E-S180E, and MyD88 –L265P, FKBPx2-NEMO, NEMO-L600-FKBPx2, and CMV-141 *etc.*

[00155] The term "transfer vector" refers to a composition of matter which comprises an isolated nucleic acid and which can be used to deliver the isolated nucleic acid to the interior of a cell. Numerous vectors are known in the art including, but not limited to, linear polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, and viruses. Thus, the term "transfer vector" includes an autonomously replicating plasmid or a virus. The term should also be construed to further include non-plasmid and non-viral compounds which facilitate transfer of nucleic acid into cells, such as, for example, a poly lysine compound, liposome, and the like. Examples of viral transfer vectors include, but are not limited to, adenoviral vectors, adena-associated virus vectors, retroviral vectors, lentiviral vectors, and the like.

[00156] "Transmembrane domain" (TMD) as used herein refers to the region of the CAR which crosses the plasma membrane. The transmembrane domain of the CAR of the invention is the transmembrane region of a transmembrane protein (for example Type I transmembrane proteins), an artificial hydrophobic sequence or a combination thereof. Other transmembrane domains will be apparent to those of skill in the art and may be used in connection with alternate embodiments of the invention. In some embodiments, the TMD encoded CAR comprising any of the backbones described herein comprises a transmembrane domain selected from the transmembrane domain of an alpha, beta or zeta chain of a T-cell receptor, CD3 γ , CD3 ϵ , CD3 δ , CD28, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, KIRDS2, OX40, CD2, CD27, LFA-1

(CD11a, CD18), ICOS (CD278), 4-1BB (CD137), GITR, CD40, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRFl), CD160, CD19, IL2R beta, IL2R gamma, IL7R a, ITGA1, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, DNAM1(CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp, NKp44, NKp30, NKp46, NKG2D, and/or NKG2C.

[00157] As used herein “Tri-functional T cell antigen coupler or Tri-TAC” refer to a next generation CAR platform described in WO 2015/117229 A1, which is incorporated herein by reference. Tri-TAC targeting different antigens can be constructed using the antigen binding domains (*e.g.*, vL and vH fragments, scFv, vHH, ligands and receptors *etc.*) described in this disclosure using techniques known in the art. Furthermore, the different accessory modules (*e.g.*, NEMO-K277A, mNEMO-K270A *etc.*) described in this disclosure can be expressed in the Tri-TAC expressing immune cells, *e.g.*, T cells, *e.g.*, CAR-T cells.

[00158] As used herein, the terms “treat,” “treatment,” “treating,” or “amelioration” refer to therapeutic treatments, wherein the object is to reverse, alleviate, ameliorate, inhibit, slow down or stop the progression or severity of a condition associated with, a disease or disorder. The term “treating” includes reducing or alleviating at least one adverse effect or symptom of a condition, disease or disorder, such as cancer. Treatment is generally “effective” if one or more symptoms or clinical markers are reduced. Alternatively, treatment is “effective” if the progression of a disease is reduced or halted. That is, “treatment” includes not just the improvement of symptoms or markers, but also a cessation of at least slowing of progress or worsening of symptoms that would be expected in absence of treatment. Beneficial or desired clinical results include, but are not limited to, alleviation of one or more symptom(s), diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. The term “treatment” of a disease also includes providing relief from the symptoms or side-effects of the disease (including palliative treatment). In some embodiments, treatment of cancer includes decreasing tumor volume, decreasing the number of cancer cells, inhibiting cancer metastases, increasing life expectancy, decreasing cancer cell proliferation, decreasing

cancer cell survival, or amelioration of various physiological symptoms associated with the cancerous condition.

[00159] "Tumor," as used herein refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues.

[00160] "Vector", "cloning vector" and "expression vector" as used herein refer to the vehicle by which a polynucleotide sequence (*e.g.* a foreign gene) can be introduced into a host cell, so as to transform the host and promote expression (*e.g.* transcription and translation) of the introduced sequence. Vectors include plasmids, phages, viruses, *etc.*

[00161] The term "zeta" or alternatively "zeta chain", "CD3-zeta" or "TCR-zeta" is defined as the protein provided as GenBank Ace. No. BAG36664.1, or the equivalent residues from a non-human species, *e.g.*, mouse, rodent, monkey, ape and the like, and a "zeta stimulatory domain" or alternatively a "CD3-zeta stimulatory domain" or a "TCR-zeta stimulatory domain" is defined as the amino acid residues from the cytoplasmic domain of the zeta chain, or functional derivatives thereof, that are sufficient to functionally transmit an initial signal necessary for T cell activation. In one aspect the cytoplasmic domain of zeta comprises residues 52 through 164 of GenBank Ace. No. BAG36664.1 or the equivalent residues from a non-human species, *e.g.*, mouse, rodent, monkey, ape and the like, that are functional orthologs thereof. In one aspect, the "zeta stimulatory domain" or a "CD3-zeta stimulatory domain" is the sequence provided as SEQ ID NO: 4853 (**Table 6D**).

[00162] The binding domain of the CAR is selected to bind to a desired epitope. For example, the epitope recognized by a CAR can be also determined from the epitope recognized by the scFv comprising the CAR. For example, since the antigen specific domain of the CAR CD8SP-MPL-161-(vL-vH)-Myc-BBz-T2A-PAC (SEQ ID NO: 1509 and SEQ ID NO: 5422) targeting MPL is comprised of scFv MPL-161-(vL-vH) (SEQ ID NO: 808 and SEQ ID NO: 4721), it is expected that the CAR would target the same epitope as the scFv and the parental antibody from which the scFv is derived. The epitope recognized by the scFv MPL-161-(vL-vH) (SEQ ID NO: 808 and SEQ ID NO: 4721) is provided in SEQ ID NO: 15160. The epitopes recognized by several scFv and/or their parental antibodies used in the construction of the CARs and backbones of this disclosure are known in the art. Alternatively, the epitope targeted by a CAR (including the CARs that are present as part of backbones) can be determined by generating a series of mutants of its target antigen and testing the ability of the mutants to bind to the CAR-expressing cells. As an example, the epitope recognized by the CAR CD8SP-MPL-161-(vL-vH)-Myc-BBz-T2A-PAC targeting MPL can be determined by generating a panel of mutants of the MPL-ECD-GGSG-Nluc-

AcV5 fusion construct (DNA SEQ ID NO: 1015 and PRT SEQ ID NO: 4928). The mutant constructs would be transfected into a suitable cell line (*e.g.*, 293FT cells) and the supernatant containing the fusion protein collected and assayed for NLuc activity to assure that the different mutant MPL-ECD-GGSG-Nluc-AcV5 fusion proteins are being secreted in the supernatant. Subsequently, the fusion proteins would be tested for their ability to bind to cells (*e.g.*, Jurkat cells or T cells) expressing the CD8SP-MPL-161-(vL-vH)-Myc-BBz-T2A-PAC CAR construct. The mutant that fails to bind to the CAR-expressing cells is a candidate for containing the epitope targeted by the MPL-specific CAR. An alternate approach to determine the epitope recognized by a particular CAR could include a functional competitive assay with different test antibodies. For example, T cells expressing the CD8SP-MPL-161-(vL-vH)-Myc-BBz-T2A-PAC CAR could be co-cultured with a cell line expressing MPL (*e.g.*, HEL cells) in the absence and presence of increasing concentrations of different test MPL antibodies. In case the epitope recognized by a test MPL antibody overlaps with the epitope recognized by the CD8SP-MPL-161-(vL-vH)-Myc-BBz-T2A-PAC CAR, then the test antibody would be expected to block target-cell killing and cytokine production induced by T cells expressing the CD8SP-MPL-161-(vL-vH)-Myc-BBz-T2A-PAC CAR in a dose-dependent manner. A non-specific antibody of the same isotype as the test antibody would be included as a control and would be expected to have no effect on the target-cell killing and cytokine production induced by T cells expressing the CAR. Similarly, a specific CAR can be expressed in Jurkat-NFAT-EGFP cells and the ability of a test antibody to block EGFP induction by the CAR-expressing Jurkat-NFAT-GFP cells upon coculture with a target cell line can be used to determine whether the epitope recognized by the test antibody overlaps with the epitope recognized by the said CAR.

[00163] Also provided herein are compositions comprising a non-naturally occurring immune receptor, *e.g.*, a CAR, and an accessory module (including NF- κ B stimulatory molecules and selective NF- κ B activators) and method of using same to treat diseases, including cancer. As described herein, specific combinations of conventional CARs (**Table 1**) and accessory modules as described in **Table 2** are provided.

[00164] **Table 1:** Conventional CAR architectures. First generation conventional CARs (Conventional CAR I) have an intracellular signaling (ISD) domain (*e.g.* CD3z) and no costimulatory domain. The TCR fusion proteins (TFP) are another example of conventional CAR 1. Second generation conventional CARs (Conventional CAR 2 or CAR II) have one costimulatory domain (*e.g.* 41BB or CD28) and an intracellular signaling (ISD) domain (*e.g.* CD3z). Third generation conventional CARs (Conventional CAR 3 or CAR III)

have two costimulatory domains (*e.g.* 41BB and CD28) and an intracellular signaling (ISD) domain (*e.g.* CD3z). Ab-TCRs are dual chain receptors and have been described in PCT/US2016/058305. cTCRs are single chain, one-and-half, or double chain receptors consisting of antigen binding domain derived from a vL and vH fragment that are fused to one or more TCR constant chain and result in activation of T cell signaling. Synthetic immune receptors are next generation CARs and are described in US 62/429,597 and WO 2018/102795 A1:

Table 1 Conventional CAR Architectures							
1	CAR 1 or CAR I (including TFP)	ASD	HR	TMD	ISD		
2	CAR 2 (CAR II)	ASD	HR	TMD	CSD	ISD	
3	CAR 3 (CAR III)	ASD	HR	TMD	CSD-I	CSD-II	ISD
4	Ab-TCR	vL-cL	TCRD(I)	2A	vH-CH1	TCRD (II)	
5	Double Chain cTCR/SIR-1	vL	TCR-C(I)	2A	vH	TCR-C (II)	
6	One & Half Chain cTCR/SIR-3		TCR-C(I)	2A	ASD	TCR-C (II)	

[00165] **Table 2: Exemplary Backbones**

Backbone No.	CAR Component	Accessory Module		
		NAME	SEQ ID (DNA)	SEQ ID (PRT)
Backbone 1	CAR I	K13-vFLIP	972	4885
Backbone 2	CAR I	hNEMO-K277A	979	4892
Backbone 3	CAR I	FKBPx2-hNEMO-K277A	1006	4919
Backbone 4	CAR I	FKBPx2-hNEMO-L753(251)	1007	4920
Backbone 5	CAR I	FKBPx2-hNEMO-L600(200)	1008	4921
Backbone 6	CAR I	FKBPx2-RIP-ID	1009	4922
Backbone 7	CAR I	IKK2-S177E-S181E	1002	4915
Backbone 8	CAR I	IKK1-S176E-S180E	1004	4917
Backbone 9	CAR I	MYD88-L265P	1000	4913
Backbone 10	CAR I	TCL-1A	1005	4918
Backbone 11	CAR I	IgSP-[hTRAC-opt2]	1010	4923
Backbone 12	CAR I	IgSP-[hTRBC-opt2]	1011	4924
Backbone 13	CAR II	K13-vFLIP	972	4885
Backbone 14	CAR II	hNEMO-K277A	979	4892
Backbone 15	CAR II	FKBPx2-hNEMO-K277A	1006	4919
Backbone 16	CAR II	FKBPx2-hNEMO-L753(251)	1007	4920
Backbone 17	CAR II	FKBPx2-hNEMO-L600(200)	1008	4921
Backbone 18	CAR II	FKBPx2-RIP-ID	1009	4922
Backbone 19	CAR II	IKK2-S177E-S181E	1002	4915
Backbone 20	CAR II	IKK1-S176E-S180E	1004	4917
Backbone 21	CAR II	MYD88-L265P	1000	4913
Backbone 22	CAR II	TCL-1A	1005	4918
Backbone 23	CAR II	IgSP-[hTRAC-opt2]	1010	4923

Backbone No.	CAR Component	Accessory Module		
		NAME	SEQ ID (DNA)	SEQ ID (PRT)
Backbone 24	CAR II	IgSP-[hTRBC-opt2]	1011	4924
Backbone 25	CAR III	K13-vFLIP	972	4885
Backbone 26	CAR III	hNEMO-K277A	979	4892
Backbone 27	CAR III	FKBPx2-hNEMO-K277A	1006	4919
Backbone 28	CAR III	FKBPx2-hNEMO-L753(251)	1007	4920
Backbone 29	CAR III	FKBPx2-hNEMO-L600(200)	1008	4921
Backbone 30	CAR III	FKBPx2-RIP-ID	1009	4922
Backbone 31	CAR III	IKK2-S177E-S181E	1002	4915
Backbone 32	CAR III	IKK1-S176E-S180E	1004	4917
Backbone 33	CAR III	MYD88-L265P	1000	4913
Backbone 34	CAR III	TCL-1A	1005	4918
Backbone 35	CAR III	IgSP-[hTRAC-opt2]	1010	4923
Backbone 36	CAR III	IgSP-[hTRBC-opt2]	1011	4924
Backbone 37	Ab-TCR	K13-vFLIP	972	4885
Backbone 38	Ab-TCR	hNEMO-K277A	979	4892
Backbone 39	Ab-TCR	FKBPx2-hNEMO-K277A	1006	4919
Backbone 40	Ab-TCR	FKBPx2-hNEMO-L753(251)	1007	4920
Backbone 41	Ab-TCR	FKBPx2-hNEMO-L600(200)	1008	4921
Backbone 42	Ab-TCR	FKBPx2-RIP-ID	1009	4922
Backbone 43	Ab-TCR	IKK2-S177E-S181E (IKK2-SS/EE)	1002	4915
Backbone 44	Ab-TCR	IKK1-S176E-S180E (IKK1-SS/EE)	1004	4917
Backbone 45	Ab-TCR	MYD88-L265P	1000	4913
Backbone 46	Ab-TCR	TCL-1A	1005	4918
Backbone 47	Ab-TCR	IgSP-[hTRAC-opt2]	1010	4923
Backbone 48	Ab-TCR	IgSP-[hTRBC-opt2]	1011	4924
Backbone 49	DC-cTCR/SIR	K13-vFLIP	972	4885
Backbone 50	DC-cTCR/SIR	hNEMO-K277A	979	4892
Backbone 51	DC-cTCR/SIR	FKBPx2-hNEMO-K277A	1006	4919
Backbone 52	DC-cTCR/SIR	FKBPx2-hNEMO-L753(251)	1007	4920
Backbone 53	DC-cTCR/SIR	FKBPx2-hNEMO-L600(200)	1008	4921
Backbone 54	DC-cTCR/SIR	FKBPx2-RIP-ID	1009	4922
Backbone 55	DC-cTCR/SIR	IKK2-S177E-S181E	1002	4915
Backbone 56	DC-cTCR/SIR	IKK1-S176E-S180E	1004	4917
Backbone 57	DC-cTCR/SIR	MYD88-L265P	1000	4913
Backbone 58	DC-cTCR/SIR	TCL-1A	1005	4918
Backbone 59	DC-cTCR/SIR	IgSP-[hTRAC-opt2]	1010	4923
Backbone 60	DC-cTCR/SIR	IgSP-[hTRBC-opt2]	1011	4924
Backbone 61	OHC-cTCR/SIR	K13-vFLIP	972	4885
Backbone 62	OHC-cTCR/SIR	hNEMO-K277A	979	4892
Backbone 63	OHC-cTCR/SIR	FKBPx2-hNEMO-K277A	1006	4919
Backbone 64	OHC-cTCR/SIR	FKBPx2-hNEMO-L753(251)	1007	4920
Backbone 65	OHC-cTCR/SIR	FKBPx2-hNEMO-L600(200)	1008	4921
Backbone 66	OHC-cTCR/SIR	FKBPx2-RIP-ID	1009	4922
Backbone 67	OHC-cTCR/SIR	IKK2-S177E-S181E	1002	4915
Backbone 68	OHC-cTCR/SIR	IKK1-S176E-S180E	1004	4917
Backbone 69	OHC-cTCR/SIR	MYD88-L265P	1000	4913

Backbone No.	CAR Component	Accessory Module		
		NAME	SEQ ID (DNA)	SEQ ID (PRT)
Backbone 70	OHC-cTCR/SIR	TCL-1A	1005	4918
Backbone 71	OHC-cTCR/SIR	IgSP-[hTRAC-opt2]	1010	4923
Backbone 72	OHC-cTCR/SIR	IgSP-[hTRBC-opt2]	1011	4924

[00166] TABLE 6A: TARGET ANTIGENS, NAMES AND SEQ IDs OF vL

FRAGMENTS AND SEQ IDs of CDR1-3

TARGET	NAME of vL	SEQ ID vL (DNA)	SEQ ID vL (PRT)	SEQ ID-vL CDR1	SEQ ID-vL CDR2	SEQ ID-vL CDR3
ALK	Alk-48-vL	7792	10553	13204	13510	13816
ALK	Alk-58-vL	7793	10554	13205	13511	13817
Amyloid	Amyloid-158-vL	7794	10555	13206	13512	13818
BCMA	BCMA-ET-40-vL	7795	10556	13207	13513	13819
BCMA	BCMA-ET-54-vL	7796	10557	13208	13514	13820
BCMA	BCMA-huC12A3-vL	7797	10558	13209	13515	13821
BCMA	BCMA-J6M0-vL	7798	10559	13210	13516	13822
CCR4	CCR4-humAb1567-vL	7799	10560	13211	13517	13823
CD123	CD123-CSL362-vL	7800	10561	13212	13518	13824
CD138	CD138-vL	7801	10562	13213	13519	13825
CD179b	CD179b-vL	7802	10563	13214	13520	13826
CD19	CD19-4G7-vL	7803	10564	13215	13521	13827
CD19	CD19Bu12-vL	7804	10565	13216	13522	13828
CD19	CD19MM-vL	7805	10566	13217	13523	13829
CD19	FMC63-vL	7806	10567	13218	13524	13830
CD19	FMC63-[2]-vL	7807	10568	13219	13525	13831
CD19	FMC63-[3]-vL	7808	10569	13220	13526	13832
CD19	huFMC63-11-vL	7809	10570	13221	13527	13833
CD20	CD20-2F2-vL	7810	10571	13222	13528	13834
CD20	CD20-GA101-vL	7811	10572	13223	13529	13835
CD22	CD22-h10F4-vL	7812	10573	13224	13530	13836
CD22	CD22-H22RhoV2ACDRKA-vL	7813	10574	13225	13531	13837
CD22	CD22m971-vL	7814	10575	13226	13532	13838
CD276	CD276-17-vL	7815	10576	13227	13533	13839
CD30	CD30-5F11-vL	7816	10577	13228	13534	13840
CD30	CD30-Ac10-vL	7817	10578	13229	13535	13841
CD32	CD32-Med9-vL	7818	10579	13230	13536	13842
CD324	CD324-hSC10-17-vL	7819	10580	13231	13537	13843

TARGET	NAME of vL	SEQ ID vL (DNA)	SEQ ID vL (PRT)	SEQ ID-vL CDR1	SEQ ID-vL CDR2	SEQ ID-vL CDR3
CD324	CD324-SC10-6-vL	7820	10581	13232	13538	13844
CD33	CD33-huMyc9-vL	7821	10582	13233	13539	13845
CD33	CD33-AF5-vL	7822	10583	13234	13540	13846
CD34	CD34-hu4C7-[2]-vL	7823	10584	13235	13541	13847
CD34	CD34-hu4C7-vL	7824	10585	13236	13542	13848
CD44v6	CD44v6-Biwa8-vL	7825	10586	13237	13543	13849
CD5	CD5-18-vL	7826	10587	13238	13544	13850
CD5	CD5-9-vL	7827	10588	13239	13545	13851
CD70	CD70-h1F6-vL	7828	10589	13240	13546	13852
CD79b	CD79b-2F2-vL	7829	10590	13241	13547	13853
CD79b	huMA79bv28-vL	7830	10591	13242	13548	13854
CDH17	CDH17-PTA001A4-vL	7831	10592	13243	13549	13855
CDH19	CDH19-16A4-vL	7832	10593	13244	13550	13856
CDH6	CDH6-NOV710-vL	7833	10594	13245	13551	13857
CDH6	CDH6-NOV712-vL	7834	10595	13246	13552	13858
CLEC5A	CLEC5A-3E12A2-vL	7835	10596	13247	13553	13859
CLEC5A	CLEC5A-8H8F5-vL	7836	10597	13248	13554	13860
CLL1	CLL1-M26-vL	7837	10598	13249	13555	13861
CLL1	CLL1-M32-vL	7838	10599	13250	13556	13862
CMVpp65/MHC class I	CMVpp65-F5-vL	7839	10600	13251	13557	13863
CS1	huLuc63-vL	7840	10601	13252	13558	13864
CS1	HuLuc64-[2]-vL	7841	10602	13253	13559	13865
CS1	HuLuc64-vL	7842	10603	13254	13560	13866
CS1	huLuc90-vL	7843	10604	13255	13561	13867
CSF2RA	CSF2RA-Ab1-vL	7844	10605	13256	13562	13868
CSF2RA	CSF2RA-Ab6-vL	7845	10606	13257	13563	13869
DLL3	DLL3-hSC16-13-vL	7846	10607	13258	13564	13870
DLL3	DLL3-hSC16-56-vL	7847	10608	13259	13565	13871
EBNA3c/MHC class I	EBNA3c-315-vL	7848	10609	13260	13566	13872
EGFR	Cetuximab-vL	7849	10610	13261	13567	13873
EGFR	Nimotuzumab-vL	7850	10611	13262	13568	13874
EGFRviii	EGFRviii-139-vL	7851	10612	13263	13569	13875
EGFRviii	EGFRviii-2173-vL	7852	10613	13264	13570	13876
EpCam1	EpCam1-D5K5-vL	7853	10614	13265	13571	13877
EpCam1	Epcam1-MM1-vL	7854	10615	13266	13572	13878
FITC	FITC-vL	7855	10616	13267	13573	13879
FLT3	FLT3-NC7-vL	7856	10617	13268	13574	13880

TARGET	NAME of vL	SEQ ID vL (DNA)	SEQ ID vL (PRT)	SEQ ID-vL CDR1	SEQ ID-vL CDR2	SEQ ID-vL CDR3
HIV1-envelop glycoprotein	HIV1-N6-vL	7857	10618	13269	13575	13881
Folate Receptor 1 (FR1)	FR1-huMov19-vL	7858	10619	13270	13576	13882
GAD	GAD-G3H8-vL	7859	10620	13271	13577	13883
GD2	GD2-hu14-18-vL	7860	10621	13272	13578	13884
GD2	GD2-hu3F8-vL	7861	10622	13273	13579	13885
GD3	GD3-KM-641-vL	7862	10623	13274	13580	13886
GFRa4	GFRa4-P4-10-2-vL	7863	10624	13275	13581	13887
GFRa4	GFRa4-P4-10-vL	7864	10625	13276	13582	13888
GFRa4	GFRAlpha4-P4-6-vL	7865	10626	13277	13583	13889
GM1	GM1-5B2-vL	7866	10627	13278	13584	13890
GM1	GM1-7E5-vL	7867	10628	13279	13585	13891
gp100/MHC class I	gp100-G2D12-vL	7868	10629	13280	13586	13892
gp100/MHC class I	gp100-vL	7869	10630	13281	13587	13893
GPC3	GPC3-4E5-vL	7870	10631	13282	13588	13894
gpNMB	gpNMB-115-vL	7871	10632	13283	13589	13895
GPRC5D	GPRC5D-ET150-18- vL	7872	10633	13284	13590	13896
GPRC5D	GPRC5D-ET150-5- vL	7873	10634	13285	13591	13897
Her2	Her2-Hu4D5-vL	7874	10635	13286	13592	13898
HIV1-gag (77- 85)/MHC	HIV1-E5-vL	7875	10636	13287	13593	13899
HIV1-envelop glycoprotein	HIV1-3BNC117-vL	7876	10637	13288	13594	13900
HIV1-envelop glycoprotein	HIV1-PGT-128-vL	7877	10638	13289	13595	13901
HIV1-envelop glycoprotein	HIV1-VR-C01-vL	7878	10639	13290	13596	13902
HIV1-envelop glycoprotein	HIV1-X5-vL	7879	10640	13291	13597	13903
HMW-MAA	HMW-MAA-hIND- vL	7880	10641	13292	13598	13904
HTLV1- TAX/MHC class I	TAX-T3E3-vL	7881	10642	13293	13599	13905
HTLV1- TAX/MHC class I	TAX-T3F2-vL	7882	10643	13294	13600	13906
IL11Ra	IL11Ra-8E2-vL	7883	10644	13295	13601	13907
IL13Ra2	IL13Ra2-hu107-vL	7884	10645	13296	13602	13908

TARGET	NAME of vL	SEQ ID vL (DNA)	SEQ ID vL (PRT)	SEQ ID-vL CDR1	SEQ ID-vL CDR2	SEQ ID-vL CDR3
IL13Ra2	IL13Ra2-Hu108-vL	7885	10646	13297	13603	13909
IL6R	IL6R-M83-vL	7886	10647	13298	13604	13910
Influenza A HA	FLU-MEDI-8852-vL	7887	10648	13299	13605	13911
KSHV-gH	YC15-vL	7888	10649	13300	13606	13912
KSHV-K8.1	4C3-vL	7889	10650	13301	13607	13913
L1CAM	L1CAM-9-3-HU3-vL	7890	10651	13302	13608	13914
LAMP1	LAMP1-humab1-2-vL	7891	10652	13303	13609	13915
LAMP1	LAMP1-Mb4-vL	7892	10653	13304	13610	13916
LewisY	LewisY-huS193-vL	7893	10654	13305	13611	13917
Lym1	Lym1-vL	7894	10655	13306	13612	13918
Lym2	Lym2-vL	7895	10656	13307	13613	13919
MART1/MHC class I	MART1-CAG10-vL	7896	10657	13308	13614	13920
MART1/MHC class I	MART1-CLA12-vL	7897	10658	13309	13615	13921
Mesothelin	Mesothelin-m912-vL	7898	10659	13310	13616	13922
MPL (TPO-R)	MPL-111-vL	7899	10660	13311	13617	13923
MPL (TPO-R)	MPL-161-HL-vL	7900	10661	13312	13618	13924
MPL (TPO-R)	MPL-161-vL	7901	10662	13313	13619	13925
MPL (TPO-R)	MPL-175-vL	7902	10663	13314	13620	13926
MPL (TPO-R)	MPL-178-vL	7903	10664	13315	13621	13927
MPL (TPO-R)	MPL-huVB22Bw5-vL	7904	10665	13316	13622	13928
MPL (TPO-R)	MPL-12E10-vL	7905	10666	13317	13623	13929
MPL (TPO-R)	MPL-AB317-vL	7906	10667	13318	13624	13930
Muc1/MHC class I	MUC1-D6-M3A1-vL	7907	10668	13319	13625	13931
Muc1/MHC class I	Muc1-D6-M3B8-vL	7908	10669	13320	13626	13932
Muc16	Muc16-4H11-vL	7909	10670	13321	13627	13933
NKG2D	NKG2D-MS-vL	7910	10671	13322	13628	13934
NYBR1	NYBR1-vL	7911	10672	13323	13629	13935
NY-ESO/MHC class I	NY-ESO-T1-vL	7912	10673	13324	13630	13936
PD1	PD1-4H1-vL	7913	10674	13325	13631	13937
PD1	PD1-5C4-vL	7914	10675	13326	13632	13938
PDL1	PDL1-10A5-vL	7915	10676	13327	13633	13939
PDL1	PDL1-Atezoli-vL	7916	10677	13328	13634	13940
PDL1	PDL1-SP142-vL	7917	10678	13329	13635	13941
PR1/MHC class I	PR1-vL	7918	10679	13330	13636	13942

TARGET	NAME of vL	SEQ ID vL (DNA)	SEQ ID vL (PRT)	SEQ ID-vL CDR1	SEQ ID-vL CDR2	SEQ ID-vL CDR3
PSCA	PSCA-Ha14-117-vL	7919	10680	13331	13637	13943
PSCA	PSCA-Ha14-121-vL	7920	10681	13332	13638	13944
PSMA	PSMA-006-vL	7921	10682	13333	13639	13945
PSMA	PSMA-J591-vL	7922	10683	13334	13640	13946
PTK7	PTK7-hSC6-23-vL	7923	10684	13335	13641	13947
PTK7	PTK7-SC6-10-2-vL	7924	10685	13336	13642	13948
ROR1	ROR1-4A5-vL	7925	10686	13337	13643	13949
ROR1	ROR1-4C10-vL	7926	10687	13338	13644	13950
SLea	SLea-5B1-vL	7927	10688	13339	13645	13951
SLea	SLea-7E3-vL	7928	10689	13340	13646	13952
SSEA4	SSEA4-vL	7929	10690	13341	13647	13953
TCRB1	TCRB1-E09-vL	7930	10691	13342	13648	13954
TCRB1	TCRB1-Jovi1-vL	7931	10692	13343	13649	13955
TCRB2	TCRB2-CP01-D05- vL	7932	10693	13344	13650	13956
TCRB2	TCRB2-CP01-E05- vL	7933	10694	13345	13651	13957
TCRgd	TCRgd-G5-4-vL	7934	10695	13346	13652	13958
TERT/MHC class I	TERT-3G3-T865-vL	7935	10696	13347	13653	13959
TERT/MHC class I	TERT-4A9-T540-vL	7936	10697	13348	13654	13960
TGFBR2	TGFBR2-Ab1-vL	7937	10698	13349	13655	13961
TIM1	TIM1-HVCR1-270- 2-vL	7938	10699	13350	13656	13962
TIM1	Tim1HVCR1-ARD5- vL	7939	10700	13351	13657	13963
TnAg	TnAg-vL	7940	10701	13352	13658	13964
Tn-Muc1	Tn-Muc1-hu5E5-vL	7941	10702	13353	13659	13965
TROP2	TROP2-ARA47- HV3KV3-vL	7942	10703	13354	13660	13966
TROP2	TROP2-h7E6-SVG- vL	7943	10704	13355	13661	13967
TSHR	TSHR-5C9-vL	7944	10705	13356	13662	13968
TSHR	TSHR-K1-70-vL	7945	10706	13357	13663	13969
TSHR	TSHR-KB1-vL	7946	10707	13358	13664	13970
TSLRP	TSLRP-vL	7947	10708	13359	13665	13971
Tyrosinase/MH C class I	Tyro-B2-vL	7948	10709	13360	13666	13972
Tyrosinase/MH C class I	Tyro-Mc1-vL	7949	10710	13361	13667	13973
Tyrosinase/MH C class I	TA2-vL	7950	10711	13362	13668	13974

TARGET	NAME of vL	SEQ ID vL (DNA)	SEQ ID vL (PRT)	SEQ ID-vL CDR1	SEQ ID-vL CDR2	SEQ ID-vL CDR3
VEGFR3	VEGFR3-Ab1-vL	7951	10712	13363	13669	13975
WT1/MHC class I	WT1-Ab13-vL	7952	10713	13364	13670	13976
WT1/MHC class I	WT1-Ab15-vL	7953	10714	13365	13671	13977
WT1/MHC class I	WT1-Ab1-vL	7954	10715	13366	13672	13978
WT1/MHC class I	WT1-Ab5-vL	7955	10716	13367	13673	13979
EBV-gp350	EBV-gp350-vL	7956	10717	13368	13674	13980
CD123	CD123-1172-vL	7957	10718	13369	13675	13981
CDH19	CDH19-4B10-vL	7958	10719	13370	13676	13982
Folate Receptor Beta	FRbeta-m923-vL	7959	10720	13371	13677	13983
LHR	LHR-8B7-vL	7960	10721	13372	13678	13984
LHR	LHR-5F4-21-vL	7961	10722	13373	13679	13985
B7H4	B7H4-hu22C10-vL	7962	10723	13374	13680	13986
B7H4	B7H4-hu1D11-vL	7963	10724	13375	13681	13987
IgE	IgE-omalizumab-vL	7964	10725	13376	13682	13988
CD23	CD23-p5E8-vL	7965	10726	13377	13683	13989
GCC	GCC-5F9-vL	7966	10727	13378	13684	13990
GCC	GCC-Ab229-vL	7967	10728	13379	13685	13991
CD200R	CD200R-huDx182- vL	7968	10729	13380	13686	13992
AFP/MHC class I	AFP-61-vL	7969	10730	13381	13687	13993
AFP/MHC class I	AFP-76-vL	7970	10731	13382	13688	13994
AFP/MHC class I	AFP-79-vL	7971	10732	13383	13689	13995
BCMA	BCMA-ET-03-vL	7972	10733	13384	13690	13996
BCMA	BCMA- huC11.D5.3L1H3-vL	7973	10734	13385	13691	13997
BCMA	BCMA-huC13-F12- vL	7974	10735	13386	13692	13998
CD123	CD123-DART-1-vL	7975	10736	13387	13693	13999
CD123	CD123-DART-2-vL	7976	10737	13388	13694	14000
CD123	CD123-I3RB18-vL	7977	10738	13389	13695	14001
CD123	CD123-hu3E3-vL	7978	10739	13390	13696	14002
CD123	CD123-9F6-vL	7979	10740	13391	13697	14003
CD123	CD123-I3RB2-vL	7980	10741	13392	13698	14004
CD123	CD123-1176-vL	7981	10742	13393	13699	14005
CD123	CD123-8B11-vL	7982	10743	13394	13700	14006
CD123	CD123-2B8-vL	7983	10744	13395	13701	14007

TARGET	NAME of vL	SEQ ID vL (DNA)	SEQ ID vL (PRT)	SEQ ID-vL CDR1	SEQ ID-vL CDR2	SEQ ID-vL CDR3
CD123	CD123-9D7-vL	7984	10745	13396	13702	14008
CD123	CD123-3B10-vL	7985	10746	13397	13703	14009
CD19	CD19-MEDI-3649- vL	7986	10747	13398	13704	14010
CD19	CD19-Medrex-24D1- vL	7987	10748	13399	13705	14011
CD19	CD19-MOR0028-vL	7988	10749	13400	13706	14012
CD19	CD19-HD37-H2L1- vL	7989	10750	13401	13707	14013
CD19	CD19-huBly3-vL	7990	10751	13402	13708	14014
CD19	CD19-huSJ25C1-vL	7991	10752	13403	13709	14015
CD19	CD19-hB4-vL	7992	10753	13404	13710	14016
CD19	CD19-hu-mROO5-1- vL	7993	10754	13405	13711	14017
CD19	CD19-hA19-vL	7994	10755	13406	13712	14018
CD20	CD20-Leu16-vL	7995	10756	13407	13713	14019
CD20	CD20-11B8-vL	7996	10757	13408	13714	14020
CD20	CD20-2C6-vL	7997	10758	13409	13715	14021
CD20	CD20-2H7-vL	7998	10759	13410	13716	14022
CD20	CD20-hA20-vL	7999	10760	13411	13717	14023
CD20	CD20-BM-CA-1925- v4-vL	8000	10761	13412	13718	14024
CD20	CD20-Ubli-v4-vL	8001	10762	13413	13719	14025
CD20	CD20-h1F5-vL	8002	10763	13414	13720	14026
CD20	CD20-7D8-vL	8003	10764	13415	13721	14027
CD20	CD20-AME-33-vL	8004	10765	13416	13722	14028
CD33	CD33- Boehr2800308-vL	8005	10766	13417	13723	14029
CD33	CD33-Him3-4-vL	8006	10767	13418	13724	14030
CD33	CD33-SGNh2H12- vL	8007	10768	13419	13725	14031
CD33	CD33-15G15-33-vL	8008	10769	13420	13726	14032
CD33	CD33-33H4-vL	8009	10770	13421	13727	14033
CD33	CD33-9C3-2-vL	8010	10771	13422	13728	14034
CD99	CD99-hu12E7-vL	8011	10772	13423	13729	14035
CLL1	CLL1-21C9-L2H3- vL	8012	10773	13424	13730	14036
CLL1	CLL1-6E7L4H1e-vL	8013	10774	13425	13731	14037
CLL1	CLL1-hu1075-v1-vL	8014	10775	13426	13732	14038
CLL1	CLL1-hu1075-v2-vL	8015	10776	13427	13733	14039
CS1	CS1-PDL241-vL	8016	10777	13428	13734	14040
CS1	CS1-Hu27A-vL	8017	10778	13429	13735	14041

TARGET	NAME of vL	SEQ ID vL (DNA)	SEQ ID vL (PRT)	SEQ ID-vL CDR1	SEQ ID-vL CDR2	SEQ ID-vL CDR3
CS1	CS1-ScHu34C3-vL	8018	10779	13430	13736	14042
CS1	CS1-Hu31-D2-vL	8019	10780	13431	13737	14043
CS1	CS1-Luc34-vL	8020	10781	13432	13738	14044
CS1	CS1-LucX2-vL	8021	10782	13433	13739	14045
FITC	FITC-4M-53-vL	8022	10783	13434	13740	14046
FITC	FITC-E2-vL	8023	10784	13435	13741	14047
GPRC5D	GPRC5D-ET150-1-vL	8024	10785	13436	13742	14048
GPRC5D	GPRC5D-ET150-2-vL	8025	10786	13437	13743	14049
HLA-A2	HLA-A2-3PB2-vL	8026	10787	13438	13744	14050
HPV16-E7/MHC class I	HPV16-7-8-vL	8027	10788	13439	13745	14051
HPV16-E7/MHC class I	HPV16-2-vL	8028	10789	13440	13746	14052
Tissue Factor I (TFI)	TFI-98-vL	8029	10790	13441	13747	14053
Tn-Muc1	Tn-Muc1-5E5-vL	8030	10791	13442	13748	14054
Ig Kappa-Light Chain	Kappa-LC1-vL	8031	10792	13443	13749	14055
PTK7	PTK7-7C8-vL	8032	10793	13444	13750	14056
PTK7	PTK7-12C6a-vL	8033	10794	13445	13751	14057
CD19	hCD19-EUK5-13-vL	8034	10795	13446	13752	14058
Ras/MHC class I	Ras-Ab2-vL	8035	10796	13447	13753	14059
Ras/MHC class I	Ras-Ab4-vL	8036	10797	13448	13754	14060
CLD18A2	CLD18A2-43A11-vL	8037	10798	13449	13755	14061
CLD18A2	CLD18A2-175D10-vL	8038	10799	13450	13756	14062
CD43	CD43-huJL-1-257-10-vL	8039	10800	13451	13757	14063
CD69L	CD69L-DREG200-vL	8040	10801	13452	13758	14064
NY-ESO	NYESO-35-15-vL	8041	10802	13453	13759	14065
P-glycoprotein (MDR1)	Pgp-9F11-vL	8042	10803	13454	13760	14066
Streptag	Streptag-vL	8043	10804	13455	13761	14067
BCMA	BCMA-huC13-F12-L1H2-vL	8044	10805	13456	13762	14068
BCMA	BCMA-huC12A3-L3H3-vL	8045	10806	13457	13763	14069
MPL/TPO-R	Hu-161-2-vL	8046	10807	13458	13764	14070
P-glycoprotein (MDR1)	Pgp-MRK16-vL	8047	10808	13459	13765	14071
CD22	CD22-5-vL	8048	10809	13460	13766	14072

TARGET	NAME of vL	SEQ ID vL (DNA)	SEQ ID vL (PRT)	SEQ ID-vL CDR1	SEQ ID-vL CDR2	SEQ ID-vL CDR3
CD22	CD22-10-vL	8049	10810	13461	13767	14073
CD22	CD22-31-vL	8050	10811	13462	13768	14074
CD22	CD22-53-vL	8051	10812	13463	13769	14075
CD22	CD22-65-vL	8052	10813	13464	13770	14076
CD19	hu-FMC65-1-vL	8053	10814	13465	13771	14077
MPL/TPO-R	MPL-hu-175-2-vL	8054	10815	13466	13772	14078
MPL/TPO-R	MPL-hu-111-2-vL	8055	10816	13467	13773	14079
CD179a	CD179a-2460-B04-vL	8056	10817	13468	13774	14080
CD179a	CD179a-2462-E07-vL	8057	10818	13469	13775	14081
CD37	CD37-TRU-HL-vL	8058	10819	13470	13776	14082
CD37	huCD37-Boeh-vL	8059	10820	13471	13777	14083
CD70	CD70-13D-vL	8060	10821	13472	13778	14084
CD70	CD70-16D-vL	8061	10822	13473	13779	14085
CD70	CD70-21D-vL	8062	10823	13474	13780	14086
CD70	CD70-1G2D-vL	8063	10824	13475	13781	14087
CD70	CD70-hu2H5-vL	8064	10825	13476	13782	14088
CD70	CD70-69A7-vL	8065	10826	13477	13783	14089
CD70	CD70-10B4-vL	8066	10827	13478	13784	14090
CD70	CD70-24D-vL	8067	10828	13479	13785	14091
CD70	CD70-25D-vL	8068	10829	13480	13786	14092
HIV1-envelop glycoprotein	HIV1-N49P6-vL	8069	10830	13481	13787	14093
HIV1-envelop glycoprotein	HIV1-N49P7-vL	8070	10831	13482	13788	14094
HIV1-envelop glycoprotein	HIV1-N49P11-vL	8071	10832	13483	13789	14095
HIV1-envelop glycoprotein	HIV1-N60P1-1	8072	10833	13484	13790	14096
HIV1-envelop glycoprotein	HIV1-N60P25-vL	8073	10834	13485	13791	14097
HIV1-envelop glycoprotein	HIV1-N49P9-vL	8074	10835	13486	13792	14098
HIV1-envelop glycoprotein	HIV1-N60P2-1-vL	8075	10836	13487	13793	14099
HIV1-envelop glycoprotein	HIV1-N60P31-1-vL	8076	10837	13488	13794	14100
HIV1-envelop glycoprotein	HIV1-N60P22-vL	8077	10838	13489	13795	14101
HIV1-envelop glycoprotein	HIV1-N60P38-vL	8078	10839	13490	13796	14102

TARGET	NAME of vL	SEQ ID vL (DNA)	SEQ ID vL (PRT)	SEQ ID-vL CDR1	SEQ ID-vL CDR2	SEQ ID-vL CDR3
HIV1-envelop glycoprotein	HIV1-N60P30-vL	8079	10840	13491	13797	14103
HIV1-envelop glycoprotein	HIV1-N60P36-vL	8080	10841	13492	13798	14104
HIV1-envelop glycoprotein	HIV1-N60P39-vL	8081	10842	13493	13799	14105
HIV1-envelop glycoprotein	HIV1-N6039-1-vL	8082	10843	13494	13800	14106
HIV1-envelop glycoprotein	HIV1-N60P47-vL	8083	10844	13495	13801	14107
HIV1-envelop glycoprotein	HIV1-N60P48-vL	8084	10845	13496	13802	14108
HIV1-envelop glycoprotein	HIV1-N60P51-vL	8085	10846	13497	13803	14109
HIV1-envelop glycoprotein	HIV1-N60P35-vL	8086	10847	13498	13804	14110
HIV1-envelop glycoprotein	HIV1-N60P37-vL	8087	10848	13499	13805	14111
Lym1	Hu-Lym1-vL	8088	10849	13500	13806	14112
Lym2	Hu-Lym2-vL	8089	10850	13501	13807	14113
BCMA	BCMA-USC1-vL	8090	10851	13502	13808	14114
BCMA	BCMA-USC2-vL	8091	10852	13503	13809	14115
BCMA	BCMA-USC3-vL	8092	10853	13504	13810	14116
BCMA	BCMA-USC4-vL	8093	10854	13505	13811	14117
BCMA	BCMA-USC5-vL	8094	10855	13506	13812	14118
BCMA	BCMA-USC6-vL	8095	10856	13507	13813	14119
BCMA	BCMA-USC7-vL	8096	10857	13508	13814	14120
CD43	CD43-huJL-1-257- 10-vL	8097	10858	13509	13815	14121

[00167] **TABLE 6B: TARGET ANTIGENS, NAMES AND SEQ IDS OF vH
FRAGMENTS AND SEQ IDs of CDR1-3**

TARGET	NAME of vH	SEQ ID vH (DNA)	SEQ ID vH (PRT)	SEQ ID- vH CDR1	SEQ ID- vH CDR2	SEQ ID-vH CDR3
ALK	Alk-48-vH	8098	10859	14122	14428	14734
ALK	Alk-58-vH	8099	10860	14123	14429	14735
Amyloid	Amyloid-158-vH	8100	10861	14124	14430	14736
BCMA	BCMA-ET-40-vH	8101	10862	14125	14431	14737
BCMA	BCMA-ET-54-vH	8102	10863	14126	14432	14738
BCMA	BCMA-huC12A3-vH	8103	10864	14127	14433	14739
BCMA	BCMA-J6M0-vH	8104	10865	14128	14434	14740

TARGET	NAME of vH	SEQ ID vH (DNA)	SEQ ID vH (PRT)	SEQ ID- vH CDR1	SEQ ID- vH CDR2	SEQ ID-vH CDR3
CCR4	CCR4-humAb1567- vH	8105	10866	14129	14435	14741
CD123	CD123-CSL362-vH	8106	10867	14130	14436	14742
CD138	CD138-vH	8107	10868	14131	14437	14743
CD179b	CD179b-vH	8108	10869	14132	14438	14744
CD19	CD19-4G7-vH	8109	10870	14133	14439	14745
CD19	CD19Bu12-vH	8110	10871	14134	14440	14746
CD19	CD19Bu12-[2]-vH	8111	10872	14135	14441	14747
CD19	CD19MM-vH	8112	10873	14136	14442	14748
CD19	FMC63-vH	8113	10874	14137	14443	14749
CD19	FMC-63-vH	8114	10875	14138	14444	14750
CD19	huFMC63-11-vH	8115	10876	14139	14445	14751
CD20	CD20-2F2-vH	8116	10877	14140	14446	14752
CD20	CD20-GA101-vH	8117	10878	14141	14447	14753
CD22	CD22-h10F4-vH	8118	10879	14142	14448	14754
CD22	CD22- H22Rhov2ACDRKA -vH	8119	10880	14143	14449	14755
CD22	CD22m971-vH	8120	10881	14144	14450	14756
CD276	CD276-17-vH	8121	10882	14145	14451	14757
CD30	CD30-5F11-vH	8122	10883	14146	14452	14758
CD30	CD30-Ac10-vH	8123	10884	14147	14453	14759
CD32	CD32-Med9-vH	8124	10885	14148	14454	14760
CD324	CD324-hSC10-17-vH	8125	10886	14149	14455	14761
CD324	CD324-SC10-6-vH	8126	10887	14150	14456	14762
CD33	CD33-huMyc9-vH	8127	10888	14151	14457	14763
CD33	CD33-AF5-vH	8128	10889	14152	14458	14764
CD34	CD34-hu4C7-vH	8129	10890	14153	14459	14765
CD44v6	CD44v6-Biwa8-vH	8130	10891	14154	14460	14766
CD5	CD5-18-vH	8131	10892	14155	14461	14767
CD5	CD5-9-vH	8132	10893	14156	14462	14768
CD70	CD70-h1F6-vH	8133	10894	14157	14463	14769
CD79b	CD79b-2F2-vH	8134	10895	14158	14464	14770
CD79b	huMA79bv28-vH	8135	10896	14159	14465	14771
CDH17	CDH17-PTA001A4- vH	8136	10897	14160	14466	14772
CDH19	CDH19-16A4-vH	8137	10898	14161	14467	14773
CDH6	CDH6-NOV710-vH	8138	10899	14162	14468	14774
CDH6	CDH6-NOV712-vH	8139	10900	14163	14469	14775
CLEC5A	CLEC5A-3E12A2- vH	8140	10901	14164	14470	14776
CLEC5A	CLEC5A-8H8F5-vH	8141	10902	14165	14471	14777

TARGET	NAME of vH	SEQ ID vH (DNA)	SEQ ID vH (PRT)	SEQ ID- vH CDR1	SEQ ID- vH CDR2	SEQ ID-vH CDR3
CLL1	CLL1-M26-vH	8142	10903	14166	14472	14778
CLL1	CLL1-M32-vH	8143	10904	14167	14473	14779
CMVpp65/MHC class I	CMVpp65-F5-vH	8144	10905	14168	14474	14780
CS1	huLuc63-vH	8145	10906	14169	14475	14781
CS1	HuLuc64-vH	8146	10907	14170	14476	14782
CS1	huLuc90-vH	8147	10908	14171	14477	14783
CSF2RA	CSF2RA-Ab1-vH	8148	10909	14172	14478	14784
CSF2RA	CSF2RA-Ab6-vH	8149	10910	14173	14479	14785
DLL3	DLL3-hSC16-13-vH	8150	10911	14174	14480	14786
DLL3	DLL3-hSC16-56-vH	8151	10912	14175	14481	14787
EBNA3c/MHC class I	EBNA3c-315-vH	8152	10913	14176	14482	14788
EGFR	Cetuximab-vH	8153	10914	14177	14483	14789
EGFR	Nimotuzumab-vH	8154	10915	14178	14484	14790
EGFRviii	EGFRviii-139-vH	8155	10916	14179	14485	14791
EGFRviii	EGFRviii-2173-vH	8156	10917	14180	14486	14792
EpCam1	EpCam1-D5K5-vH	8157	10918	14181	14487	14793
EpCam1	Epcam1-MM1-vH	8158	10919	14182	14488	14794
FITC	FITC-vH	8159	10920	14183	14489	14795
FLT3	FLT3-NC7-vH	8160	10921	14184	14490	14796
HIV1-envelop glycoprotein	HIV1-N6-vH	8161	10922	14185	14491	14797
Folate Receptor 1 (FR1)	FR1-huMov19-vH	8162	10923	14186	14492	14798
GAD	GAD-G3H8-vH	8163	10924	14187	14493	14799
GD2	GD2-hu14-18-vH	8164	10925	14188	14494	14800
GD2	GD2-hu3F8-vH	8165	10926	14189	14495	14801
GD3	GD3-KM-641-vH	8166	10927	14190	14496	14802
GFRa4	GFRa4-P4-10-vH	8167	10928	14191	14497	14803
GFRa4	GFRAlpha4-P4-6-vH	8168	10929	14192	14498	14804
GM1	GM1-5B2-vH	8169	10930	14193	14499	14805
GM1	GM1-7E5-vH	8170	10931	14194	14500	14806
gp100/MHC class I	gp100-G2D12-vH	8171	10932	14195	14501	14807
gp100/MHC class I	gp100-vH	8172	10933	14196	14502	14808
GPC3	GPC3-4E5-vH	8173	10934	14197	14503	14809
gpNMB	gpNMB-115-vH	8174	10935	14198	14504	14810
GPRC5D	GPRC5D-ET150-18- vH	8175	10936	14199	14505	14811

TARGET	NAME of vH	SEQ ID vH (DNA)	SEQ ID vH (PRT)	SEQ ID- vH CDR1	SEQ ID- vH CDR2	SEQ ID-vH CDR3
GPRC5D	GPRC5D-ET150-5- vH	8176	10937	14200	14506	14812
Her2	Her2-Hu4D5-vH	8177	10938	14201	14507	14813
HIV1-gag (77- 85)/MHC	HIV1-E5-vH	8178	10939	14202	14508	14814
HIV1-envelop glycoprotein	HIV1-3BNC117-vH	8179	10940	14203	14509	14815
HIV1-envelop glycoprotein	HIV1-PGT-128-vH	8180	10941	14204	14510	14816
HIV1-envelop glycoprotein	HIV1-VR-C01-vH	8181	10942	14205	14511	14817
HIV1-envelop glycoprotein	HIV1-X5-vH	8182	10943	14206	14512	14818
HMW-MAA	HMW-MAA-hIND- vH	8183	10944	14207	14513	14819
HTLV1- TAX/MHC class I	TAX-T3E3-vH	8184	10945	14208	14514	14820
HTLV1- TAX/MHC class I	TAX-T3F2-vH	8185	10946	14209	14515	14821
IL11Ra	IL11Ra-8E2-vH	8186	10947	14210	14516	14822
IL13Ra2	IL13Ra2-hu107-vH	8187	10948	14211	14517	14823
IL13Ra2	IL13Ra2-Hu108-vH	8188	10949	14212	14518	14824
IL6R	IL6R-M83-vH	8189	10950	14213	14519	14825
Influenza A HA	FLU-MEDI-8852-vH	8190	10951	14214	14520	14826
KSHV-gH	YC15-vH	8191	10952	14215	14521	14827
KSHV-K8.1	4C3-vH	8192	10953	14216	14522	14828
L1CAM	L1CAM-9-3-HU3- vH	8193	10954	14217	14523	14829
LAMP1	LAMP1-humab1-2- vH	8194	10955	14218	14524	14830
LAMP1	LAMP1-Mb4-vH	8195	10956	14219	14525	14831
LewisY	LewisY-huS193-vH	8196	10957	14220	14526	14832
Lym1	Lym1-vH	8197	10958	14221	14527	14833
Lym2	Lym2-vH	8198	10959	14222	14528	14834
MART1/MHC class I	MART1-CAG10-vH	8199	10960	14223	14529	14835
MART1/MHC class I	MART1-CLA12-vH	8200	10961	14224	14530	14836
Mesothelin	Mesothelin-m912- [2]-vH	8201	10962	14225	14531	14837
Mesothelin	Mesothelin-m912-vH	8202	10963	14226	14532	14838
MPL (TPO-R)	MPL-111-vH	8203	10964	14227	14533	14839
MPL (TPO-R)	MPL-161-HL-vH	8204	10965	14228	14534	14840

TARGET	NAME of vH	SEQ ID vH (DNA)	SEQ ID vH (PRT)	SEQ ID- vH CDR1	SEQ ID- vH CDR2	SEQ ID-vH CDR3
MPL (TPO-R)	MPL-161-vH	8205	10966	14229	14535	14841
MPL (TPO-R)	MPL-175-vH	8206	10967	14230	14536	14842
MPL (TPO-R)	MPL-178-vH	8207	10968	14231	14537	14843
MPL (TPO-R)	MPL-huVB22Bw5- vH	8208	10969	14232	14538	14844
MPL (TPO-R)	MPL-12E10-vH	8209	10970	14233	14539	14845
MPL (TPO-R)	MPL-AB317-vH	8210	10971	14234	14540	14846
Muc1/MHC class I	MUC1-D6-M3A1-vH	8211	10972	14235	14541	14847
Muc1/MHC class I	Muc1-D6-M3B8-vH	8212	10973	14236	14542	14848
Muc16	Muc16-4H11-vH	8213	10974	14237	14543	14849
NKG2D	NKG2D-MS-vH	8214	10975	14238	14544	14850
NYBR1	NYBR1-vH	8215	10976	14239	14545	14851
NY-ESO/MHC class I	NY-ESO-T1-vH	8216	10977	14240	14546	14852
NY-ESO/MHC class I	NY-ESO-T2-vH	8217	10978	14241	14547	14853
PD1	PD1-4H1-vH	8218	10979	14242	14548	14854
PD1	PD1-5C4-vH	8219	10980	14243	14549	14855
PDL1	PDL1-Atezoli-vH	8220	10981	14244	14550	14856
PDL1	PDL1-SP142-vH	8221	10982	14245	14551	14857
PR1/MHC class I	PR1-vH	8222	10983	14246	14552	14858
PSCA	PSCA-Ha14-117-vH	8223	10984	14247	14553	14859
PSCA	PSCA-Ha14-121-vH	8224	10985	14248	14554	14860
PSMA	PSMA-006-vH	8225	10986	14249	14555	14861
PSMA	PSMA-J591-vH	8226	10987	14250	14556	14862
PTK7	PTK7-hSC6-23-vH	8227	10988	14251	14557	14863
PTK7	PTK7-SC6-10-2-vH	8228	10989	14252	14558	14864
ROR1	ROR1-4A5-vH	8229	10990	14253	14559	14865
ROR1	ROR1-4C10-vH	8230	10991	14254	14560	14866
SLea	SLea-5B1-vH	8231	10992	14255	14561	14867
SLea	SLea-7E3-vH	8232	10993	14256	14562	14868
SSEA4	SSEA4-vH	8233	10994	14257	14563	14869
TCRB1	TCRB1-E09-vH	8234	10995	14258	14564	14870
TCRB1	TCRB1-Jovi1-vH	8235	10996	14259	14565	14871
TCRB2	TCRB2-CP01-D05- vH	8236	10997	14260	14566	14872
TCRB2	TCRB2-CP01-E05- vH	8237	10998	14261	14567	14873
TCRgd	TCRgd-G5-4-vH	8238	10999	14262	14568	14874

TARGET	NAME of vH	SEQ ID vH (DNA)	SEQ ID vH (PRT)	SEQ ID- vH CDR1	SEQ ID- vH CDR2	SEQ ID-vH CDR3
TERT/MHC class I	TERT-3G3-T865-vH	8239	11000	14263	14569	14875
TERT/MHC class I	TERT-4A9-T540-vH	8240	11001	14264	14570	14876
TGFBR2	TGFBR2-Ab1-vH	8241	11002	14265	14571	14877
TIM1	TIM1-HVCR1-270- 2-vH	8242	11003	14266	14572	14878
TIM1	Tim1HVCR1-ARD5- vH	8243	11004	14267	14573	14879
TnAg	TnAg-vH	8244	11005	14268	14574	14880
Tn-Muc1	Tn-Muc1-hu5E5-vH	8245	11006	14269	14575	14881
TROP2	TROP2-ARA47- HV3KV3-vH	8246	11007	14270	14576	14882
TROP2	TROP2-h7E6-SVG- vH	8247	11008	14271	14577	14883
TSHR	TSHR-5C9-vH	8248	11009	14272	14578	14884
TSHR	TSHR-K1-70-vH	8249	11010	14273	14579	14885
TSHR	TSHR-KB1-vH	8250	11011	14274	14580	14886
TSLRP	TSLRP-vH	8251	11012	14275	14581	14887
Tyrosinase/MH C class I	Tyro-B2-vH	8252	11013	14276	14582	14888
Tyrosinase/MH C class I	Tyro-Mc1-vH	8253	11014	14277	14583	14889
Tyrosinase/MH C class I	TA2-vH	8254	11015	14278	14584	14890
VEGFR3	VEGFR3-Ab1-vH	8255	11016	14279	14585	14891
WT1/MHC class I	WT1-Ab13-vH	8256	11017	14280	14586	14892
WT1/MHC class I	WT1-Ab15-vH	8257	11018	14281	14587	14893
WT1/MHC class I	WT1-Ab1-vH	8258	11019	14282	14588	14894
WT1/MHC class I	WT1-Ab5-[2]-vH	8259	11020	14283	14589	14895
WT1/MHC class I	WT1-Ab5-vH	8260	11021	14284	14590	14896
EBV-gp350	EBV-gp350-vH	8261	11022	14285	14591	14897
CD123	CD123-1172-vH	8262	11023	14286	14592	14898
CDH19	CDH19-4B10-vH	8263	11024	14287	14593	14899
Folate Receptor Beta	FRbeta-m923-vH	8264	11025	14288	14594	14900
LHR	LHR-8B7-vH	8265	11026	14289	14595	14901
LHR	LHR-5F4-21-vH	8266	11027	14290	14596	14902
B7H4	B7H4-hu22C10-vH	8267	11028	14291	14597	14903
B7H4	B7H4-hu1D11-vH	8268	11029	14292	14598	14904

TARGET	NAME of vH	SEQ ID vH (DNA)	SEQ ID vH (PRT)	SEQ ID- vH CDR1	SEQ ID- vH CDR2	SEQ ID-vH CDR3
IgE	IgE-omalizumab-vH	8269	11030	14293	14599	14905
CD23	CD23-p5E8-vH	8270	11031	14294	14600	14906
GCC	GCC-5F9-vH	8271	11032	14295	14601	14907
GCC	GCC-Ab229-vH	8272	11033	14296	14602	14908
CD200R	CD200R-huDx182- vH	8273	11034	14297	14603	14909
AFP/MHC class I	AFP-61-vH	8274	11035	14298	14604	14910
AFP/MHC class I	AFP-76-vH	8275	11036	14299	14605	14911
AFP/MHC class I	AFP-79-vH	8276	11037	14300	14606	14912
BCMA	BCMA-ET-03-vH	8277	11038	14301	14607	14913
BCMA	BCMA- huC11.D5.3L1H3-vH	8278	11039	14302	14608	14914
BCMA	BCMA-huC13-F12- vH	8279	11040	14303	14609	14915
CD123	CD123-DART-1-vH	8280	11041	14304	14610	14916
CD123	CD123-DART-2-vH	8281	11042	14305	14611	14917
CD123	CD123-13RB18-vH	8282	11043	14306	14612	14918
CD123	CD123-hu3E3-vH	8283	11044	14307	14613	14919
CD123	CD123-9F6-vH	8284	11045	14308	14614	14920
CD123	CD123-13RB2-vH	8285	11046	14309	14615	14921
CD123	CD123-1176-vH	8286	11047	14310	14616	14922
CD123	CD123-8B11-vH	8287	11048	14311	14617	14923
CD123	CD123-2B8-vH	8288	11049	14312	14618	14924
CD123	CD123-9D7-vH	8289	11050	14313	14619	14925
CD123	CD123-3B10-vH	8290	11051	14314	14620	14926
CD19	CD19-MEDI-3649- vH	8291	11052	14315	14621	14927
CD19	CD19-Medrex-24D1- vH	8292	11053	14316	14622	14928
CD19	CD19-MOR0028-vH	8293	11054	14317	14623	14929
CD19	CD19-HD37-H2L1- vH	8294	11055	14318	14624	14930
CD19	CD19-huBly3-vH	8295	11056	14319	14625	14931
CD19	CD19-huSJ25C1-vH	8296	11057	14320	14626	14932
CD19	CD19-hB4-vH	8297	11058	14321	14627	14933
CD19	CD19-hu-mROO5-1- vH	8298	11059	14322	14628	14934
CD19	CD19-hA19-vH	8299	11060	14323	14629	14935
CD20	CD20-Leu16-vH	8300	11061	14324	14630	14936
CD20	CD20-11B8-vH	8301	11062	14325	14631	14937
CD20	CD20-2C6-vH	8302	11063	14326	14632	14938

TARGET	NAME of vH	SEQ ID vH (DNA)	SEQ ID vH (PRT)	SEQ ID- vH CDR1	SEQ ID- vH CDR2	SEQ ID-vH CDR3
CD20	CD20-2H7-vH	8303	11064	14327	14633	14939
CD20	CD20-hA20-vH	8304	11065	14328	14634	14940
CD20	CD20-BM-CA-1925- v4-vH	8305	11066	14329	14635	14941
CD20	CD20-Ubli-v4-vH	8306	11067	14330	14636	14942
CD20	CD20-h1F5-vH	8307	11068	14331	14637	14943
CD20	CD20-7D8-vH	8308	11069	14332	14638	14944
CD20	CD20-AME-33-vH	8309	11070	14333	14639	14945
CD33	CD33- Boehr2800308-vH	8310	11071	14334	14640	14946
CD33	CD33-Him3-4-vH	8311	11072	14335	14641	14947
CD33	CD33-SGNh2H12- vH	8312	11073	14336	14642	14948
CD33	CD33-15G15-33-vH	8313	11074	14337	14643	14949
CD33	CD33-33H4-vH	8314	11075	14338	14644	14950
CD33	CD33-33H4-2-vH	8315	11076	14339	14645	14951
CD33	CD33-9C3-2-vH	8316	11077	14340	14646	14952
CD99	CD99-hu12E7-vH	8317	11078	14341	14647	14953
CLL1	CLL1-21C9-L2H3- vH	8318	11079	14342	14648	14954
CLL1	CLL1-6E7L4H1e-vH	8319	11080	14343	14649	14955
CLL1	CLL1-hu1075-v1-vH	8320	11081	14344	14650	14956
CLL1	CLL1-hu1075-v2-vH	8321	11082	14345	14651	14957
CS1	CS1-PDL241-vH	8322	11083	14346	14652	14958
CS1	CS1-Hu27A-vH	8323	11084	14347	14653	14959
CS1	CS1-ScHu34C3-vH	8324	11085	14348	14654	14960
CS1	CS1-Hu31-D2-vH	8325	11086	14349	14655	14961
CS1	CS1-Luc34-vH	8326	11087	14350	14656	14962
CS1	CS1-LucX2-vH	8327	11088	14351	14657	14963
FITC	FITC-4M-53-vH	8328	11089	14352	14658	14964
FITC	FITC-E2-vH	8329	11090	14353	14659	14965
GPRC5D	GPRC5D-ET150-1- vH	8330	11091	14354	14660	14966
GPRC5D	GPRC5D-ET150-2- vH	8331	11092	14355	14661	14967
HLA-A2	HLA-A2-3PB2-vH	8332	11093	14356	14662	14968
HPV16- E7/MHC class I	HPV16-7-8-vH	8333	11094	14357	14663	14969
HPV16- E7/MHC class I	HPV16-2-vH	8334	11095	14358	14664	14970
Tissue Factor 1 (TF1)	TF1-98-vH	8335	11096	14359	14665	14971

TARGET	NAME of vH	SEQ ID vH (DNA)	SEQ ID vH (PRT)	SEQ ID- vH CDR1	SEQ ID- vH CDR2	SEQ ID-vH CDR3
Tn-Muc1	Tn-Muc1-5E5-vH	8336	11097	14360	14666	14972
Ig Kappa-Light Chain	Kappa-LC1-vH	8337	11098	14361	14667	14973
PTK7	PTK7-7C8-vH	8338	11099	14362	14668	14974
PTK7	PTK7-12C6a-vH	8339	11100	14363	14669	14975
CD19	hCD19-EUK5-13-vH	8340	11101	14364	14670	14976
Ras/MHC class I	Ras-Ab2-vH	8341	11102	14365	14671	14977
Ras/MHC class I	Ras-Ab4-vH	8342	11103	14366	14672	14978
CLD18A2	CLD18A2-43A11-vH	8343	11104	14367	14673	14979
CLD18A2	CLD18A2-175D10-vH	8344	11105	14368	14674	14980
CD43	CD43-huJL-1-257-10-vH	8345	11106	14369	14675	14981
CD69L	CD69L-DREG200-vH	8346	11107	14370	14676	14982
NY-ESO	NYESO-35-15-vH	8347	11108	14371	14677	14983
P-glycoprotein (MDR1)	Pgp-9F11-vH	8348	11109	14372	14678	14984
Streptag	Streptag-vH	8349	11110	14373	14679	14985
BCMA	BCMA-huC13-F12-L1H2-v2-vH	8350	11111	14374	14680	14986
BCMA	BCMA-huC12A3-L3H3-v2-vH	8351	11112	14375	14681	14987
MPL/TPO-R	Hu-161-2-vH	8352	11113	14376	14682	14988
P-glycoprotein (MDR1)	Pgp-MRK16-vH	8353	11114	14377	14683	14989
CD22	CD22-5-vH	8354	11115	14378	14684	14990
CD22	CD22-10-vH	8355	11116	14379	14685	14991
CD22	CD22-31-vH	8356	11117	14380	14686	14992
CD22	CD22-53-vH	8357	11118	14381	14687	14993
CD22	CD22-65-vH	8358	11119	14382	14688	14994
CD19	hu-FMC65-1-vH	8359	11120	14383	14689	14995
MPL/TPO-R	MPL-hu-175-2-vH	8360	11121	14384	14690	14996
MPL/TPO-R	MPL-hu-111-2-vH	8361	11122	14385	14691	14997
CD179a	CD179a-2460-B04-vH	8362	11123	14386	14692	14998
CD179a	CD179a-2462-E07-vH	8363	11124	14387	14693	14999
CD37	CD37-TRU-HL-vH	8364	11125	14388	14694	15000
CD37	huCD37-Boeh-vH	8365	11126	14389	14695	15001
CD70	CD70-13D-vH	8366	11127	14390	14696	15002
CD70	CD70-16D-vH	8367	11128	14391	14697	15003
CD70	CD70-21D-vH	8368	11129	14392	14698	15004
CD70	CD70-1G2D-vH	8369	11130	14393	14699	15005

TARGET	NAME of vH	SEQ ID vH (DNA)	SEQ ID vH (PRT)	SEQ ID- vH CDR1	SEQ ID- vH CDR2	SEQ ID-vH CDR3
CD70	CD70-hu-2H5-vH	8370	11131	14394	14700	15006
CD70	CD70-69A7-vH	8371	11132	14395	14701	15007
CD70	CD70-10B4-vH	8372	11133	14396	14702	15008
CD70	CD70-24D-vH	8373	11134	14397	14703	15009
CD70	CD70-25D-vH	8374	11135	14398	14704	15010
HIV1-env glycoprotein	HIV1-N49P6-vH	8375	11136	14399	14705	15011
HIV1-env glycoprotein	HIV1-N49P7-vH	8376	11137	14400	14706	15012
HIV1-env glycoprotein	HIV1-N49P11-vH	8377	11138	14401	14707	15013
HIV1-env glycoprotein	HIV1-N60P1-1-vH	8378	11139	14402	14708	15014
HIV1-env glycoprotein	HIV1-N60P25-vH	8379	11140	14403	14709	15015
HIV1-env glycoprotein	HIV1-N49P9-vH	8380	11141	14404	14710	15016
HIV1-env glycoprotein	HIV1-N60P2-1-vH	8381	11142	14405	14711	15017
HIV1-env glycoprotein	HIV1-N60P31-1-vH	8382	11143	14406	14712	15018
HIV1-env glycoprotein	HIV1-N60P22-vH	8383	11144	14407	14713	15019
HIV1-env glycoprotein	HIV1-N60P38-vH	8384	11145	14408	14714	15020
HIV1-env glycoprotein	HIV1-N60P30-vH	8385	11146	14409	14715	15021
HIV1-env glycoprotein	HIV1-N60P36-vH	8386	11147	14410	14716	15022
HIV1-env glycoprotein	HIV1-N60P39-vH	8387	11148	14411	14717	15023
HIV1-env glycoprotein	HIV1-N6039-1-vH	8388	11149	14412	14718	15024
HIV1-env glycoprotein	HIV1-N60P47-vH	8389	11150	14413	14719	15025
HIV1-env glycoprotein	HIV1-N60P48-vH	8390	11151	14414	14720	15026
HIV1-env glycoprotein	HIV1-N60P51-vH	8391	11152	14415	14721	15027
HIV1-env glycoprotein	HIV1-N60P35-vH	8392	11153	14416	14722	15028
HIV1-env glycoprotein	HIV1-N60P37-vH	8393	11154	14417	14723	15029
Lym1	hu-Lym1-vH	8394	11155	14418	14724	15030
Lym2	hu-Lym2-vH	8395	11156	14419	14725	15031
BCMA	BCMA-USC1-vH	8396	11157	14420	14726	15032
BCMA	BCMA-USC2-vH	8397	11158	14421	14727	15033

TARGET	NAME of vH	SEQ ID vH (DNA)	SEQ ID vH (PRT)	SEQ ID- vH CDR1	SEQ ID- vH CDR2	SEQ ID-vH CDR3
BCMA	BCMA-USC3-vH	8398	11159	14422	14728	15034
BCMA	BCMA-USC4-vH	8399	11160	14423	14729	15035
BCMA	BCMA-USC5-vH	8400	11161	14424	14730	15036
BCMA	BCMA-USC6-vH	8401	11162	14425	14731	15037
BCMA	BCMA-USC7-vH	8402	11163	14426	14732	15038
CD43	CD43-huJL-1-257- 10-vH	8403	11164	14427	14733	15039

[00168] TABLE 6C: scFV Fragments

Target	NAME	SEQ ID- DNA	SEQ ID- PRT	Target	NAME	SEQ ID- DNA	SEQ ID- PRT
CD19	FMC63	8404	11165	CDH17	CDH17- PTA001A4	8443	11204
CD19	huFMC63- 11	8405	11166	CDH19	CDH19- 16A4	8444	11205
CD19	CD19Bu12	8406	11167	EGFR	Cetuximab	8445	11206
CD19	CD19MM	8407	11168	CLEC5A	CLEC5A- 8H8F5	8446	11207
CD19	CD19-4G7	8408	11169	CLEC5A	CLEC5A- 3E12A2	8447	11208
HIV1-env	HIV1-N6	8409	11170	CLL1	CLL1-M26	8448	11209
ALK	Alk-48	8410	11171	CLL1	CLL1-M32	8449	11210
ALK	Alk-58	8411	11172	CMVpp65	CMVpp65- F5	8450	11211
Amyloid	Amyloid- 158	8412	11173	CS1	CS1- huLuc63	8451	11212
CD45	BC8-CD45	8413	11174	CS1	CS1- HuLuc64	8452	11213
BCMA	BCMA- J6M0	8414	11175	CS1	CS1- huLuc90	8453	11214
BCMA	BCMA- huC12A3- L3H3	8415	11176	CSF2RA	CSF2RA- Ab6	8454	11215
BCMA	BCMA- ET-40	8416	11177	CSF2RA	CSF2RA- Ab1	8455	11216
BCMA	BCMA- ET-54	8417	11178	DLL3	DLL3- hSC16-13	8456	11217
CCR4	CCR4- humAb156 7	8418	11179	DLL3	DLL3- hSC16-56	8457	11218
CD5	CD5-9	8419	11180	EBNA3c	EBNA3c- 315	8458	11219
CD5	CD5-18	8420	11181	Ebv-gp350	EBV- gp350	8459	11220
CD20	CD20-2F2	8421	11182	EGFRviii	EGFRvIII- 139	8460	11221

CD20	CD20-GA101	8422	11183	EGFRviii	EGFRvIII-2173	8461	11222
CD22	CD22-h10F4v2	8423	11184	EpCam1	Epcam1-MM1	8462	11223
CD22	CD22-H22Rhov2 ACDRKA	8424	11185	EpCam1	Epcam1-D5K5	8463	11224
CD22	CD22-m971	8425	11186	FLT3	FLT3-NC7	8464	11225
CD30	CD30-5F11	8426	11187	FITC	FITC	8465	11226
CD30	CD30-Ac10	8427	11188	Influenza A HA	FLU-MEDI-8852	8466	11227
CD32	CD32-Med9	8428	11189	FR1	FR1-huMov19	8467	11228
CD33	CD33-AF5	8429	11190	GAD	GAD-G3H8	8468	11229
CD33	CD33-huMyc9	8430	11191	GD2	GD2-hu14-18	8469	11230
CD34	CD34-hu4C7	8431	11192	GD2	GD2-hu3F8	8470	11231
CD44v6	CD44v6-Biwa8	8432	11193	GD3	GD3-KM-641	8471	11232
CD70	CD70-h1F6	8433	11194	GFRa4	GFRAlpha4-P4-6	8472	11233
CD79b	CD79b-2F2	8434	11195	GFRa4	GFRa4-P4-10	8473	11234
CD123	CD123-CSL362	8435	11196	GM1	GM1-5B2	8474	11235
CD138	CD138	8436	11197	GM1	GM1-7E5	8475	11236
CD179b	CD179b	8437	11198	GPRC5D	GPRC5D-ET150-5	8476	11237
CD276	CD276-17	8438	11199	GPRC5D	GPRC5D-ET150-18	8477	11238
CD324	CD324-SC10-6	8439	11200	gp100	gp100	8478	11239
CD324	CD324-hSC10-17	8440	11201	gp100	gp100-G2D12	8479	11240
CDH6	CDH6-NOV710	8441	11202	GPC3	GPC3-4E5	8480	11241
CDH6	CDH6-NOV712	8442	11203	gpNMB	gpNMB-115	8481	11242
GRP78	GRP78-GC18	8482	11243	PDL1	PDL1-SP142	8522	11283
HIV1-gag(77-85)	HIV1-E5	8483	11244	PDL1	PDL1-10A5	8523	11284
HIV1-env	HIV1-3BNC117	8484	11245	PSCA	PSCA-Ha14-121	8524	11285

HIV1-env	HIV1-PGT-128	8485	11246	PSCA	PSCA-Ha14-117	8525	11286
HIV1-env	HIV1-VR-C01	8486	11247	PR1	PR1	8526	11287
HIV1-env	HIV1-X5	8487	11248	PSMA	PSMA-006	8527	11288
HMW-MAA	HMW-MAA-hIND	8488	11249	PSMA	PSMA-J591	8528	11289
HTLV1-TAX	HTLV-TAX-T3F2	8489	11250	PTK7	PTK7-hSC6-23	8529	11290
HTLV1-TAX	HTLV-TAX-T3E3	8490	11251	PTK7	PTK7-SC6-10-2	8530	11291
IL11Ra	IL11Ra-8E2-Ts107	8491	11252	ROR1	ROR1-4A5	8531	11292
IL13Ra2	IL13Ra2-hu107	8492	11253	ROR1	ROR1-4C10	8532	11293
IL13Ra2	IL13Ra2-Hu108	8493	11254	Mesothelin	SD1-vHH-Linker-SD2-vHH	8533	11294
KSHV-K8.1	KSHV-4C3	8494	11255	SLea	SLea-7E3	8534	11295
LAMP1	LAMP1-humab1-2	8495	11256	SLea	SLea-5B1	8535	11296
LAMP1	LAMP1-Mb4	8496	11257	SSEA4	SSEA4	8536	11297
LewisY	LewisY-huS193	8497	11258	TCRB1	TCRB1-CP01-E09	8537	11298
L1CAM	L1CAM-9-3-HU3	8498	11259	TCRB1	TCRB1-Jovi1	8538	11299
Lym1	Lym1	8499	11260	TCRB2	TCRB2-CP01-D05	8539	11300
Lym2	Lym2	8500	11261	TCRB2	TCRB2-CP01-E05	8540	11301
CD79b	huMA79bv28	8501	11262	TCRgd	TCRgd-G5-4	8541	11302
MART1	MART1-CAG10	8502	11263	TERT	TERT-4A9-T540	8542	11303
MART1	MART1-CLA12	8503	11264	TERT	TERT-3G3-T865	8543	11304
Mesothelin	Mesothelin-m912	8504	11265	TGFBR2	TGFBR2-Ab1	8544	11305
MPL	MPL-175	8505	11266	TIM1	TIM1-HVCR1-270-2	8545	11306
MPL	MPL-161	8506	11267	TIM1	TIM1-HVCR1-ARD5	8546	11307
MPL	MPL-161-HL	8507	11268	TnAg	TnAg	8547	11308

MPL	MPL-111	8508	11269	Tn-Muc1	TnMuc1-hu5E5-RHA8-RKA-2	8548	11309
MPL	MPL-178	8509	11270	TROP2	TROP2-ARA47-HV3KV3	8549	11310
MPL	MPL-AB317	8510	11271	TROP2	TROP2-h7E6-SVG	8550	11311
MPL	MPL-12E10	8511	11272	TSHR	TSHR-K1-70	8551	11312
MPL	MPL-huVB22Bw5	8512	11273	TSHR	TSHR-KB1	8552	11313
Muc1	Muc1-D6-M3B8	8513	11274	TSHR	TSHR-5C9	8553	11314
Muc1	MUC1-D6-M3A1	8514	11275	TSLRP	TSLRP	8554	11315
Muc16	Muc16-4H11	8515	11276	Tyrosinase	Tyros-B2	8555	11316
EGFR	Nimotuzu mab	8516	11277	Tyrosinase	Tyros-MC1	8556	11317
NKG2D	NKG2D-MS	8517	11278	Tyrosinase	Tyros-TA2	8557	11318
NYBR1	NYBR1	8518	11279	VEGFR3	VEGFR3-Ab1	8558	11319
NY-ESO	NYESO-T1	8519	11280	WT1	WT1-Ab1	8559	11320
NY-ESO	NYESO-T1	8520	11281	WT1	WT1-Ab5	8560	11321
PDL1	PDL1-Atezoli	8521	11282	WT1	WT1-Ab13	8561	11322
WT1	WT1-Ab15	8562	11323	CD22	CD22-65	8658	11356
CD123	CD123-1172	8563	11324	CD19	hu-FMC65	8659	11357
CDH19	CDH19-4B10	8564	11325	MPL	MPL-hu-175-2	8660	11358
FRbeta	FRbeta-m923	8565	11326	MPL	MPL-hu-111-2	8661	11359
LHR-8B7	LHR-8B7	8566	11327	CD179a	CD179a-2460-B04	8662	11360
LHR-5F4-21	LHR-5F4-21	8567	11328	CD179a	CD179a-2462-E07	8663	11361
B7H4	B7H4-hu22C10	8568	11329	CD37	CD37-TRU-HL	8664	11362
B7H4-hu1D11	B7H4-hu1D11	8569	11330	CD37	huCD37-Boeh	8665	11363
IgE	IgE-omalizuma b	8570	11331	CD70	CD70-13D	8666	11364

CD23	CD23-p5E8	8571	11332	CD70	CD70-16D	8667	11365
GCC	GCC-5F9	8572	11333	CD70	CD70-21D	8668	11366
GCC	GCC-Ab229	8573	11334	CD70	CD70-1G2D	8669	11367
CD200R	CD200R-huDx182	8637	11335	CD70	CD70-hu2H5	8670	11368
Tn-Muc1-5E5	Tn-Muc1-5E5	8638	11336	CD70	CD70-69A7	8671	11369
Igk-Light Chain	Kappa-LC1	8639	11337	CD70	CD70-10B4	8672	11370
PTK7	PTK7-7C8	8640	11338	CD70	CD70-24D	8673	11371
PTK7	PTK7-12C6a	8641	11339	CD70	CD70-25D	8674	11372
CD19	hCD19-EUK5-13	8642	11340	HIV1-env	HIV1-N49P6	8675	11373
Ras	Ras-Ab2	8643	11341	HIV1-env	HIV1-N49P7	8676	11374
Ras	Ras-Ab4	8644	11342	HIV1-env	HIV1-N49P11	8677	11375
CLD18A2	CLD18A2-43A11	8645	11343	HIV1-env	HIV1-N60P1-1	8678	11376
CLD18A2	CLD18A2-175D10	8646	11344	HIV1-env	HIV1-N60P25	8679	11377
CD43	CD43-huJL-1-257-10	8647	11345	HIV1-env	HIV1-N49P9	8680	11378
CD69L	CD69L-DREG200	8648	11346	HIV1-env	HIV1-N60P2-1	8681	11379
NY-ESO	NYESO-35-15	8649	11347	HIV1-env	HIV1-N60P31-1	8682	11380
Pgp	Pgp-9F11	8650	11348	HIV1-env	HIV1-N60P22	8683	11381
Streptag	Streptag	8651	11349	HIV1-env	HIV1-N60P38	8684	11382
MPL	Hu-161-2	8652	11350	HIV1-env	HIV1-N60P30	8685	11383
Pgp	Pgp-MRK16	8653	11351	HIV1-env	HIV1-N60P36	8686	11384
CD22	CD22-5	8654	11352	HIV1-env	HIV1-N60P39	8687	11385
CD22	CD22-10	8655	11353	HIV1-env	HIV1-N6039.1	8688	11386
CD22	CD22-31	8656	11354	HIV1-env	HIV1-N60P47	8689	11387
CD22	CD22-53	8657	11355	HIV1-env	HIV1-N60P48	8690	11388
HIV1-env	HIV1-N60P51	8691	11389	BCMA	BCMA-USC3	8698	11396
HIV1-env	HIV1-N60P35	8692	11390	BCMA	BCMA-USC4	8699	11397

HIV1-env	HIV1-N60P37	8693	11391	BCMA	BCMA-USC5	8700	11398
Lym1	hu-Lym1	8694	11392	BCMA	BCMA-USC6	8701	11399
Lym2	hu-Lym2	8695	11393	BCMA	BCMA-USC7	8702	11400
BCMA	BCMA-USC1	8696	11394	CD33	CD33-SGNh2H12	8727	15099
BCMA	BCMA-USC2	8697	11395	CD33	CD33-15G15-33	8728	15100
CD19	CD19-MEDI-3649	8698	15070	CD33	CD33-33H4	8729	15101
CD19	CD19-Medrex-24D1	8699	15071	CD33	CD33-9C3-2	8730	15102
CD19	CD8SP-Ritx-CD19-MOR0028	8700	15072	CD99	CD99-hu12E7	8731	15103
CD19	CD19-HD37-H2L1	8701	15073	CD123	CD123-DART1-1	8732	15104
CD19	CD19-huBly3	8702	15074	CD123	CD123-DART1-2	8733	15105
CD19	CD19-huSJ25C1	8703	15075	CD123	CD123-I3RB18	8734	15106
CD19	CD8SP-Ritx-CD19-hB4	8704	15076	CD123	CD123-hu3E3	8735	15107
CD19	CD19-hu-mROO5-1	8705	15077	CD123	CD123-9F6	8736	15108
CD19	CD19-hA19	8706	15078	CD123	CD123-I3RB2	8737	15109
AFP/MHC I	AFP-61	8707	15079	CD123	CD123-1176	8738	15110
AFP/MHC I	AFP-76	8708	15080	CD123	CD8SP-Ritx2-CD123-8B11	8739	15111
AFP/MHC I	AFP-79	8709	15081	CD123	CD123-2B8	8740	15112
BCMA	BCMA-ET-03	8710	15082	CD123	CD123-9D7	8741	15113
BCMA	BCMA-huC11.D5.3L1H3	8711	15083	CD123	CD123-3B10	8742	15114
BCMA	BCMA-huC13-F12	8712	15084	CLL1	CLL1-21C9-L2H3	8743	15115
CD20	CD20-11B8	8713	15085	CLL1	CLL1-6E7L4H1e	8744	15116

CD20	CD20-2C6	8714	15086	CLL1	CLL1-hu1075-v1	8745	15117
CD20	CD20-2H7	8715	15087	CLL1	CLL1-hu1075-v2	8746	15118
CD20	CD20-hA20	8716	15088	CS1	CS1-PDL241	8747	15119
CD20	CD20-BM-CA-1925-v4	8717	15089	CS1	CS1-Hu27A	8748	15120
CD20	CD20-Ubli-v4	8718	15090	CS1	CS1-ScHu34C3	8749	15121
CD20	CD20-2H7	8719	15091	CS1	CS1-Hu31-D2	8750	15122
CD20	CD20-h1F5	8720	15092	CS1	CS1-Luc34	8751	15123
CD20	CD20-7D8	8721	15093	CS1	CS1-LucX2	8752	15124
CD20	CD20-7D8-vL-linker-GA-Tag-VH	8722	15094	FITC	FITC-4M-53	8753	15125
CD20	CD20-AME-33	8723	15095	FITC	FITC-E2-HL	8754	15126
CD43	CD43-huJL-1-257-10	8703	11401	GPRC5D	GPRC5D-ET150-1	8755	15127
CD22	CD22-m971-HL	8724	15096	GPRC5D	GPRC5D-ET150-2	8756	15128
CD33	CD8SP-Ritx2-BC33-Boehr2800308	8725	15097	HLA-A2	HLA-A2-3PB2	8757	15129
CD33	CD8SP-Ritx2-CD33-Him3-4	8726	15098	HPV16/MHC I	HPV16-7-8	8758	15130
TF1	TF1-98	8760	15132	HPV16/MHC I	HPV16-2	8759	15131

[00169] TABLE 6D: CAR COMPONENTS

CAR component	SEQ ID NO (DNA)	SEQ ID NO (PRT)	CAR component	SEQ ID NO (DNA)	SEQ ID NO (PRT)
F2A	925	4838	IgG1-CH1-TCRd-6MD	962	4875
T2A	926	4839	IgG1-CH1-TCRa-SDVP-6MD	963	4876
P2A	928	4841	IgG1-CH1-TCRa-wt2-opt-6MD	964	4877
E2A	930	4843	hTCRa-WT	3885	15041

SGSG Linker	931	4844	hTCRa-CSDVP	3886	15042
FURINE SITE	933	4846	hTCRa-opt2	3887	15043
hCD8-Hinge-TM	936	4849	hTCRa-T48C-opt	3889	15045
hCD8-Hinge-TM-BBz	937	4850	hTCRa-S61R	3892	15048
4-IBB-cytosolic-domain	939	4852	hTCR-b1-constant	3895	15051
CD3z-cytosolic-domain	940	4853	hTCR-b2-constant	3896	15052
CD28-Hinge-TM-CP	942	4855	hTCRb-WT	3897	15053
CD3d-ECDTMCP-opt2	944	4857	hTCRb-S57C-opt1	3898	15054
CD3eECDTMCP-opt2	948	4861	hTCRb-KACIAH	3899	15055
CD3g-ECDTMCP-opt2	949	4862	hTCRb-opt2	3900	15056
CD3zECDTMCP-opt2	958	4871	hTCRb-R79G	3910	15066
IgCL-TCRg-6MD	959	4872	hTCRg-(hTCR-gamma)	3912	15068
IgCL-TCRb-IAH-6MD	960	4873	hTCR-(hTCR-delta)	3913	15069
IgCL-TCRb-wt2-opt-6MD	961	4874	CD8-Signal-Peptide	1	3914
IgH-Signal Peptide	5	3918	(GGGGS)x3_LINKER	278	4191
Myc-Tag	903	4816	V5 Tag	908	4821
RiTX2-TAG	918	4831	RITX4 TAG	919	4832
PG4SP	288	4201	EAAAK	292	4205
PG4SP-v2-U	289	4202	EAAAK-v2	293	4206
E-coil	290	4203	K-coil	291	4204
TCRa-opt-6MD	15141	15133	TCRg-6MD	15143	15135
TCRb-opt-6MD	15142	15134	TCRd-6MD	15144	15136

[00170] **TABLE 7: EXEMPLARY ACCESSORY MODULES**

Accessory Module	SEQ ID NO (DNA)	SEQ ID NO (PRT)	Accessory Module	SEQ ID NO (DNA)	SEQ ID NO (PRT)
K13-opt	7768	10538	IKK1-S176E-S180E	1004	4917
K13-vFLIP	972	4885	FKBPx2-hNEMO-K277A	1006	4919
FKBP-K13	973	4886	FKBPx2-hNEMO-L753(251)	1007	4920
FKBPX2-K13	974	4887	FKBPx2-hNEMO-L600(200)	1008	4921
Myr-FKBPx2-K13	975	4888	FKBPx2-RIP-ID	1009	4922
FKBPx2-HTLV2-Tax-RS	976	4889	hNEMO-FL-GS-FKBPv36X2	7763	10533
FKBPx2-Flag-HTLV2-Tax-RS	977	4890	hNEMO-L825-GS-FKBPv36x2	7764	10534
hNEMO-K277A	979	4892	hNEMO-L753-GS-FKBPv36x2	7765	10535
hNEMO-D23V-K277A	980	4893	hNEMO-L600-GS-FKBPv36x2	7766	10536
hNEMO-K277A-L1161	986	4899	hNEMO-K277A-Delta-V249-K255	7767	10537
hNEMO-K277A-L1014	989	4902	IKK1-delta-SCD-FKBPv36x2	7781	10541

Accessory Module	SEQ ID NO (DNA)	SEQ ID NO (PRT)	Accessory Module	SEQ ID NO (DNA)	SEQ ID NO (PRT)
mNEMO-K270A	992	4905	IKK2-delta-SCD-FKBPv36x2	7782	10542
RIP-ID	998	4911	TCL-1A	1005	4918
MyD88	999	4912	MTCP-1	7769	10539
MYD88-L265P	1000	4913	CMV-141	7770	10540
IKK2	1001	4914	IgSP-[hTRAC-opt2]	1010	4923
IKK2-S177E-S181E	1002	4915	IgSP-[hTRBC-opt2]	1011	4924
IKK1	1003	4916			
IgSP-TCRa-opt-6MD	15145	15137	IgSP-TCRg-6MD	15147	15139
IgSP-TCRb-opt-6MD	15146	15138	IgSP-TCRd-6MD	15148	15140

[00171] **TABLE 8: MHC I (HLA-A2) restricted peptides used for generation of****CARs**

Protein/Epitope	SEQ ID NO:	Protein/Epitope	SEQ ID NO:
gp100	10511	MART (26-35)	10521
gp100	10512	EBNA-3A (596-604)	10522
gp100	10513	EBNA-3c	10523
MUC1-A7 (130-138)	10514	WT1	10524
MUC1-D6 (13-21)	10515	PR1	10525
TAX (11-19)	10516	Ras9-G12V	10526
hTERT(540-548)	10517	HPV16-E7	10527
hTERT (865-873)	10518	NY-ESO-1-(155-163)	10528
HIV1 gag (77-85)	10519	NY-ESO-1-(157-165)	10529
CMV-pp65(495-503)	10520	NY-ESO-1-(157-167)	10530

[00172] **Table 9:**

CAR/BI TE "X" TARGET	EXEMPLARY DISEASE TARGETED BY CARs (i.e. conventional CARs and next generation CARs. E.g., SIR, Ab-TCR, and TFP) and Bispecific T Cell Engagers (BiTE)
CD19	ALL, CLL, lymphoma, lymphoid blast crisis of CML, multiple myeloma, immune disorders
ALK	Non Small Cell Lung Cancer (NSCLC), ALCL (anaplastic large cell lymphoma), IMT (inflammatory myofibroblastic tumor), or neuroblastoma
CD45	Blood cancers
BCMA	Myeloma, PEL, plasma cell leukemia, Waldenstrom's macroglobinemia
CD5	Blood cancer, T cell leukemia, T cell lymphoma
CD20	Blood cancers, Leukemia, ALL, CLL, lymphoma, immune disorders
CD22	Blood cancers, Leukemia, ALL, CLL, lymphoma, lymphoid blast crisis of CML, immune disorders
CD23	Blood cancers, Leukemia, ALL, CLL, lymphoma, autoimmune disorders
CD30	Hodgkins's lymphoma, Cutaneous T cell lymphoma
CD32	Solid tumors
CD33	Blood cancers, AML, MDS

CAR/BiTE "X" TARGET	EXEMPLARY DISEASE TARGETED BY CARs (<i>i.e.</i> conventional CARs and next generation CARs. <i>E.g.</i>, SIR, Ab-TCR, and TFP) and Bispecific T Cell Engagers (BiTE)
CD34	Blood cancers, AML, MDS
CD44v6	Blood cancers, AML, MDS
CD70	Blood cancers, lymphoma, myeloma, Waldenstrom's macroglobulinemia, Kidney cancer
CD79b	Blood cancers, ALL, Lymphoma
CD123	Blood cancers, AML, MDS
CD138	Blood cancers, Myeloma, PEL, plasma cell leukemia, waldenstrom's macroglobulinemia
CD179b	Blood cancers, ALL, Lymphoma
CD276/B7-H3	Ewing's sarcoma, neuroblastoma, rhabdomyosarcoma, ovarian, colorectal and lung cancers
CD324	Solid tumors, esophageal, prostate, colorectal, breast, lung cancers
CDH6	Solid tumors, renal, ovarian, thyroid cancers
CDH17	Adenocarcinomas, gastrointestinal, lung, ovarian, endometrial cancers
CDH19	Solid tumor, Melanoma
EGFR	Colon cancer, lung cancer
CLEC5A	Blood cancers, Leukemia, AML
GR/LHR	Prostate cancer, ovarian cancer or breast cancer
CLL1	Blood cancer, Leukemia
CMVpp65	CMV infection, CMV colitis, CMV pneumonitis
CS1	Blood cancers, myeloma, PEL, plasma cell leukemia
CSF2RA	AML, CML, MDS
CD123	Blood cancers, AML, MDS
DLL3	Melanoma, lung cancer or ovarian cancer
EBNA3c/MHC I	Epstein Barr virus infection and related diseases including cancers
EBV-gp350	Epstein Barr virus infection and related diseases
EGFR	Solid tumors, Colon cancer, lung cancer
EGFRvIII	Solid tumors, glioblastoma
EpCam1	Gastrointestinal cancer
FLT3	Blood cancers, AML, MDS, ALL
Folate Receptor alpha(FR1 or FOLR1)	Ovarian cancer, NSCLC, endometrial cancer, renal cancer, or other solid tumors
FSHR	Prostate cancer, ovarian cancer or breast cancer
GD2	Neuroblastoma
GD3	Melanoma
GFRa4	Cancer, thyroid medullary cancer
Fucosyl-GM1(GM1)	Small cell lung cancer
GPRC5D	Myeloma, PEL, plasma cell leukemia, waldenstrom's macroglobulinemia
gp100	Melanoma
GPC3	Solid tumors, Lung cancer
gpNMB	Melanoma, brain tumors, gastric cancers
GRP78	Myeloma

CAR/BiTE "X" TARGET	EXEMPLARY DISEASE TARGETED BY CARs (<i>i.e.</i> conventional CARs and next generation CARs. <i>E.g.</i>, SIR, Ab-TCR, and TFP) and Bispecific T Cell Engagers (BiTE)
Her2	Solid tumors, breast cancer, stomach cancer
Her3	Colorectal, breast cancer
HMW-MAA	Melanoma
HTLV1-TAX/MHC I	HTLV1 infection associated diseases, Adult T cell leukemia-lymphoma
IL11Ra	Blood cancers, AML, ALL, CML, MDS, sarcomas
IL6Ra	Solid tumors, Liver cancer
IL13Ra2	Glioblastomas
KSHV-K8.1	Kaposi's sarcoma, PEL, Multicentric Castleman's disease
LAMP1	Blood cancers, AML, ALL, MDS, CLL, CML
LewisY	Cancers
L1CAM	Solid tumors, ovarian, breast, endometrial cancers, melanoma
LHR	Prostate cancer, ovarian cancer or breast cancer
Lym1	Blood cancer, Leukemia, Lymphoma
Lym2	Blood cancer, Leukemia, Lymphoma
CD79b	Blood cancers, lymphoma
MART1/MHC I	Melanoma
Mesothelin	Mesothelioma, ovarian cancer, pancreatic cancer
Muc1/MHC I	Breast cancer, gastric cancer, colorectal cancer, lung cancer, or other solid tumors
Muc16	Ovarian cancer
NKG2D	Leukemia, lymphoma or myeloma
NYBR1	Breast cancer
PSCA	Prostate cancer
PR1/MHC I	Blood cancer, Leukemia
Prolactin Receptor	Breast cancer, chromophobe renal cell cancer
PSMA	Prostate cancer
PTK7	Melanoma, lung cancer or ovarian cancer
ROR1	Blood cancer, B cell malignancy, lymphoma, CLL
SLea	Pancreatic cancer, colon cancer
SSEA4	Pancreatic cancer
Tyrosinase/MHC I	Melanoma
TCRB1	T cell leukemias and lymphomas, autoimmune disorders
TCRB2	T cell leukemias and lymphomas, autoimmune disorders
TCRgd	T cell leukemias and lymphomas, autoimmune disorders
hTERT	Solid tumors, blood cancers
TGFBR2	Solid tumors, keloid
TIM1/HAVCR1	Kidney cancer, liver cancer
TROP2	Solid tumors, Breast cancer, prostate cancer
TSHR	Thyroid cancer, T cell leukemia, T cell Lymphoma
TSLPR	Blood cancers, Leukemias, AML, MDS

CAR/BiTE "X" TARGET	EXEMPLARY DISEASE TARGETED BY CARs (<i>i.e.</i> conventional CARs and next generation CARs. <i>E.g.</i>, SIR, Ab-TCR, and TFP) and Bispecific T Cell Engagers (BiTE)
Tyrosinase/MHC I	Melanoma
VEGFR3	Solid tumors
WT1/MHC I	Blood cancers, AML
Folate Receptor β	AML, Myeloma
B7H4	Breast cancer or ovarian cancer
CD23	Blood cancers, Leukemias, CLL
GCC	Gastrointestinal cancer
CD200R	Blood cancers, AML, MDS
AFP/MHC I	Solid tumors, Liver cancer
CD99	Liver cancer
GPRC5D	Myeloma, waldenstrom's macroglobinemia
HPV16-E7/MHC I	HPV16 associated cancers, cervical cancer, head and neck cancers
Tissue Factor 1 (TF1)	Solid tumors
Tn-Muc1	Solid tumors and blood cancers
Igk-Light Chain	Myeloma, plasma cell leukemia
Ras G12V/ MHC I	Solid tumors and blood cancers
CLD18A2 (Claudin 18.2)	Gastric, pancreatic, esophageal, ovarian, or lung cancer
CD43	Blood cancers, AML
NY-ESO-1/MHC I	Myeloma
MPL/TPO-R	Blood cancer, AML, MDS, CML, ALL
P-glycoprotein (MDR1)	Renal cancer, liver cancer, Myeloma
CD179a	Blood cancers, Acute Leukemia, CLL, ALL, Lymphoma
STEAP1	Gastric or prostate cancer, or lymphoma
Liv1 (SLC39A6)	Breast or prostate cancer
Nectin4 (PVRL4)	Bladder, renal, cervical, lung, head and neck or breast cancer
Cripto (TDGF1)	Colorectal or endometrial or ovarian cancer
gpA33	Colorectal or endometrial or ovarian cancer
FLT3	Blood cancers, AML, ALL, MDS
BST1/CD157	Blood cancers, AML, MDS
IL1RAP	Liver, colorectal, cervical, lung or ovarian cancer
Chloride channel	Glioma
IgE	Allergy
HLA-A2	Graft vs host disease, tissue rejection (SIR Expressed in regulatory T cells)
Amyloid	Amyloidoses, alzheimer's disease
HIV1-env	HIV/AIDS and related conditions
HIV1-gag	HIV/AIDS and related conditions
Influenza A HA	Influenza A infection

[00173] TABLE 10: Exemplary CARs Targeting HIV-1 Envelop Glycoprotein

Based on HIV1-N49P6 vL and vH binding domains

CAR TYPE	Accessory Module	CAR NAME	SEQ ID NO (DNA)	SEQ ID NO (PRT)
2nd Gen CAR	None	CD8SP-HIV1-N49P6-vL-Gly-Ser-Linker-HIV1-N49P6-vH-Myc-CD8TM-BBz	8704	11402
2nd Gen CAR	None	CD8SP-HIV1-N49P6-vH-Gly-Ser-Linker-vL-Myc-CD8TM-BBz	8705	11403
Ist Gen CAR	vFLIP-K13	CD8SP-HIV1-N49P6-vL-Gly-Ser-Linker-HIV1-N49P6-vH-Myc-CD8TM-z-P2A-K13	8706	11404
Ist Gen CAR	hNEMO-K277A	CD8SP-HIV1-N49P6-(vL-vH)-Myc-z-P2A-hNEMO-K277A	8707	11405
TFP	hNEMO-K277A	CD8SP-HIV1-N49P6-(vL-vH)-CD3e-ECDTMCP-opt2-P2A-hNEMO-K277A	8708	11406
TFP	hNEMO-K277A	CD8SP-HIV1-N49P6-(vL-vH)-CD3d-ECDTMCP-opt2-P2A-hNEMO-K277A	8709	11407
TFP	hNEMO-K277A	CD8SP-HIV1-N49P6-(vL-vH)-CD3g-ECDTMCP-opt2-P2A-hNEMO-K277A	8710	11408
TFP	hNEMO-K277A	CD8SP-HIV1-N49P6-(vL-vH)-CD3z-ECDTMCP-opt2-P2A-hNEMO-K277A	8711	11409
TFP	hNEMO-K277A	CD8SP-HIV1-N49P6-(vH-vL)-CD3e-ECDTMCP-opt2-P2A-hNEMO-K277A	8712	11410
TFP	hNEMO-K277A	CD8SP-HIV1-N49P6-(vH-vL)-CD3d-ECDTMCP-opt2-P2A-hNEMO-K277A	8713	11411
TFP	hNEMO-K277A	CD8SP-HIV1-N49P6-(vH-vL)-CD3g-ECDTMCP-opt2-P2A-hNEMO-K277A	8714	11412
TFP	hNEMO-K277A	CD8SP-HIV1-N49P6-(vH-vL)-CD3z-ECDTMCP-opt2-P2A-hNEMO-K277A	8715	11413
DC SIR	None	CD8SP-HIV1-N49P6-vL-[hTCRb-KACIAH]-F-P2A-SP-HIV1-N49P6-vH-[hTCRa-CSDVP]	8716	11414
DC SIR	None	CD8SP-HIV1-N49P6-vL-[hTCRa-CSDVP]-F-F2A-SP-HIV1-N49P6-vH-[hTCRb-KACIAH]	8717	11415
DC SIR	None	CD8SP-HIV1-N49P6-vL-PG4SP-v2-[hTCRb-KACIAH]-F-P2A-SP-HIV1-N49P6-vH-PG4SP-[hTCRa-CSDVP]	8718	11416
DC SIR	None	CD8SP-HIV1-N49P6-vL-E-Coil-[hTCRb-KACIAH]-F-P2A-SP-HIV1-N49P6-vH-K-Coil-[hTCRa-CSDVP]	8719	11417
DC SIR	None	CD8SP-HIV1-N49P6-vL-EAAAK-[hTCRb-KACIAH]-F-P2A-SP-HIV1-N49P6-vH-EAAAK-v2-[hTCRa-CSDVP]	8720	11418

CAR TYPE	Accessory Module	CAR NAME	SEQ ID NO (DNA)	SEQ ID NO (PRT)
DC SIR	None	CD8SP-HIV1-N49P6-vL-V5-[hTCRb-KACIAH]-F-P2A-SP-HIV1-N49P6-vH-Myc4-[hTCRa-CSDVP]	8721	11419
DC SIR	hNEMO-K277A	CD8SP-HIV1-N49P6-vL-[hTCRb-KACIAH]-F-P2A-SP-HIV1-N49P6-vH-[hTCRa-CSDVP]-F-F2A-hNEMO-K277A	8722	11420
DC SIR	hNEMO-K277A	CD8SP-HIV1-N49P6-vL-[hTCRa-CSDVP]-F-F2A-SP-HIV1-N49P6-vH-[hTCRb-KACIAH]-F-P2A-hNEMO-K277A	8723	11421
Ab-TCR	hNEMO-K277A	CD8SP-HIV1-N49P6-vL-[IgCL-TCRg-6MD]-F-P2A-SP-HIV1-N49P6-vH-[IgG1-CH1-TCRd-6MD]-F-F2A-hNEMO-K277A	8724	11422
Ab-TCR	hNEMO-K277A	CD8SP-HIV1-N49P6-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-HIV1-N49P6-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	8725	11423
Ab-TCR	hNEMO-K277A	CD8SP-HIV1-N49P6-vL-[IgCL-TCRb-wt2-opt-6MD]-F-P2A-SP-HIV1-N49P6-vH-[IgG1-CH1-TCRa-wt2-opt-6MD]-F-F2A-hNEMO-K277A	8726	11424
Ist Gen CAR	hNEMO-K277A-Delta-V249-K255	CD8SP-HIV1-N49P6-vL-Gly-Ser-Linker-HIV1-N49P6-vH--CD8TM-z-P2A-hNEMO-K277A-Delta-V249-K255	8727	11425
Ist Gen CAR	IKK2-S177E-S181E	CD8SP-HIV1-N49P6-vL-Gly-Ser-Linker-HIV1-N49P6-vH--CD8TM-z-P2A-IKK2-S177E-S181E	8728	11426
DC SIR	hNEMO-K277A	CD8SP-HIV1-N49P6-vL-[hTCRa-T48C]-F-F2A-SP-HIV1-N49P6-vH-[hTCRb-S57C]-F-P2A-hNEMO-K277A	8729	11427
DC SIR	IKK1-S176E-S180E	CD8SP-HIV1-N49P6-vL-[hTCRb-S57C]-F-P2A-SP-HIV1-N49P6-vH-[hTCRa-T48C]-F-F2A-IKK1-S176E-S180E	8730	11428
DC SIR	hNEMO-K277A-Delta-V249-K255	CD8SP-HIV1-N49P6-vL-[hTCRb-S57C]-F-P2A-SP-HIV1-N49P6-vH-[hTCRa-T48C]-F-F2A-hNEMO-K277A-Delta-V249-K255	8731	11429
OHC SIR	None	CD8SP-MYC-[hTCRa-T48C-opt1]-F-F2A-SP-HIV1-N49P6-vL-Gly-Ser-Linker-HIV1-N49P6-vH-V5-[hTCRb-S57C-opt1]	8732	11430
DC SIR	None	CD8SP-HIV1-N49P6-vL-V5-[hTCRb-S57C-opt]-F-P2A-SP-HIV1-N49P6-vH-Myc-[hTCRa-T48C-opt]	8733	11431

CAR TYPE	Accessory Module	CAR NAME	SEQ ID NO (DNA)	SEQ ID NO (PRT)
DC SIR	None	CD8SP-HIV1-N49P6-vL-[hTCRb-opt2]-F-P2A-SP-HIV1-N49P6-vH-[hTCRa-opt2]	8734	11432
DC SIR	None	CD8SP-HIV1-N49P6-vL-[hTCRb-opt2]-F-P2A-SP-HIV1-N49P6-vH-Myc-[preTCRa-Del48]	8735	11433
OHC SIR	None	CD8SP-[hTCRb-opt2]-F-P2A-CD8SP-HIV1-N49P6-vL-Gly-Ser-Linker-HIV1-N49P6-vH-Myc4-[preTCRa-Del48]	8736	11434
DC SIR	None	CD8SP-HIV1-N49P6-vL-V5-[hTCRg1-opt]-F-P2A-SP-HIV1-N49P6-vH-Myc-[hTCRd-opt]	8737	11435

[00174] Abbreviations; 1st Gen CAR, First Generation CAR; 2nd Gen CAR, 2nd

Generation CAR; DC SIR, Double Chain SIR; OHC SIR, One half chain SIR.

[00175] The accessory modules in the above exemplary constructs in Table 10 are optional and can be deleted or replaced by other accessory modules.

[00176] **TABLE 11: SEQ ID NOs OF CARs CONTAINING DIFFERENT ANTIGEN BINDING DOMAINS USING SEQ ID NOs OF CARs WITH HIV1-N49P6 AS REFERENCE**

Target Antigen	Antigen binding domain	CAR SEQ ID NOs (DNA)	CAR SEQ ID NO (PRT)
HIV1 Env	HIV1-N49P6	8704-8737	11402-11435
HIV1 Env	HIV1-N49P7	8738-8771	11436-11469
HIV1 Env	HIV1-N49P11	8806-8839	11504-11537
HIV1 Env	HIV1-N60P1-1	8840-8873	11538-11571
HIV1 Env	HIV1-N60P25	8942-8975	11640-11673
HIV1 Env	HIV1-N49P9	8772-8805	11470-11503
HIV1 Env	HIV1-N60P2-1	8874-8907	11572-11605
HIV1 Env	HIV1-N60P31-1	9010-9043	11708-11741
HIV1 Env	HIV1-N60P22	8908-8941	11606-11639
HIV1 Env	HIV1-N60P38	9146-9179	11844-11877
HIV1 Env	HIV1-N60P30	8976-9009	11674-11707
HIV1 Env	HIV1-N60P36	9078-9111	11776-11809
HIV1 Env	HIV1-N60P39	9180-9213	11878-11911
HIV1 Env	HIV1-N6039-1	9316-9349	12014-12047
HIV1 Env	HIV1-N60P47	9214-9247	11912-11945
HIV1 Env	HIV1-N60P48	9248-9281	11946-11979
HIV1 Env	HIV1-N60P51	9282-9315	11980-12013
HIV1 Env	HIV1-N60P35	9044-9077	11742-11775
HIV1 Env	HIV1-N60P37	9112-9145	11810-11843
Lym1	hu-Lym1	10370-10403	13068-13101

Target Antigen	Antigen binding domain	CAR SEQ ID NOS (DNA)	CAR SEQ ID NO (PRT)
Lym2	hu-Lym2	10404-10437	13102-13135
BCMA	BCMA-USC1	9418-9451	12116-12149
BCMA	BCMA-USC2	9452-9485	12150-12183
BCMA	BCMA-USC3	9486-9519	12184-12217
BCMA	BCMA-USC4	9520-9554	12218-12252
BCMA	BCMA-USC5	9555-9587	12253-12285
BCMA	BCMA-USC6	9588-9621	12286-12319
BCMA	BCMA-USC7	9622-9655	12320-12353
CD43	CD43-huJL-1-257-10	9758-9791	12456-12489
BCMA	BCMA-huC11.D5.3L1H3	9350-9383	12048-12081
BCMA	BCMA-huC13-F12	9384-9417	12082-12115
CD20	CD20-Ubli-v4	9656-9689	12354-12387
CD37	CD37-TRU-HL	9724-9757	12422-12455
CD70	CD70-1G2D	9792-9825	12490-12523
CD70	CD70-10B4	9826-9859	12524-12557
CD70	CD70-13D	9860-9893	12558-12591
CD70	CD70-16D	9894-9927	12592-12625
CD70	CD70-21D	9928-9961	12626-12659
CD70	CD70-24D	9962-9995	12660-12693
CD70	CD70-25D	9996-10029	12694-12727
CD70	CD70-69A7	10030-10063	12728-12761
CD70	CD70-hu-2H5	10064-10097	12762-12795
CD123	CD123-DART-1	10098-10131	12796-12829
CD123	CD123-DART-2	10132-10165	12830-12863
CD179a	CD179a-2460-B04	10166-10199	12864-12897
CD179a	CD179a-2462-E07	10200-10233	12898-12931
FITC	FITC-4M-53	10234-10267	12932-12965
FITC	FITC-E2	10268-10301	12966-12999
MPL	Hu-161-2	10302-10335	13000-13033
CD37	huCD37-Boeh	10336-10369	13034-13067
Kappa-Light Chain	Kappa-LC1	10438-10471	13136-13169
MPL	MPL-hu-111-2	10472-10505	13170-13203

[00177] **TABLE 12. Exemplary 1st Generation CAR constructs coexpressing hNEMO-K277A and PAC accessory modules. Both accessory modules are optional.**

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
CD19	CD8SP-FMC63-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1594	5507
CD19	CD8SP-huFMC63-11-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1595	5508

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
CD19	CD8SP-huFMC63-11-N203Q-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1596	5509
CD19	CD8SP-CD19Bu12-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1597	5510
CD19	CD8SP-2-CD19MM-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1598	5511
CD19	CD8SP-CD19-4G7-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1599	5512
CD19	CD8SP-CD19-MEDI-3649-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1600	5513
CD19	CD8SP-CD19-Medrex-24D1-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1601	5514
CD19	CD8SP-Ritx-CD19-MOR0028-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1602	5515
CD19	CD8SP-CD19-HD37-H2L1-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1603	5516
CD19	CD8SP-CD19-huBly3-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1604	5517
CD19	CD8SP-CD19-huSJ25C1-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1605	5518
CD19	CD8SP-Ritx-CD19-hB4-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1606	5519
CD19	CD8SP-CD19-hu-mROO5-1-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1607	5520
CD19	CD8SP-CD19-hA19-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1608	5521
AFP/MHC class I complex	CD8SP-AFP-61-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1609	5522
AFP/MHC class I complex	CD8SP-AFP-76-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1610	5523
AFP/MHC class I complex	CD8SP-AFP-79-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1611	5524
HIV1-envelop glycoprotein	CD8SP-HIV1-N6-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1612	5525
ALK	CD8SP-Alk-48-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1613	5526
ALK	CD8SP-Alk-58-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1614	5527
Amyloid	SP-Amyloid-158-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1615	5528
Biotin	CD8SP-dc-Avidin-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1616	5529

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
CD45	CD8SP-BC8-CD45-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1617	5530
BCMA	CD8SP-BCMA-J6M0-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1618	5531
BCMA	CD8SP-BCMA-huC12A3-L3H3-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1619	5532
BCMA	CD8SP-BCMA-ET-40-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1620	5533
BCMA	CD8SP-BCMA-ET-54-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1621	5534
BCMA	CD8SP-BCMA-ET-03-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1622	5535
BCMA	CD8SP-BCMA-huC11.D5.3L1H3-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1623	5536
BCMA	CD8SP-BCMA-huC13-F12-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1624	5537
CCR4	CD8SP-CCR4-humAb1567-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1625	5538
HIV1-envelop glycoprotein	CD8SP-CD4-ECD-Linker-DC-SIGN-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1626	5539
CD5	CD8SP-CD5-9-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1627	5540
CD5	CD8SP-CD5-18-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1628	5541
Ig Fc	CD8SP-CD16A-V158-ECD-v2-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1629	5542
Ig Fc	CD8SP-CD16A-V158-ECD-v1-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1630	5543
CD20	CD8SP-CD20-2F2-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1631	5544
CD20	CD8SP-CD20-GA101-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1632	5545
CD20	CD8SP-CD20-Leu16-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1633	5546
CD20	CD8SP-CD20-11B8-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1634	5547
CD20	CD8SP-CD20-2C6-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1635	5548
CD20	CD8SP-CD20-2H7-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1636	5549

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
CD20	CD8SP-CD20-hA20-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1637	5550
CD20	CD8SP-CD20-BM-CA-1925-v4-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1638	5551
CD20	CD8SP-CD20-Ubli-v4-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1639	5552
CD20	CD8SP-CD20-2H7-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1640	5553
CD20	CD8SP-CD20-h1F5-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1641	5554
CD20	CD8SP-CD20-7D8-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1642	5555
CD20	CD8SP-CD20-AME-33-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1643	5556
CD22	CD8SP-CD22-h10F4v2-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1644	5557
CD22	CD8SP-CD22-H22RhoV2ACDRKA-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1645	5558
CD22	CD8SP-CD22-m971-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1646	5559
CD22	CD8SP-CD22-m971-HL-(vH-vL)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1647	5560
CD30	CD8SP-CD30-5F11-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1648	5561
CD30	CD8SP-CD30-Ac10-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1649	5562
CD32	CD8SP-CD32-Med9-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1650	5563
CD33	CD8SP-CD33-AF5-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1651	5564
CD33	CD8SP-CD33-huMyc9-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1652	5565
CD33	CD8SP-CD33-Bochr2800308-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1653	5566
CD33	CD8SP-CD33-Him3-4-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1654	5567
CD33	CD8SP-CD33-SGNh2H12-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1655	5568
CD33	CD8SP-CD33-15G15-33-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1656	5569
CD33	CD8SP-CD33-33H4-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1657	5570

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
CD33	CD8SP-CD33-9C3-2-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1658	5571
CD34	CD8SP-CD34-hu4C7-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1659	5572
CD44v6	CD8SP-CD44v6-Biwa8-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1660	5573
CD70	CD8SP-CD70-h1F6-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1661	5574
CD79b	CD8SP-CD79b-2F2-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1662	5575
CD79b	CD8SP-huMA79bv28-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1663	5576
CD99	CD8SP-CD99-hu12E7-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1664	5577
CD123	CD8SP-CD123-CSL362-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1665	5578
CD123	CD8SP-CD123-1172-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1666	5579
CD123	CD8SP-CD123-DART-1-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1667	5580
CD123	CD8SP-CD123-DART-2-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1668	5581
CD123	CD8SP-CD123-I3RB18-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1669	5582
CD123	CD8SP-CD123-hu3E3-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1670	5583
CD123	CD8SP-CD123-9F6-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1671	5584
CD123	CD8SP-CD123-I3RB2-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1672	5585
CD123	CD8SP-CD123-1176-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1673	5586
CD123	CD8SP-Ritx2-CD123-8B11-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1674	5587
CD123	CD8SP-CD123-2B8-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1675	5588
CD123	CD8SP-CD123-9D7-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1676	5589
CD123	CD8SP-CD123-3B10-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1677	5590
CD138	CD8SP-CD138-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1678	5591

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
CD179b	CD8SP-CD179b-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1679	5592
CD276	CD8SP-CD276-17-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1680	5593
CD324	CD8SP-CD324-SC10-6-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1681	5594
CD324	CD8SP-CD324-hSC10-17-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1682	5595
CDH6	CD8SP-CDH6-NOV710-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1683	5596
CDH6	CD8SP-CDH6-NOV712-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1684	5597
CDH17	CD8SP-CDH17-PTA001A4-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1685	5598
CDH19	CD8SP-CDH19-16A4-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1686	5599
EGFR	CD8SP-Cetuximab-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1687	5600
CLEC5A	CD8SP-CLEC5A-8H8F5-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1688	5601
CLEC5A	CD8SP-CLEC5A-3E12A2-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1689	5602
GR/LHR (Gonadotropin Receptor)	SP-CGHb-Linker-CGHa-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1690	5603
CLL1	CD8SP-CLL1-M26-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1691	5604
CLL1	CD8SP-CLL1-M32-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1692	5605
CLL1	CD8SP-CLL1-21C9-L2H3-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1693	5606
CLL1	CD8SP-CLL1-6E7L4H1e-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1694	5607
CLL1	CD8SP-CLL1-hu1075-v1-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1695	5608
CLL1	CD8SP-CLL1-hu1075-v2-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1696	5609
CMVpp65/MHC class I complex	CD8SP-CMVpp65-F5-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1697	5610
CS1 (SLAMF7)	CD8SP-CS1-huLuc63-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1698	5611
CS1 (SLAMF7)	CD8SP-CS1-HuLuc64-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1699	5612

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
CS1 (SLAMF7)	CD8SP-CS1-huLuc90-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1700	5613
CS1 (SLAMF7)	CD8SP-CS1-PDL241-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1701	5614
CS1 (SLAMF7)	CD8SP-CS1-Hu27A-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1702	5615
CS1 (SLAMF7)	CD8SP-CS1-ScHu34C3-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1703	5616
CS1 (SLAMF7)	CD8SP-CS1-Hu31-D2-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1704	5617
CS1(SLAMF7)	CD8SP-CS1-Luc34-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1705	5618
CS1 (SLAMF7)	CD8SP-CS1-LucX2-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1706	5619
CSF2RA	CD8SP-CSF2RA-Ab6-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1707	5620
CSF2RA	CD8SP-CSF2RA-Ab1-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1708	5621
CXCR4 and CD123	CD8SP-CXCR4-1-vHH-Linker-CD123-1-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1709	5622
CXCR4 and CD123	CD8SP-CXCR4-2-VHH-Linker-CD123-2-VHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1710	5623
DLL3 (Delta Like Ligand 3)	CD8SP-DLL3-hSC16-13-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1711	5624
DLL3	CD8SP-DLL3-hSC16-56-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1712	5625
EBNA3c/MHC class I complex	CD8SP-EBNA3c-315-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1713	5626
EBV-gp350	CD8SP-EBV-gp350-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1714	5627
EGFR	CD8SP-EGFR1-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1715	5628
EGFR & CEA	CD8SP-EGFR1-vHH-Linker-CEA1-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1716	5629
EGFR & CEA	CD8SP-EGFR33-vHH-Linker-CEA5-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1717	5630
EGFRvIII	CD8SP-EGFRvIII-139-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1718	5631
EGFRvIII	CD8SP-EGFRvIII-2173-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1719	5632
EpCam1	CD8SP-Epcam1-MM1-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1720	5633
EpCam1	CD8SP-Epcam1-D5K5-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1721	5634

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
FLT3	CD8SP-FLT3-NC7-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1722	5635
FITC	CD8SP-FITC-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1723	5636
FITC	CD8SP-FITC-4M-53-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1724	5637
FITC	CD8SP-FITC-E2-HL-(vH-vL)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1725	5638
Influenza A HA	CD8SP-FLU-MEDI-8852-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1726	5639
FR1 (Folate Receptor alpha)	CD8SP-FR1-huMov19-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1727	5640
FSHR (Follicle Stimulating Hormone Receptor)	CD8SP-FSHb-Linker-CGHa-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1728	5641
GAD (Glutamic Acid Decarboxylase)/MHC class I complex	CD8SP-GAD-G3H8-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1729	5642
GD2	CD8SP-GD2-hu14-18-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1730	5643
GD2	CD8SP-GD2-hu3F8-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1731	5644
GD3	CD8SP-GD3-KM-641-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1732	5645
GFRA4 (GDNF Family Receptor Alpha 4)	CD8SP-GFRA4-P4-6-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1733	5646
GFRA4	CD8SP-GFRA4-P4-10-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1734	5647
GM1	CD8SP-GM1-5B2-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1735	5648
GM1	CD8SP-GM1-7E5-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1736	5649
GPRC5D (G-protein coupled receptor family C group 5 member D)	CD8SP-GPRC5D-ET150-5-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1737	5650
GPRC5D	CD8SP-GPRC5D-ET150-18-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1738	5651
GPRC5D	CD8SP-GPRC5D-ET150-1-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1739	5652
GPRC5D	CD8SP-GPRC5D-ET150-2-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1740	5653

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
gp100/MHC class I complex	CD8SP-gp100-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1741	5654
gp100/MHC class I complex	CD8SP-gp100-G2D12-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1742	5655
GPC3 (Glypican 3)	CD8SP-GPC3-4E5-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1743	5656
gpNMB (Glycoprotein Nmb)	CD8SP-gpNMB-115-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1744	5657
GRP78	CD8SP-GRP78-GC18-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1745	5658
Her2	CD8SP-Her2-5F7-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1746	5659
Her2	IgHSP-Her2-Affi-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1747	5660
Her2	CD8SP-Her2-1-Darpin-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1748	5661
Her2	IgHSP-Her2-2-Darpin-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1749	5662
Her2	CD8SP-Her2-5F7-vHH-Linker-Her2-47D5-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1750	5663
Her2	CD8SP-Her2-Hu4D5-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1751	5664
Her3	CD8SP-Her3-17B05So-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1752	5665
Her3	CD8SP-Her3-Affi-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1753	5666
Her2 and Her3	CD8SP-Her3-17B05So-vHH-Linker-Her2-2D3-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1754	5667
HIV1-gag/MHC class I complex	CD8SP-HIV1-E5-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1755	5668
HIV1-envelop glycoprotein	CD8SP-HIV1-3BNC117-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1756	5669
HIV1-envelop glycoprotein	CD8SP-HIV1-PGT-128-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1757	5670
HIV1-envelop glycoprotein	CD8SP-HIV1-VR-C01-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1758	5671
HIV1-envelop glycoprotein	CD8SP-HIV1-X5-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1759	5672
HLA-A2	CD8SP-HLA-A2-3PB2-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1760	5673
HMW-MAA	CD8SP-HMW-MAA-hIND-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1761	5674

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
HPV16-E7/MHC class I complex	CD8SP-HPV16-7-8-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1762	5675
HPV16-E7/MHC class I complex	CD8SP-HPV16-2-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1763	5676
HTLV1-TAX/MHC class I complex	CD8SP-HTLV-TAX-T3F2-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1764	5677
HTLV1-TAX/MHC class I complex	CD8SP-HTLV-TAX-T3E3-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1765	5678
IL11Ra	CD8SP-IL11Ra-8E2-Ts107-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1766	5679
IL6Ra	IgHSP-IL6R-304-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1767	5680
IL13Ra2	CD8SP-IL13Ra2-hu107-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1768	5681
IL13Ra2	CD8SP-IL13Ra2-Hu108-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1769	5682
KSHV-K8.1	CD8SP-KSHV-4C3-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1770	5683
LAMP1 (Lysosomal-associated membrane protein 1)	CD8SP-LAMP1-humab1-2-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1771	5684
LAMP1	CD8SP-LAMP1-Mb4-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1772	5685
LewisY	CD8SP-LewisY-huS193-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1773	5686
L1CAM	CD8SP-L1CAM-9-3-HU3-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1774	5687
LHR	SP-LHb-Linker-CGHa-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1775	5688
Lym1	CD8SP-Lym1-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1776	5689
Lym2	CD8SP-Lym2-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1777	5690
CD79b	CD8SP-huMA79bv28-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1778	5691
MART1/MHC class I complex	CD8SP-MART1-CAG10-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1779	5692
MART1/MHC class I complex	CD8SP-MART1-CLA12-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1780	5693
Mesothelin	CD8SP-Mesothelin-m912-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1781	5694

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
cMet	CD8SP-cMet-171-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1782	5695
cMet and Her3	CD8SP-cMET-171-vHH-Linker-Her3-21F06-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1783	5696
MPL	CD8SP-MPL-175-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1784	5697
MPL	CD8SP-MPL-161-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1785	5698
MPL	CD8SP-MPL-161-HL-(vH-vL)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1786	5699
MPL	CD8SP-2-MPL-111-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1787	5700
MPL	CD8SP-MPL-178-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1788	5701
MPL	CD8SP-MPL-AB317-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1789	5702
MPL	CD8SP-MPL-12E10-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1790	5703
MPL	CD8SP-MPL-huVB22Bw5-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1791	5704
Muc1/MHC class I complex	CD8SP-Muc1-D6-M3B8-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1792	5705
Muc1/MHC class I complex	CD8SP-MUC1-D6-M3A1-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1793	5706
Muc16	CD8SP-Muc16-4H11-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1794	5707
EGFR	CD8SP-Nimotuzumab-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1795	5708
NKG2D Ligand	CD8SP-NKG2D-(GGGGS-GGGGD)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1796	5709
NKG2D	CD8SP-NKG2D-MS-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1797	5710
NY-BR1	CD8SP-NYBR1-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1798	5711
NY-ESO/MHC class I complex	CD8SP-NYESO-T1-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1799	5712
NY-ESO/MHC class I complex	CD8SP-NYESO-T1-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1800	5713
PD1 ligand (e.g., PDL1)	CD8SP-PD1-ECD-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1801	5714
PDL1	CD8SP-PDL1-Atezoli-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1802	5715
PDL1	CD8SP-PDL1-SP142-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1803	5716

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
PDL1	CD8SP-PDL1-10A5-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1804	5717
PSCA (Prostate stem cell antigen)	CD8SP-PSCA-Ha14-121-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1805	5718
PSCA (Prostate stem cell antigen)	CD8SP-PSCA-Ha14-117-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1806	5719
PR1/MHC class I complex	CD8SP-PR1-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1807	5720
PSMA (Prostate Specific Membrane Antigen)	CD8SP-PSMA-006-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1808	5721
PSMA	CD8SP-PSMA-J591-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1809	5722
PTK7 (Tyrosine-protein kinase-like 7)	CD8SP-PTK7-hSC6-23-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1810	5723
PTK7	CD8SP-PTK7-SC6-10-2-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1811	5724
ROR1	CD8SP-ROR1-4A5-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1812	5725
ROR1	CD8SP-ROR1-4C10-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1813	5726
Mesothelin	CD8SP-SD1-vHH-Linker-SD2-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1814	5727
SLea	CD8SP-SLea-7E3-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1815	5728
SLea	CD8SP-SLea-5B1-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1816	5729
SSEA4 (stage-specific embryonic antigen 4)	CD8SP-SSEA4-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1817	5730
TCRB1 (TCR beta 1 constant chain)	CD8SP-TCRB1-CP01-E09-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1818	5731
TCRB1	CD8SP-TCRB1-Jovi1-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1819	5732
TCRB2 (TCR beta 2 constant chain)	CD8SP-TCRB2-CP01-D05-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1820	5733
TCRB2	CD8SP-TCRB2-CP01-E05-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1821	5734
TCRgd (TCR gamma/delta)	CD8SP-TCRgd-G5-4-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1822	5735
hTERT/MHC class I complex	CD8SP-TERT-4A9-T540-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1823	5736

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
hTERT/MHC class I complex	CD8SP-TERT-3G3-T865-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1824	5737
Tissue Factor-1	CD8SP-TGFBR2-Ab1-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1825	5738
TGFBR2	CD8SP-TF1-98-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1826	5739
TIM1/HAOCR	CD8SP-TIM1-HVCR1-270-2-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1827	5740
TIM1/HAOCR	CD8SP-TIM1-HVCR1-ARD5-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1828	5741
TnAg	CD8SP-TnAg-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1829	5742
Tn-Muc1	CD8SP-TnMuc1-hu5E5-RHA8-RKA-2-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1830	5743
MPL	CD8SP-hTPO-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1831	5744
TROP2 (Trophoblast cell-surface antigen-2)	CD8SP-TROP2-ARA47-HV3KV3-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1832	5745
TROP2	CD8SP-TROP2-h7E6-SVG-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1833	5746
TSHR	SP-TSHb-Linker-CGHa-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1834	5747
TSHR	CD8SP-TSHR-K1-70-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1835	5748
TSHR	CD8SP-TSHR-KB1-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1836	5749
TSHR	CD8SP-TSHR-5C9-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1837	5750
TSLPR (thymic stromal lymphopoietin receptor)	CD8SP-TSLPR-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1838	5751
Tyrosinase/MHC class I complex	CD8SP-Tyros-B2-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1839	5752
Tyrosinase/MHC class I complex	CD8SP-Tyros-MC1-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1840	5753
Tyrosinase/MHC class I complex	CD8SP-Tyros-TA2-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1841	5754
VEGFR3	CD8SP-VEGFR3-Ab1-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1842	5755
WT1/MHC class I complex	CD8SP-WT1-Ab1-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1843	5756

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
WT1/MHC class I complex	CD8SP-WT1-Ab5-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1844	5757
WT1/MHC class I complex	CD8SP-MYC3-WT1-Ab13-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1845	5758
WT1/MHC class I complex	CD8SP-MYC3-WT1-Ab15-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1846	5759
CDH19	CD8SP-CDH19-4B10-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1847	5760
Folate Receptor beta	CD8SP-FRbeta-m923-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1848	5761
LHR (Luteinizing hormone Receptor)	CD8SP-LHR-8B7-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1849	5762
LHR	CD8SP-LHR-5F4-21-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1850	5763
B7H4	CD8SP-B7H4-hu22C10-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1851	5764
B7H4	CD8SP-B7H4-hu1D11-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1852	5765
IgE	CD8SP-IgE-omalizumab-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1853	5766
CD23	CD8SP-CD23-p5E8-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1854	5767
GCC (Guanylyl cyclase C)	CD8SP-GCC-5F9-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1855	5768
GCC	CD8SP-GCC-Ab229-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1856	5769
CD200R	CD8SP-CD200R-huDx182-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1857	5770
Tn-Muc1	CD8SP-Tn-Muc1-5E5-HL-(vH-vL)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1858	5771
CD22	CD8SP-CD22-5-HL-(vH-vL)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1859	5772
CD22	CD8SP-CD22-10-HL-(vH-vL)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1860	5773
CD22	CD8SP-CD22-31-HL-(vH-vL)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1861	5774
CD22	CD8SP-CD22-53-HL-(vH-vL)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1862	5775
CD22	CD8SP-CD22-65-HL-(vH-vL)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1863	5776
Tn-Muc1	CD8SP-Tn-Muc1-5E5-(vH-vL)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1864	5777

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
Kappa Light Chain	CD8SP-Kappa-LC1-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1865	5778
PTK7	CD8SP-PTK7-7C8-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1866	5779
PTK7	CD8SP-PTK7-12C6a-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1867	5780
CD19	CD8SP-hCD19-EUK5-13-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1868	5781
Ras	CD8SP-Ras-Ab2-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1869	5782
Ras	CD8SP-Ras-Ab4-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1870	5783
Claudin 18.2	CD8SP-CLD18A2-43A11-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1871	5784
Claudin 18.2	CD8SP-CLD18A2-175D10-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1872	5785
CD43	CD8SP-CD43-huJL-1-257-10-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1873	5786
CD69L	CD8SP-CD69L-DREG200-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1874	5787
NY-ESO-1/MHC I complex	CD8SP-NYESO-35-15-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1875	5788
Pgp	CD8SP-Pgp-9F11-(vH-vL)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1876	5789
Streptag	CD8SP-Streptag-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1877	5790
MPL	CD8SP-MPL-Hu-161-2-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1878	5791
Pgp	CD8SP-Pgp-MRK16-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1879	5792
BCMA	CD8SP-BCMA-353-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1880	5793
BCMA	CD8SP-BCMA-917-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1881	5794
BCMA	CD8SP-BCMA-353-vHH-Linker-BCMA917-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1882	5795
CD38	CD8SP-CD38-717-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1883	5796
BCMA	CD8SP-BCMA-346-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1884	5797
CD38-BCMA	CD8SP-CD38-717-vHH-Ecoil-BCMA-346-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1885	5798
BCMA	CD8SP-BCMA-348-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1886	5799

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
CD38	CD8SP-CD38-331-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1887	5800
BCMA-CD38	CD8SP-BCMA-vHH-348-Ecoil-CD38-331-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1888	5801
CD19	CD8SP-CD19-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1889	5802
CD20	CD8SP-CD20-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1890	5803
CD19	CD8SP-CD19-vHH-Linker-CD20-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1891	5804
BCMA	CD8SP-BCMA-948-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1892	5805
BCMA	CD8SP-BCMA-972-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1893	5806
BCMA	CD8SP-BCMA-948-vHH-PG4SP-BCMA-972-vHH-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1894	5807
BCMA	CD8SP-BCMA-948-vHH-PG4SP-BCMA-972-vHH-Ecoilx4-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1895	5808
MPL	CD8SP-MPL-hu-175-2-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1896	5809
MPL	CD8SP-MPL-hu-111-2-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1897	5810
CD179a	CD8SP-CD179a-2460-B04-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1898	5811
CD179a	CD8SP-CD179a-2462-E07-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1899	5812

[00178] **TABLE 13. SEQ ID IDENTIFICATION OF CARS/BITES USING ANTIGEN BINDING DOMAINS DESCRIBED FOR zCAR-NEMO-K277A (TABLE 12) AS A TEMPLATE**

CAR ARCHITECTURE	EXEMPLARY CAR/Bispecific T cell Engager	SEQ ID NO DNA		SEQ ID NO PRT	
zCAR-NEMO-K277A	CD8SP-FMC63-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	1594-1857	1858-1899	5507-5770	5771-5812
zCAR-K13	CD8SP-FMC63-(vL-vH)-Myc-z-P2A-K13-Flag-T2A-PAC	1016-1285		4929-5192	
BBz CAR	CD8SP-FMC63-(vL-vH)-Myc-BBz-T2A-PAC	1318-1581		5231-5494	

CAR ARCHITECTURE	EXEMPLARY CAR/Bispecific T cell Engager	SEQ ID NO DNA		SEQ ID NO PRT	
CD3 ϵ -TFP-NEMO-K277A	CD8SP-FMC63-(vL-vH)-CD3 ϵ -ECDTMCP-opt2-P2A-hNEMO-K277A-Flag-T2A-PAC	1900-2163	2164-2205	5813-6076	6077-6118
CD3 δ -TFP-NEMO-K277A	CD8SP-FMC63-(vL-vH)-CD3 δ -ECDTMCP-opt2-P2A-hNEMO-K277A-Flag-T2A-PAC	2206-2469	2470-2511	6119-6382	6383-6424
CD3 γ -TFP-NEMO-K277A	CD8SP-FMC63-(vL-vH)-CD3 γ -ECDTMCP-opt2-P2A-hNEMO-K277A-Flag-T2A-PAC	2512-2775	2776-2817	6425-6688	6689-6730
CD3 ζ -TFP-NEMO-K277A	CD8SP-FMC63-(vL-vH)-CD3 ζ -ECDTMCP-opt2-P2A-hNEMO-K277A-Flag-T2A-PAC	2818-3081	3082-3123	6731-6994	6995-7036
Bispecific T cell Engager	CD8SP-FMC63-scFv-Linker-CD3-scFv-Myc-His	3545-3814		7458-7721	

[00179] **TABLE 14: Ab-TCR CONSTRUCTS WITH DIFFERENT ANTIGEN BINDING DOMAINS.**

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
CD19	CD8SP-FMC63-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-FMC63-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3124	7037
CD19	CD8SP-huFMC63-11-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-huFMC63-11-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3125	7038
CD19	CD8SP-CD19Bu12-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD19Bu12-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3126	7039
CD19	CD8SP2-CD19MM-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD19MM-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3127	7040
CD19	CD8SP-CD19-4G7-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD19-4G7-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3128	7041
HIV1-env	CD8SP-HIV1-N6-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-HIV1-N6-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3129	7042
ALK	CD8SP-Alk-48-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-Alk-48-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3130	7043

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
ALK	CD8SP-ALK-58-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-ALK-58-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3131	7044
Amyloid	SP-Amyloid-158-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-Amyloid-158-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3132	7045
Biotin	CD8SP-dc-Avidin-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-dc-Avidin-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3133	7046
CD45	CD8SP-BC8-CD45-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-BC8-CD45-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3134	7047
BCMA	CD8SP-BCMA-J6M0-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-BCMA-J6M0-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3135	7048
BCMA	CD8SP-BCMA-huC12A3-L3H3-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-BCMA-huC12A3-L3H3-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3136	7049
BCMA	CD8SP-BCMA-ET-40-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-BCMA-ET-40-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3137	7050
BCMA	CD8SP-BCMA-ET-54-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-BCMA-ET-54-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3138	7051
CCR4	CD8SP-CCR4-humAb1567-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CCR4-humAb1567-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3139	7052
HIV1-env	CD8SP-CD4-ECD-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-DC-SIGN-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3140	7053
CD5	CD8SP-CD5-9-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD5-9-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3141	7054
CD5	CD8SP-CD5-18-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD5-18-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3142	7055
IgFc	CD8SP-CD16A-V158-ECD-v1-[IgCL-TCRb-IAH-6MD]-P2A-CD8SP2-CD16A-V158-ECD-v2-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3143	7056
IgFc	CD8SP-CD16A-V158-ECD-v1-[IgCL-TCRb-IAH-6MD]-P2A-SP-CD123-1-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3144	7057

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
CD20	CD8SP-CD20-2F2-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD20-2F2-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3145	7058
CD20	CD8SP-CD20-GA101-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD20-GA101-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3146	7059
CD22	CD8SP-CD22-h10F4v2-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD22-h10F4v2-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3147	7060
CD22	CD8SP-CD22-H22RhoV2ACDRKA-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD22-H22RhoV2ACDRKA-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3148	7061
CD22	CD8SP-CD22-m971-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD22-m971-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3149	7062
CD30	CD8SP-CD30-5F11-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD30-5F11-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3150	7063
CD30	CD8SP-CD30-Ac10-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD30-Ac10-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3151	7064
CD32	CD8SP-CD32-Med9-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD32-Med9-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3152	7065
CD33	CD8SP-CD33-AF5-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD33-AF5-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3153	7066
CD33	CD8SP-CD33-huMyc9-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD33-huMyc9-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3154	7067
CD34	CD8SP-CD34-hu4C7-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD34-hu4C7-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3155	7068
CD44v6	CD8SP-CD44v6-Biwa8-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD44v6-Biwa8-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3156	7069
CD70	CD8SP-CD70-h1F6-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD70-h1F6-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3157	7070
CD79b	CD8SP-CD79b-2F2-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD79b-2F2-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3158	7071

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
CD123	CD8SP-CD123-CSL362-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD123-CSL362-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3159	7072
CD138	CD8SP-CD138-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD138-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3160	7073
CD179b	CD8SP-CD179b-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD179b-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3161	7074
CD276	CD8SP-CD276-17-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD276-17-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3162	7075
CD324	CD8SP-CD324-SC10-6-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD324-SC10-6-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3163	7076
CD324	CD8SP-CD324-hSC10-17-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD324-hSC10-17-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3164	7077
CDH6	CD8SP-CDH6-NOV710-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CDH6-NOV710-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3165	7078
CDH6	CD8SP-CDH6-NOV712-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CDH6-NOV712-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3166	7079
CDH17	CD8SP-CDH17-PTA001A4-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CDH17-PTA001A4-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3167	7080
CDH19	CD8SP-CDH19-16A4-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CDH19-16A4-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3168	7081
EGFR	CD8SP-Cetuximab-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-Cetuximab-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3169	7082
CLEC5A	CD8SP-CLEC5A-8H8F5-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CLEC5A-8H8F5-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3170	7083
CLEC5A	CD8SP-CLEC5A-3E12A2-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CLEC5A-3E12A2-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3171	7084
GR/LHR	SP-CGHb-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CGHa-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3172	7085

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
CLL1	CD8SP-CLL1-M26-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CLL1-M26-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3173	7086
CLL1	CD8SP-CLL1-M32-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CLL1-M32-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3174	7087
CMVpp65	CD8SP-CMVpp65-F5-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CMVpp65-F5-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3175	7088
CS1	CD8SP-CS1-huLuc63-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-huLuc63-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3176	7089
CS1	CD8SP-HuLuc64-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-HuLuc64-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3177	7090
CS1	CD8SP-CS1-huLuc90-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-huLuc90-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3178	7091
CSF2RA	CD8SP-CSF2RA-Ab6-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CSF2RA-Ab6-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3179	7092
CSF2RA	CD8SP-CSF2RA-Ab1-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CSF2RA-Ab1-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3180	7093
CD123	IgHSP-CD123-2-vHH-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD123-1-vHH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3181	7094
CD123 & IgFc	IgHSP-CD123-2-vHH-[IgCL-TCRb-IAH-6MD]-F-P2A-CD8SP1-CD16A-V158-ECD-v1-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3182	7095
CD123 & IgFc	IgHSP-CD123-2-vHH-[IgCL-TCRb-IAH-6MD]-F-P2A-CD8SP2-CD16A-V158-ECD-v2-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3183	7096
CD123 & MPL	IgHSP-CD123-2-vHH-[IgCL-TCRb-IAH-6MD]-F-P2A-CD8SP-MPL-161-HL-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3184	7097
CXCR4 & CD123	CD8SP-CXCR4-1-vHH-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD123-1-vHH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3185	7098
CXCR4 & CD123	CD8SP-CXCR4-2-VHH-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD123-2-VHH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3186	7099

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
DLL3	CD8SP-DLL3-hSC16-13-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-DLL3-hSC16-13-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3187	7100
DLL3	CD8SP-DLL3-hSC16-56-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-DLL3-hSC16-56-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3188	7101
EBNA3c	CD8SP-EBNA3c-315-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-EBNA3c-315-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3189	7102
EBV-gp350	CD8SP-EBV-gp350-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-EBV-gp350-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3190	7103
EGFR	CD8SP-EGFR1-vHH-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CEA1-vHH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3191	7104
EGFR	CD8SP-EGFR33-vHH-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CEA5-vHH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3192	7105
EGFRvIII	CD8SP-EGFRvIII-139-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-EGFRvIII-139-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3193	7106
EGFRvIII	CD8SP-EGFRvIII-2173-vH-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-EGFRvIII-2173-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3194	7107
EpCam1	CD8SP-Epcam1-MM1-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-Epcam1-MM1-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3195	7108
EpCam1	CD8SP-Epcam1-D5K5-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-Epcam1-D5K5-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3196	7109
FLT3	CD8SP-FLT3-NC7-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-FLT3-NC7-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3197	7110
FITC	CD8SP-FITC-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-FITC-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3198	7111
Influenza A HA	CD8SP-FLU-MEDI-8852-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-FLU-MEDI-8852-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3199	7112
Folate Receptor 1	CD8SP-FR1-huMov19-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-FR1-huMov19-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3200	7113

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
FSHR	CD8SP-FSHb-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CGHa-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3201	7114
GD2	CD8SP-GD2-hu14-18-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-GD2-hu14-18-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3202	7115
GD2	CD8SP-GD2-hu3F8-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-GD2-hu3F8-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3203	7116
GD3	CD8SP-GD3-KM-641-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-GD3-KM-641-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3204	7117
GFRa4	CD8SP-GFRAlpha4-P4-6-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-GFRAlpha4-P4-6-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3205	7118
GFRa4	CD8SP-GFRa4-P4-10-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-GFRa4-P4-10-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3206	7119
FUCOSY L-GM1	CD8SP-GM1-5B2-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-GM1-5B2-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3207	7120
FUCOSY L-GM1	CD8SP-GM1-7E5-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-GM1-7E5-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3208	7121
GPRC5D	CD8SP-GPRC5D-ET150-5-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-GPRC5D-ET150-5-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3209	7122
GPRC5D	CD8SP-GPRC5D-ET150-18-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-GPRC5D-ET150-18-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3210	7123
gp100	CD8SP-gp100-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-gp100-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3211	7124
gp100	CD8SP-gp100-G2D12-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-gp100-G2D12-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3212	7125
GPC3	CD8SP-GPC3-4E5-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-GPC3-4E5-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3213	7126
gpNMB	CD8SP-gpNMB-115-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-gpNMB-115-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3214	7127

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
GRP78	CD8SP-GRP78-GC18-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-GRP78-GC18-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3215	7128
Her2	CD8SP-Her2-1-Darpin-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-Her2-2-Darpin-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3216	7129
Her2	CD8SP-Her2-5F7-vHH-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-Her2-47D5-vHH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3217	7130
Her2	CD8SP-Her2-Hu4D5-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-Her2-Hu4D5-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3218	7131
Her2 & Her3	CD8SP-Her3-17B05So-vHH-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-Her2-2D3-vHH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3219	7132
HIV1-gag	CD8SP-HIV1-E5-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-HIV1-E5-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3220	7133
HIV1-env	CD8SP-HIV1-3BNC117-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-HIV1-3BNC117-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3221	7134
HIV1-env	CD8SP-HIV1-PGT-128-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3222	7135
HIV1-env	CD8SP-HIV1-VR-C01-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-HIV1-VR-C01-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3223	7136
HIV1-env	CD8SP-HIV1-X5-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-HIV1-X5-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3224	7137
HMW-MAA	CD8SP-HMW-MAA-hIND-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-HMW-MAA-hIND-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3225	7138
HTLV1-TAX	CD8SP-HTLV-TAX-T3F2-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-TAX-T3F2-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3226	7139
HTLV1-TAX	CD8SP-HTLV-TAX-T3E3-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-TAX-T3E3-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3227	7140
IL11Ra	CD8SP-IL11Ra-8E2-Ts107-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-IL11Ra-8E2-Ts107-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3228	7141

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
IL6Ra & CD19	IgHSP-IL6R-304-vHH-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-FMC63-scFV-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3229	7142
IL13Ra2	CD8SP-IL13Ra2-hu107-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-IL13Ra2-hu107vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3230	7143
IL13Ra2	CD8SP-IL13Ra2-Hu108-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-IL13Ra2-Hu108-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3231	7144
KSHV-K8.1	CD8SP-KSHV-4C3-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-4C3-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3232	7145
LAMP1	CD8SP-LAMP1-humab1-2-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-LAMP1-humab1-2vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3233	7146
LAMP1	CD8SP-LAMP1-Mb4-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-LAMP1-Mb4-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3234	7147
LewisY	CD8SP-LewisY-huS193-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-LewisY-huS193-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3235	7148
L1CAM	CD8SP-L1CAM-9-3-HU3-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-L1CAM-9-3-HU3-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3236	7149
LHR	SP-LHb-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CGHa-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3237	7150
Lym1	CD8SP-Lym1-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-Lym1-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3238	7151
Lym2	CD8SP-Lym2-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-Lym2-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3239	7152
CD79b	CD8SP-huMA79bv28-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-huMA79bv28-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3240	7153
MART1	CD8SP-MART1-CAG10-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-MART1-CAG10-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3241	7154
MART1	CD8SP-MART1-CLA12-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-MART1-CLA12-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3242	7155

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
Mesothelin	CD8SP-Mesothelin-m912-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-m912-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3243	7156
cMet	CD8SP-cMET-171-vHH-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-Her3-21F06-vHH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3244	7157
MPL	CD8SP-MPL-175-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-175-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3245	7158
MPL	CD8SP-MPL-161-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-161-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3246	7159
MPL	CD8SP2-MPL-111-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-MPL-111-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3247	7160
MPL	CD8SP-MPL-178-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-178-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3248	7161
MPL	CD8SP-MPL-AB317-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-AB317-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3249	7162
MPL	CD8SP-MPL-12E10-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-12E10-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3250	7163
MPL	CD8SP-MPL-huVB22Bw5-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-MPL-huVB22Bw5-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3251	7164
Muc1	CD8SP-Muc1-D6-M3B8-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-Muc1-D6-M3B8-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3252	7165
Muc1	CD8SP-MUC1-D6-M3A1-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-MUC1-D6-M3A1-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3253	7166
Muc16	CD8SP-Muc16-4H11-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-Muc16-4H11-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3254	7167
EGFR	CD8SP-Nimotuzumab-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-Nimotuzumab-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3255	7168
NKG2D	CD8SP-NKG2D-(G4SG4D)-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-NKG2D-(G4SG4D)-v2-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3256	7169

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
NKG2D	CD8SP-NKG2D-MS-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-NKG2D-MS-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3257	7170
NYBR1	CD8SP-NYBR1-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-NYBR1-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3258	7171
NY-ESO	CD8SP-NYESO-T1-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-NYESO-T1-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3259	7172
NY-ESO	CD8SP-NYESO-T1-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-NYESO-T2-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3260	7173
PD1 Ligand	SP-PD1-ECD-[IgCL-TCRb-IAH-6MD]-P2A-SP-PD1-opt-ECD-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3261	7174
PDL1	CD8SP-PDL1-Atezoli-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-PDL1-Atezoli-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3262	7175
PDL1	CD8SP-PDL1-SP142-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-PDL1-SP142-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3263	7176
PDL1	CD8SP-PDL1-10A5-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-PDL1-10A5-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3264	7177
PSCA	CD8SP-PSCA-Ha14-121-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-PSCA-Ha14-121-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3265	7178
PSCA	CD8SP-PSCA-Ha14-117-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-PSCA-Ha14-117-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3266	7179
PR1	CD8SP-PR1-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-PR1-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3267	7180
PSMA	CD8SP-PSMA-006-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-PSMA-006-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3268	7181
PSMA	CD8SP-PSMA-J591-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-PSMA-J591-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3269	7182
PTK7	CD8SP-PTK7-hSC6-23-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-PTK7-hSC6-23-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3270	7183

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
PTK7	CD8SP-PTK7-SC6-10-2-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-PTK7-SC6-10-2-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3271	7184
ROR1	CD8SP-ROR1-4A5-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-ROR1-4A5-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3272	7185
ROR1	CD8SP-ROR1-4C10-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-ROR1-4C10-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3273	7186
Mesothelin	CD8SP-SD1-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-SD2-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3274	7187
SLea	CD8SP-SLea-7E3-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-SLea-7E3-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3275	7188
SLea	CD8SP-SLea-5B1-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-SLea-5B1-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3276	7189
SSEA4	CD8SP-SSEA4-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-SSEA4-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3277	7190
Tyrosinase	CD8SP-TA2-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-TA2-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3278	7191
TCRB1	CD8SP-TCRB1-CP01-E09-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-TCRB1-CP01-E09-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3279	7192
TCRB1	CD8SP-TCRB1-Jovi1-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-TCRB1-Jovi1-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3280	7193
TCRB2	CD8SP-TCRB2-CP01-D05-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-TCRB2-CP01-D05-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3281	7194
TCRB2	CD8SP-TCRB2-CP01-E05-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-TCRB2-CP01-E05-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3282	7195
TCRgd	CD8SP-TCRgd-G5-4-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-TCRgd-G5-4-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3283	7196
hTERT	CD8SP-TERT-4A9-T540-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-TERT-4A9-T540-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3284	7197

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
hTERT	CD8SP-TERT-3G3-T865-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-TERT-3G3-T865-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3285	7198
TGFBR2	CD8SP-TGFBR2-Ab1-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-TGFBR2-Ab1-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3286	7199
TIM1	CD8SP-TIM1-HVCR1-270-2-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-TIM1-HVCR1-270-2-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3287	7200
TIM1	CD8SP-TIM1-HVCR1-ARD5-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-TIM1-HVCR1-ARD5vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3288	7201
TnAg	CD8SP-TnAg-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-TnAg-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3289	7202
Tn-Muc1	CD8SP-TnMuc1-hu5E5-RHA8-RKA-2-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-TnMuc1-hu5E5-RHA8-RKA-2vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3290	7203
TROP2	CD8SP-TROP2-ARA47-HV3KV3-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-TROP2-ARA47-HV3KV3-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3291	7204
TROP2	CD8SP-TROP2-h7E6-SVG-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-TROP2-h7E6-SVG-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3292	7205
TSHR	SP-TSHb-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CGHa-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3293	7206
TSHR	CD8SP-TSHR-K1-70-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-TSHR-K1-70-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3294	7207
TSHR	CD8SP-TSHR-KB1-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-TSHR-KB1-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3295	7208
TSHR	CD8SP-TSHR-5C9-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-TSHR-5C9-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3296	7209
TSLPR	CD8SP-TSLPR-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-TSLPR-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3297	7210
Tyrosinase	CD8SP-Tyros-B2-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-Tyros-B2-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3298	7211

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
Tyrosinase	CD8SP-Tyros-MC1-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-Tyros-MC1-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3299	7212
Tyrosinase	CD8SP-Tyrosinase-B2-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-Tyrosinase-B2-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3300	7213
VEGFR3	CD8SP-VEGFR3-Ab1-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-VEGFR3-Ab1-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3301	7214
WT1	CD8SP-WT1-Ab1-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-WT1-Ab1-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3302	7215
WT1	CD8SP-WT1-Ab5-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-WT1-Ab5-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3303	7216
WT1	CD8SP-MYC3-WT1-Ab13-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-WT1-Ab13-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3304	7217
WT1	CD8SP-MYC3-WT1-Ab15-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-WT1-Ab15-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3305	7218
CD123	CD8SP-CD123-1172-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD123-1172-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3306	7219
CDH19	CD8SP-CDH19-4B10-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CDH19-4B10-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3307	7220
Folate Receptor beta	CD8SP-FRbeta-m923-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-FRbeta-m923-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3308	7221
LHR	CD8SP-LHR-8B7-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-LHR-8B7-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3309	7222
LHR	CD8SP-LHR-5F4-21-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-LHR-5F4-21-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3310	7223
B7H4	CD8SP-B7H4-hu22C10-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-B7H4-hu22C10-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3311	7224
B7H4	CD8SP-B7H4-hu1D11-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-B7H4-hu1D11-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3312	7225

Target	Name of CAR constructs including the name of antigen binding domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
IgE	CD8SP-IgE-omalizumab-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-IgE-omalizumab-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3313	7226
CD23	CD8SP-CD23-p5E8-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD23-p5E8-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3314	7227
GCC	CD8SP-GCC-5F9-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-GCC-5F9-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3315	7228
GCC	CD8SP-GCC-Ab229-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-GCC-Ab229-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3316	7229
CD200R	CD8SP-CD200R-huDx182-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD200R-huDx182-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3317	7230
Tn-Muc1	CD8SP-Tn-Muc1-5E5-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-Tn-Muc1-5E5-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3318	7231
CD22	CD8SP-CD22-5-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD22-5-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3319	7232
CD22	CD8SP-CD22-10-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD22-10-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3320	7233
CD22	CD8SP-CD22-31-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD22-31-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3321	7234
CD22	CD8SP-CD22-53-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD22-53-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3322	7235
CD22	CD8SP-CD22-65-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-CD22-65-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	3323	7236

[00180] In some embodiments, the compositions comprise nucleic acids encoding conventional CARs 1-6 (**Table 1**), wherein the antigen specific domain of the CAR targets one or more specific antigens as described in **Tables 6A-C** or Tables 5-6 in PCT/US2017/064379, which are incorporated herein by reference. In some embodiments, the compositions comprise nucleic acids encoding any one or more of backbones 1-72 (**Table 2**) where the antigen specific domain of the encoded CAR targets one or more specific antigens as described in **Tables 6A-C** or Tables 5-6 in PCT/US2017/064379. In some embodiments, the compositions comprise nucleic acids encoding backbone-1, wherein the

antigen specific domain of the CAR in backbone-1 targets one or more cancer specific antigens as described herein in **Tables 6A-C** or Tables 5-6 of PCT/US2017/064379. In some embodiments, the compositions comprise nucleic acids encoding backbone-1, wherein the antigen specific domain of the CAR in backbone-2 targets one or more cancer specific antigens as described herein in **Tables 6A-C** or Tables 5-6 of PCT/US2017/064379. In some embodiments, the compositions comprise nucleic acids encoding backbone-1, wherein the antigen specific domain of the CAR in backbone-37 targets one or more cancer specific antigens as described herein in **Tables 6A-C** or Tables 5-6 of PCT/US2017/064379. In some embodiments, the compositions comprise nucleic acids encoding backbone-1, wherein the antigen specific domain of the CAR in backbone-38 targets one or more cancer specific antigens as described herein in **Tables 6A-C** or Tables 5-6 of PCT/US2017/064379. In some embodiments, the compositions comprise nucleic acids encoding backbone-1, wherein the antigen specific domain of the CAR in backbone-49 targets one or more cancer specific antigens as described herein in **Tables 6A-C** or Tables 5-6 of PCT/US2017/064379. In some embodiments, the compositions comprise nucleic acids encoding backbone-1, wherein the antigen specific domain of the CAR in backbone-50 targets one or more cancer specific antigens as described herein in **Tables 6A-C** or Tables 5-6 of PCT/US2017/064379.

[00181] In various embodiments, the isolated nucleic acid molecules encoding the non-naturally occurring immune receptor, e.g, a CAR, components of the backbones described herein, encode one, two, three or more antigen specific domains. For example, one or more ASD that binds specifically to a cancer associated antigen as described herein can be used. The sequences of the ASD are contiguous with and in the same reading frame as a nucleic acid sequence encoding the remainder of the one or more chains of CAR.

[00182] In one embodiment, each antigen specific region comprises the full-length IgG heavy chain (specific for the target antigen) having the V_H, CH1, hinge, and the CH2 and CH3 (Fc) Ig domains, if the V_H domain alone is sufficient to confer antigen-specificity ("single-domain antibodies"). The full length IgG heavy chain may be linked to a co-stimulatory domain and an optional intracellular signaling domain via the appropriate transmembrane domain. If both, the V_H and the V_L domains, are necessary to generate a fully active antigen-specific targeting region, the V_H-containing non-naturally occurring immune receptor, e.g, a CAR, and the full-length lambda light chain (IgL) are both introduced into the cells to generate an active antigen-specific targeting region.

[00183] In some embodiments, the antigen specific domain of the encoded non-naturally occurring immune receptor, e.g, a CAR, molecule comprises an antibody, an

antibody fragment, an scFv, a Fv, a Fab, a (Fab')₂, a single domain antibody (SDAB), a vH or vL domain, or a camelid vHH domain. In some embodiments, the antigen binding domain of the non-naturally occurring immune receptor, e.g., a CAR, is a scFv antibody fragment that is humanized compared to the murine sequence of the scFv from which it is derived.

[00184] In some instances, scFvs can be prepared according to methods known in the art (for example, Bird *et al.*, (1988) Science 242:423-426 and Huston *et al.*, (1988) Proc.Natl. Acad. Sci. USA 85:5879-5883). ScFv molecules can be produced by linking V_H and V_L regions together using flexible polypeptide linkers. The scFv molecules comprise a linker (e.g., a Ser-Gly linker) with an optimized length and/or amino acid composition (e.g., to optimize folding *etc.*). An scFv can comprise a linker of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, or more amino acid residues between its V_L and V_H regions. In some embodiments, the linker sequence comprises amino acids glycine and serine. In another embodiment, the linker sequence comprises sets of glycine and serine repeats. Variation in the linker length may retain or enhance activity, giving rise to superior efficacy in activity studies. In some embodiments, the antigen specific scFv antibody fragments are functional in that they bind the same antigen with the same or comparable affinity as the IgG antibody from which it is derived. In other embodiments, the antibody fragment has a lower binding affinity to the antigen compared to the antibody from which it is derived but is functional in that it provides a biological response described herein. In one embodiment, the CAR molecule comprises an antibody fragment that has a binding affinity KD of 10⁻⁴ M to 10⁻⁸ M, 10⁻⁵ M to 10⁻⁷ M, 10⁻⁶ M or 10⁻⁸ M, for the target antigen.

[00185] In one embodiment, the antigen specific domain comprises one, two or all three heavy chain (hc) CDRs, hcCDR1, hcCDR2 and hcCDR3 of an antibody or a scFv listed herein (**Table 6B**; SEQ ID NOs: 14122-15039), and/or one, two or all three light chain (lc) CDRs, lcCDR1, lcCDR2 and lcCDR3 of an antibody or a scFv listed herein (**Tables 6A**; SEQ ID NOs: 13204-14121) (also also see, Tables 5-6 of PCT/US2017/064379). In some embodiments, the ASD comprises a V_L (or vL) fragment comprising all three light chain CDRs belonging to a specific scFv (**Tables 6A**; SEQ ID NOs: 13204-14121) or a V_H (or vH) fragment comprising all three heavy chain CDRs belonging to a specific scFv (**Table 6B**; SEQ ID NOs: 14122-15039) (see also, Tables 5-6 of PCT/US2017/064379). **Table 6C** provides the names, target antigens and SEQ ID Nos of the different scFvs whose vL and vL fragments and CDRs are listed in **Tables 6A and 6B**. The vL and vH fragments and the corresponding scFvs can be used in various embodiments of the disclosure to constructs the CARs described herein.

[00186] In another embodiment, the antigen specific domain comprises a humanized antibody or an antibody fragment. In some aspects, a non-human antibody is humanized, where specific sequences or regions of the antibody are modified to increase similarity to an antibody naturally produced in a human or fragment thereof. In one aspect, the antigen binding domain is humanized. A humanized antibody can be produced using a variety of techniques known in the art, including but not limited to, CDR-grafting, veneering or resurfacing, and chain shuffling.

[00187] In a further embodiment, each antigen specific domain of the non-naturally occurring immune receptor, e.g, a CAR, may comprise a divalent (or bivalent) single-chain variable fragment (di-scFvs, bi-scFvs). In, for example, CARs comprising di-scFVs, two scFvs specific for each antigen are linked together by producing a single peptide chain with two V_H and two V_L regions, yielding tandem scFvs. (Xiong, Cheng-Yi; Natarajan, A; Shi, XB; Denardo, GL; Denardo, SJ (2006). "Development of tumor targeting anti-MUC-1 multimer: effects of di-scFv unpaired cysteine location on PEGylation and tumor binding". *Protein Engineering Design and Selection* 19 (8): 359–367; Kufer, Peter; Lutterbüse, Ralf; Baeuerle, Patrick A. (2004). "A revival of bispecific antibodies". *Trends in Biotechnology* 22 (5): 238–244). CARs comprising at least two antigen-specific targeting regions would express two scFvs specific for each of the two antigens. The resulting ASD is joined to the co-stimulatory domain and the intracellular signaling domain via a hinge region and a transmembrane domain. Alternatively, non-naturally occurring immune receptor, e.g, a CAR, comprising two antigen specific targeting regions would express two vHH specific for each of the two antigens or two epitopes of the same antigen. Exemplary CARs targeting two antigens are represented by SEQ ID NOs: 1307 and 1310.

[00188] In another embodiment, each ASD of the non-naturally occurring immune receptor, e.g, a CAR, comprises a diabody. In a diabody, the scFvs are created with linker peptides that are too short for the two variable regions to fold together, driving the scFvs to dimerize. Still shorter linkers (one or two amino acids) lead to the formation of trimers, the so-called triabodies or tribodies. Tetrabodies may also be used.

[00189] In some embodiments, the ASD of the non-naturally occurring immune receptor, e.g, a CAR, comprises V_{HH} fragments (nanobodies) as described herein (see, Tables 5-6 of PCT/US2017/064379). In some embodiments, the ASD of the non-naturally occurring immune receptor, e.g, a CAR, comprises affibodies as described herein (see, Tables 5-6 of PCT/US2017/064379).

[00190] In another embodiment, the antigen specific binding domain comprises a ligand for a cognate expressed on a target cell.

[00191] In one embodiment, an antigen specific domain of a non-naturally occurring immune receptor, e.g., a CAR, against a target antigen is an antigen binding portion, e.g., CDRs, of vHH fragments targeting this antigen (see, Tables 5-6 of PCT/US2017/064379).

[00192] In one embodiment, an antigen specific domain of a non-naturally occurring immune receptor, e.g., a CAR, against a target antigen is an antigen binding portion of a non-immunoglobulin scaffold targeting this antigen (see, Tables 5-6 of PCT/US2017/064379).

[00193] In one embodiment, an antigen specific domain of a non-naturally occurring immune receptor, e.g., a CAR, against a target antigen is an antigen binding portion of a receptor known to bind this target antigen (see, Tables 5-6 of PCT/US2017/064379).

[00194] In another aspect, the antigen binding domain is a T cell receptor ("TCR"), or a fragment thereof, for example, a single chain TCR (scTCR). Methods to make such TCRs are known in the art. See, e.g., Willemsen RA et al, *Gene Therapy* 7: 1369-1377 (2000); Zhang T et al, *Cancer Gene Ther* 11: 487-496 (2004); Aggen et al, *Gene Ther.* 19(4):365- 74 (2012) (references are incorporated herein by its entirety). For example, scTCR can be engineered that contain the V α and v β genes from a T cell clone linked by a linker (e.g., a flexible peptide). This approach is very useful to cancer associated target that itself is intracellular, however, a fragment of such antigen (peptide) is presented on the surface of the cancer cells by MHC.

[00195] In some embodiments, the antigen specific domain is a T cell receptor specific for the target antigen or a fragment of the T cell receptor, wherein the fragment retains the specificity for the target antigen.

[00196] In some embodiments, antigen specific domain of a non-naturally occurring immune receptor, e.g., a CAR, described herein binds to a MHC presented peptide. Normally, peptides derived from endogenous proteins fill the pockets of Major histocompatibility complex (MHC) class I molecules, and are recognized by T cell receptors (TCRs) on CD8+ T lymphocytes. The MHC class I complexes are constitutively expressed by all nucleated cells. In cancer, virus-specific and/or tumor-specific peptide/MHC complexes represent a unique class of cell surface targets for immunotherapy. TCR-like antibodies targeting peptides derived from viral or tumor antigens in the context of human leukocyte antigen (HLA)-A1 or HLA-A2 have been described (see, e.g., Sastry *et al.*, *J Viral.* 2011 85(5):1935-1942; Sergeeva *et al.*, *Blood*, 2011 117(16):4262-4272; Verma *et al.*, *J Immunol* 2010 184(4):2156-

2165; Willemsen *et al.*, Gene Ther 20018(21) :1601-1608; Dao *et al.*, Sci Transl Med 2013 5(176) :176ra33; Tassev *et al.*, Cancer Gene Ther 2012 19(2):84-100). For example, TCR-like antibody can be identified from screening a library, such as a human scFv phage displayed library. Exemplary CARs that are based on TCR-like antibodies targeting WT1 in association with HLA-A2 are represented by SEQ ID NO: 1266 to SEQ ID NO: 1268. In the instant invention, CARs were generated using antigen binding domain derived from TCR like antibodies against several HLA-A2 restricted intracellular peptides. The target protein antigens, the peptide fragment and the sequence of the peptide are shown in **Table 8**.

[00197] In some embodiments, the antigens specific for disease which may be targeted by the non-naturally occurring immune receptor, e.g. a CAR, when expressed alone or with the accessory modules as described herein include but are not limited to any one or more of CD5; CD19; CD123; CD22; CD30; CD171; CS1 (also referred to as CD2 subset 1, CRACC, MPL, SLAMF7, CD319, and 19A24); C-type lectin-like molecule-1 (CLL-1 or CLECL1); CD33; epidermal growth factor receptor variant III (EGFRviii); ganglioside G2 (GD2); ganglioside GD3 (aNeu5Ac(2-8)aNeu5Ac(2-3)bDGalp(l-4)bDGlc(l-l)Cer); TNF receptor family member B cell maturation (BCMA); Tn antigen ((Tn Ag) or (GalNAcα-Ser/Thr)); prostate-specific membrane antigen (PSMA); Receptor tyrosine kinase-like orphan receptor 1 (ROR1); Fms Like Tyrosine Kinase 3 (FLT3); Tumor-associated glycoprotein 72 (TAG72); CD38; CD44v6; a glycosylated CD43 epitope expressed on acute leukemia or lymphoma but not on hematopoietic progenitors, a glycosylated CD43 epitope expressed on non-hematopoietic cancers, Carcinoembryonic antigen (CEA); Epithelial cell adhesion molecule (EPCAM); B7H3 (CD276); KIT (CD117); Interleukin-13 receptor subunit alpha-2 (IL-13Ra2 or CD213A2); Mesothelin; Interleukin 11 receptor alpha (IL-11Ra); prostate stem cell antigen (PSCA); Protease Serine 21 (Testisin or PRSS21); vascular endothelial growth factor receptor 2 (VEGFR2); Lewis(Y) antigen; CD24; Platelet-derived growth factor receptor beta (PDGFR-beta); Stage-specific embryonic antigen-4 (SSEA-4); CD20; Folate receptor alpha (FRa or FR1); Folate receptor beta (FRb); Receptor tyrosine-protein kinase ERBB2 (Her2/neu); Mucin 1, cell surface associated (MUC1); epidermal growth factor receptor (EGFR); neural cell adhesion molecule (NCAM); Prostase; prostatic acid phosphatase (PAP); elongation factor 2 mutated (ELF2M); Ephrin B2; fibroblast activation protein alpha (FAP); insulin-like growth factor 1 receptor (IGF-I receptor), carbonic anhydrase IX (CAIX); Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2); glycoprotein 100 (gp100); oncogene fusion protein consisting of breakpoint cluster region (BCR) and Abelson murine leukemia viral oncogene homolog 1 (Abl) (bcr-abl); tyrosinase;

ephrin type-A receptor 2 (EphA2); sialyl Lewis adhesion molecule (sLe); ganglioside GM3 (aNeu5Ac(2-3)bDCIalp(1-4)bDGlc(1-1)Cer); transglutaminase 5 (TGS5); high molecular weight-melanoma associated antigen (HMWMAA); o-acetyl-GD2 ganglioside (OAcGD2); tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related (TEM7R); claudin 6 (CLDN6); thyroid stimulating hormone receptor (TSHR); G protein coupled receptor class C group 5, member D (GPRC5D); chromosome X open reading frame 61 (CXORF61); CD97; CD179a; anaplastic lymphoma kinase (ALK); Polysialic acid; placenta-specific 1 (PLAC1); hexasaccharide portion of globoH glycosphingolipid (GloboH); mammary gland differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3 (ADRB3); pannexin 3 (PANX3); G protein-coupled receptor 20 (GPR20); lymphocyte antigen 6 complex, locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame Protein (TARP); Wilms tumor protein (WT1); Cancer/testis antigen 1 (NY-ESO-1); Cancer/testis antigen 2 (LAGE-1a); Melanoma-associated antigen 1 (MAGE-A1); ETS translocation-variant gene 6, located on chromosome 12p (ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, Member 1A (XAGE1); angiopoietin-binding cell surface receptor 2 (Tie 2); melanoma cancer testis antigen-1 (MAD-CT-1); melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; tumor protein p53 (p53); p53 mutant; prostein; survivin; telomerase; prostate carcinoma tumor antigen-1 (PCT A-1 or Galectin 8), melanoma antigen recognized by T cells 1 (MelanA or MART1); Rat sarcoma (Ras) mutant; human Telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoints; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease, serine 2 (TMPRSS2) ETS fusion gene); N-Acetyl glucosaminyl-transferase V (NA17); paired box protein Pax-3 (PAX3); Androgen receptor; Cyclin B1; v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Tyrosinase-related protein 2 (TRP-2); Cytochrome P450 1B1 (CYP1B1); CCCTC-Binding Factor (Zinc Finger Protein)-Like (BORIS or Brother of the Regulator of Imprinted Sites), Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3); Paired box protein Pax-5 (PAX5); proacrosin binding protein sp32 (OY-TES1); lymphocyte-specific protein tyrosine kinase (LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint 2 (SSX2); Receptor for Advanced Glycation Endproducts (RAGE-1); renal ubiquitous 1 (RUI); renal ubiquitous 2 (RU2); legumain; human papilloma virus E6 (HPV E6); human papilloma virus E7 (HPV E7); intestinal carboxyl esterase; heat shock protein 70-2 mutated (mut hsp70-2); CD79a; CD79b; CD72; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment

of IgA receptor (FCAR or CD89); Leukocyte immunoglobulin-like receptor subfamily A member 2 (LILRA2); CD300 molecule-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2); lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); and immunoglobulin lambda-like polypeptide 1 (IGLL1), MPL, Biotin, c-MYC epitope Tag, CD34, LAMP1 TROP2, GFRalpha4, CDH17, CDH6, NYBR1, CDH19, CD200R, SleA (CA19.9; Sialyl Lewis Antigen); Fucosyl-GM1, PTK7, gpNMB, CDH1-CD324, DLL3, CD276/B7H3, IL11Ra, IL13Ra2, CD179b-IGL11, TCRgamma-delta, NKG2D, CD32 (FCGR2A), Tn ag, Tim1-/HVCRI, CSF2RA (GM-CSFR-alpha), TGFbetaR2, , Lews Ag, TCR-beta1 chain, TCR-beta2 chain, TCR-gamma chain, TCR-delta chain, FITC, Leutenizing hormone receptor (LHR), Follicle stimulating hormone receptor (FSHR), Gonadotropin Hormone receptor (CGHR or GR), CCR4, GD3, SLAMF6, SLAMF4, HIV1 envelope glycoprotein, HTLV1-Tax, CMV pp65, EBV-EBNA3c, KSHV K8.1, KSHV-gH, influenza A hemagglutinin (HA), GAD, PDL1, Guanylyl cyclase C (GCC), auto antibody to desmoglein 3 (Dsg3), auto antibody to desmoglein 1 (Dsg1), HLA, HLA-A, HLA-A2, HLA-B, HLA-C, HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, HLA-DR, HLA-G, IgE, CD99, Ras G12V, Tissue Factor 1 (TF1), AFP, GPRC5D, Claudin18.2 (CLD18A2 or CLDN18A.2), P-glycoprotein, STEAP1, Liv1, Nectin-4, Cripto, gpA33, BST1/CD157, low conductance chloride channel, and the antigen recognized by TNT antibody or combinations thereof.

[00198] In some embodiments, the antigens specific for a disease which may be targeted by the non-naturally occurring immune receptor, e.g. a CAR, when expressed alone or with the accessory modules as described herein include but are not limited to any one or more of 4-1BB, 5T4, adenocarcinoma antigen, alpha-fetoprotein, BAFF, B-lymphoma cell, C242 antigen, CA-125, carbonic anhydrase 9 (CA-IX), C-MET, CCR4, CD152, CD19, CD20, CD200, CD22, CD221, CD23 (IgE receptor), CD28, CD30 (TNFRSF8), CD33, CD4, CD40, CD44 v6, CD51, CD52, CD56, CD74, CD80, CD123, CEA, CNTO888, CTLA-4, DR5, EGFR, EpCAM, CD3, FAP, fibronectin extra domain-B, folate receptor 1, GD2, GD3 ganglioside, glycoprotein 75, GPNMB, HER2/neu, HGF, human scatter factor receptor kinase, IGF-1 receptor, IGF-I, IgG1, L1-CAM, IL-13, IL-6, insulin-like growth factor I receptor, integrin $\alpha 5\beta 1$, integrin $\alpha v\beta 3$, LAMP1, MORAb-009, MS4A1, MUC1, mucin CanAg, N-glycolylneuraminic acid, NPC-1C, PDGF-R α , PDL192, phosphatidylserine, prostatic carcinoma cells, RANKL, RON, ROR1, SCH 900105, SDC1, SLAMF7, TAG-72, tenascin C, TGF beta 2, TGF- β , TRAIL-R1, TRAIL-R2, tumor antigen CTAA16.88, VEGF-

A, VEGFR-1, VEGFR2, vimentin or combinations thereof. Other antigens specific for cancer will be apparent to those of skill in the art and may be used in connection with alternate embodiments of the disclosure.

[00199] A CAR when used alone or with accessory modules as described herein can comprise an antigen binding domain (*e.g.*, antibody or antibody fragment) that binds to a disease-supporting antigen (*e.g.*, a disease-supporting antigen as described herein). In some embodiments, the disease-supporting antigen is an antigen present on cells that support the survival and proliferation of disease causing cells. In some embodiments, the disease-supporting antigen is an antigen present on a stromal cell or a myeloid-derived suppressor cell (MDSC). Stromal cells can secrete growth factors and cytokines to promote cell proliferation in the microenvironment. MDSC cells can block T cell proliferation and activation. Without wishing to be bound by theory, in some embodiments, the CAR-expressing cells destroy the disease-supporting cells, thereby indirectly blocking growth or survival of disease causing cells.

[00200] In certain embodiments, a stromal cell antigen is selected from one or more of: bone marrow stromal cell antigen 2 (BST2), fibroblast activation protein (FAP) and tenascin. In an embodiment, the FAP-specific antibody is, competes for binding with, or has the same CDRs as, sibrotuzumab. In embodiments, the MDSC antigen is selected from one or more of: CD33, CD11b, C14, CD15, and CD66b. Accordingly, in some embodiments, the disease supporting antigen is selected from one or more of: bone marrow stromal cell antigen 2 (BST2), fibroblast activation protein (FAP) or tenascin, CD33, CD11b, C14, CD15, and CD66b.

[00201] In another embodiment, the disclosure provides non-naturally occurring immune receptor, *e.g.*, a CAR, that bind to the same epitope on different targets described in **Tables 6A-C** as any of the non-naturally occurring immune receptors of the disclosure (*e.g.*, CARs that have the ability to cross-compete for binding to the different targets with any of the CARs of the disclosure). In some embodiments, the antigen specific domains of these non-naturally occurring immune receptors, *e.g.*, a CARs, could be derived from vL fragments, vH fragments or scFv fragments of antibodies. In some embodiments, the reference antibodies for cross-competition studies to determine the target-epitope recognized by a non-naturally occurring immune receptor, *e.g.*, a CAR, of the disclosure are scFvs described in **Table 6C** herein having sequences as shown in SEQ ID NOs: 4555-4815, 11165-11401, 15070- 15132 (**Table 6C**) or as described in Tables 5-6 of PCT/US2017/064379. In an exemplary embodiment, the reference scFv FMC63(vL-vH) represented by SEQ ID NO:

4555 can be used in cross-competition studies to determine the target-epitope recognized by FMC63-based conventional CARs and backbones of the disclosure. In some embodiments, the reference vHH fragments for cross-competition studies to determine the target-epitope recognized by a non-naturally occurring immune receptor, e.g, a CAR, of the disclosure described herein are vHH fragments having sequences as shown in SEQ ID NOs: 4474-4514. In some embodiments, the reference non-immunoglobulin antigen binding scaffolds for cross-competition studies to determine the target-epitope recognized by a non-naturally occurring immune receptor, e.g, a CAR, of the disclosure described herein are non-immunoglobulin antigen binding scaffolds having sequences as shown in SEQ ID NOs: 4515-4519. In some embodiments, the reference ligands for cross-competition studies to determine the target-epitope recognized by a CAR of the disclosure described herein are ligands having sequences whose SEQ ID Nos: 4544-4554. In some embodiments, the reference CARs for cross-competition studies against different targets are CARs whose SEQ IDs are shown in **Tables 10-14**.

[00202] In another embodiment, the reference antibodies for cross-competition studies to determine the target-epitopes recognized by the MPL-targeting CARs of the disclosure are antibodies mAb-1.6, mAb-1.111, mAb-1.75, mAb-1.78, mAb-1.169, and mAb-1.36 described in patent application US 2012/0269814 A1.

[00203] In another embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the MPL-targeting CARs of the disclosure are scFvs having sequences as shown in SEQ ID NOs: 4720-4727, in **Table 6C** or as described in Tables 5-6 of PCT/US2017/064379.

[00204] In another embodiment, the reference ligands for cross-competition studies to determine the target-epitopes recognized by the MPL-targeting CARs of the disclosure are TPO and mTPO ligands having sequences as listed in SEQ ID NOs: 4544-4545.

[00205] In another embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the MPL-targeting CARs of the disclosure are CARs having sequences as shown in SEQ ID NOs: 5120-5126.

[00206] In the preferred embodiment, the MPL-targeting CARs of the disclosure bind to an epitope corresponding to the sequences shown in SEQ ID NO: 15160.

[00207] In one embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the CD19-targeting CARs of the invention are scFvs having sequences as shown in SEQ ID NOs: 4555-4568 and in **Table 6C** or as described in Tables 5-6 of PCT/US2017/064379. In another embodiment, the reference CARs

for cross-competition studies to determine the target-epitopes recognized by the CD19-targeting CARs of the invention are CARs having sequences as shown in SEQ ID NOs: 4929-4943.

[00208] In one embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the CD20-targeting CARs of the invention are scFvs targeting CD20 and having SEQ IDs as listed in **Table 6C** or as described in Tables 5-6 of PCT/US2017/064379. In another embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the CD20-targeting CARs of the invention are CARs targeting CD20 and having SEQ IDs as listed in **Tables 12**.

[00209] In the preferred embodiment, the CD20-targeting CARs of the disclosure bind to the epitopes corresponding to one or more of the sequences shown in SEQ ID NO: 15149-15154.

[00210] In one embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the BCMA-targeting CARs of the invention are scFvs targeting CD20 and having SEQ IDs as listed in **Table 6C** or as described in Tables 5-6 of PCT/US2017/064379. In another embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the BCMA-targeting CARs of the invention are CARs targeting BCMA and having SEQ IDs as listed in **Tables 12**.

[00211]

[00212] In the preferred embodiment, the BCMA-targeting CARs of the disclosure bind to the epitopes corresponding to one or more of the sequences shown in SEQ ID NO: 15155-15159.

[00213] In one embodiment, the reference scFvs for cross-competition studies against DLL3-targeting CARs of the invention are scFvs targeting DLL3 and having SEQ IDs as listed in **Table 6C** or as described in Tables 5-6 of PCT/US2017/064379. In another embodiment, the reference CARs for cross-competition studies against DLL3-targeting CARs of the invention are CARs targeting DLL3 and having SEQ IDs as listed in **Table 12**.

[00214] In one embodiment, the reference scFvs for cross-competition studies against LAMP1-targeting CARs of the invention are scFvs targeting LAMP1 and having SEQ IDs as listed in **Table 6C** or as described in Tables 5-6 of PCT/US2017/064379. In another embodiment, the reference CARs for cross-competition studies against LAMP1-targeting CARs of the invention are CARs targeting LAMP1 and having SEQ IDs as listed in **Table 12**.

[00215] In one embodiment, the reference scFvs for cross-competition studies against TROP2-targeting CARs of the invention are scFvs targeting TROP2 and having SEQ IDs as listed in **Table 6C** or as described in Tables 5-6 of PCT/US2017/064379. In another embodiment, the reference CARs for cross-competition studies against TROP2-targeting CARs of the invention are CARs targeting TROP2 and having SEQ IDs as listed in **Table 12**.

[00216] In one embodiment, the reference scFvs for cross-competition studies against PTK7-targeting CARs of the invention are scFvs targeting PTK7 and having SEQ IDs as listed in **Table 6C** or as described in Tables 5-6 of PCT/US2017/064379. In another embodiment, the reference CARs for cross-competition studies against PTK7-targeting CARs of the invention are CARs targeting PTK7 and having SEQ IDs as listed in **Table 12**.

[00217] In one embodiment, the reference scFvs for cross-competition studies against CD22, CD123, CD33, CD37, CD70, CD138, CS1, IL13Ra2, Folate Receptor α , Folate Receptor β , TCRB1, TCRB2, TCR $\gamma\delta$, CD30, Mesothelin, Her2, EGFRviii, and HIV1-targeting CARs of the invention are scFvs targeting these antigens and having SEQ IDs as listed in **Table 6C** or as described in Tables 5-6 of PCT/US2017/064379. In another embodiment, the reference CARs for cross-competition studies against CD22, CD123, CD33, CD37, CD70, CD138, CS1, IL13Ra2, Folate Receptor α , Folate Receptor β , TCRB1, TCRB2, TCR $\gamma\delta$, CD30, Mesothelin, Her2, EGFRviii, and HIV1-targeting CARs of the invention are CARs targeting these antigens and having SEQ IDs as listed in **Table 12**.

[00218] In some embodiments, the CARs described herein comprise a hinge or linker region between the antigen specific domain and the transmembrane domain. In some embodiments, the hinge region comprises any one or more of human CD8 α or an Fc fragment of an antibody or a functional equivalent, fragment or derivative thereof, a hinge region of human CD8 α or an antibody or a functional equivalent, fragment or derivative thereof, a CH2 region of an antibody, a CH3 region of an antibody, an artificial spacer sequence and combinations thereof. In exemplary embodiments, the hinge region comprises any one or more of (i) a hinge, CH2 and CH3 region of IgG4, (ii) a hinge region of IgG4, (iii) a hinge and CH2 region of IgG4, (iv) a hinge region of CD8 α , (v) a hinge, CH2 and CH3 region of IgG1, (vi) a hinge region of IgG1, (vii) a hinge and CH2 region of IgG1, or (viii) combinations thereof.

[00219] In some embodiments, two or more functional domains of the non-naturally occurring immune receptors, *e.g.*, CARs, as described herein, are separated by one or more linkers. Linkers are oligo- or polypeptides region from about 1 to 100 amino acids in length, that link together any of the domains/regions of the non-naturally occurring immune

receptors, *e.g.*, CARs, of the disclosure. In some embodiments, the linkers may be for example, 5-12 amino acids in length, 5-15 amino acids in length or 5-20 amino acids in length. Linkers may be composed of flexible residues like glycine and serine so that the adjacent protein domains are free to move relative to one another. Longer linkers, for example those longer than 100 amino acids, may be used in connection with alternate embodiments of the disclosure, and may be selected to, for example, ensure that two adjacent domains do not sterically interfere with one another.

[00220] As described herein, the CARs described herein comprise a transmembrane domain. The transmembrane domain may comprise the transmembrane sequence from any protein which has a transmembrane domain, including any of the type I, type II or type III transmembrane proteins. The transmembrane domain of the CAR of the disclosure may also comprise an artificial hydrophobic sequence. The transmembrane domains of the CARs described herein may be selected so that the transmembrane domain do not dimerize. In some embodiments, the CAR comprises any of the backbones described herein having a transmembrane domain selected from the transmembrane domain of an alpha, beta or zeta chain of a T-cell receptor, CD3ε, CD3ζ, CD3γ, CD3δ, CD28, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, KIRDS2, OX40, CD2, CD27, LFA-1 (CD11a, CD18), ICOS (CD278), 4-1BB (CD137), GITR, CD40, BAFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRFL), CD160, CD19, IL2R beta, IL2R gamma, IL7R a, ITGA1, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp, NKp44, NKp30, NKp46, NKG2D, and/or NKG2C.

[00221] A transmembrane domain can include one or more additional amino acids (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 up to 15 amino acids) at either end of the transmembrane region (*e.g.*, one or more amino acids extending extracellularly and/or one or more amino acids extending intracellularly) to the transmembrane region. In one aspect, the transmembrane domain is contiguous with one of the other domains of the CAR. In one embodiment, the transmembrane domain may be from the same protein that the signaling domain, costimulatory domain or the hinge domain is derived from. In another aspect, the

transmembrane domain is not derived from the same protein that any other domain of the CAR is derived from.

[00222] In various embodiments, the isolated nucleic acid molecules encoding the non-naturally occurring immune receptors, *e.g.*, CAR, components of the backbones described herein, encode zero, one, two, three or more intracellular signaling domain.

[00223] As described herein, the non-naturally occurring immune receptors, *e.g.*, CARs, described herein can optionally comprise an intracellular signaling domain. This domain may be cytoplasmic and may transduce the effector function signal and direct the cell to perform its specialized function. Examples of intracellular signaling domains include, but are not limited to, ζ chain of the T-cell receptor or any of its homologs (*e.g.*, η chain, CD3 ϵ , CD3 γ , CD3 δ , Fc ϵ R1 γ and β chains, MB1 (Ig α) chain, B29 (Ig β) chain, *etc.*), CD3 polypeptides (Δ , δ and ϵ), syk family tyrosine kinases (Syk, ZAP 70, *etc.*), src family tyrosine kinases (Lck, Fyn, Lyn, *etc.*) and other molecules involved in T-cell transduction, such as CD2, CD5 and CD28. Specifically, the intracellular signaling domain may be human CD3 zeta chain, Fc γ RIII, Fc ϵ RI, cytoplasmic tails of Fc receptors, immunoreceptor tyrosine-based activation motif (ITAM) bearing cytoplasmic receptors or combinations thereof. Additional intracellular signaling domains will be apparent to those of skill in the art and may be used in connection with alternate embodiments of the invention. In some embodiments, the intracellular signaling domain comprises a signaling domain of one or more of a human CD3 zeta chain, Fc γ RIII, Fc ϵ RI, a cytoplasmic tail of a Fc receptor, an immunoreceptor tyrosine-based activation motif (ITAM) bearing cytoplasmic receptors, and combinations thereof.

[00224] In various embodiments, the isolated nucleic acid molecules encoding the non-naturally occurring immune receptor, *e.g.*, CAR, components of the backbones described herein, encode zero, one, two, three or more co-stimulatory domains. In exemplary embodiments, the co-stimulatory domain comprises a signaling domain from any one or more of CD28, CD137 (4-1BB), CD134 (OX40), Dap10, CD27, CD2, CD5, ICAM-1, LFA-1, Lck, TNFR-I, TNFR-II, Fas, CD30, CD40 and combinations thereof. Various components of non-naturally occurring immune receptors, *e.g.*, CARs, of the disclosure are provided above and elsewhere herein. Again it should be recognized that the disclosure provides, for example, CARs comprising an ecto-domain comprising an antigen specific binding domain attached via a 'hinge' of linker to a transmembrane domain, which is in-turn linked to an endo-domain comprising one or more stimulatory domains and optionally one or more intracellular signaling domains.

[00225] Provided herein are one or more polypeptides encoded by one or more nucleic acid molecules encoding conventional CARs 1 to 6 (**Table 1**) or any one or more of backbones 1-72 described herein (**Table 2**).

[00226] Also provided herein are one or more polypeptides encoded by one or more nucleic acid molecules encoding conventional CARs 1 to 6. In some embodiments, the antigen-specific domain of the CARs is specific to one, two, three or more antigens on target cells, such as cancer cells. As described herein, each component of the CAR is contiguous and in the same reading frame with each other components of the CAR. In some embodiments, in the CAR comprising backbone comprises more than one antigen specific domain, each of the antigen specific domains are contiguous and in the same reading frame as the other antigen specific domains in the same CAR.

[00227] Also provided herein are one or more polypeptides encoded by one or more nucleic acid molecules encoding backbones 1 to 10 comprising conventional CAR I and an accessory module encoding a NF- κ B stimulatory molecule (*e.g.*, vFLIP-K13, hNEMO-K277A, FKBPx2-hNEMO-K277A, FKBPx2-hNEMO-L753(251), FKBPx2-hNEMO-L600(200), FKBPx2-RIP-ID, IKK2-S177E-S181E, IKK1-S176E-S180E, MyD88-L265P, TCL-1A or their variants) as described herein. The accessory module in backbones 1-10 can be replaced by other accessory modules encoding different molecules, including different NF- κ B activators (*e.g.*, K13-opt, hNEMO-K277A-delta-V249-K255 or hNEMO-K277L *etc.*). Also provided herein are one or more polypeptides encoded by one or more nucleic acid molecules encoding backbones 11 to 12 comprising conventional CAR I and an accessory module encoding IgSP-[hTRAC-opt2] and IgSP-[hTRBC-opt2]. In some embodiments, the antigen-specific domain of the CAR comprising backbones-1-12 is specific to one, two, three or more antigens on target cells, such as cancer cells. As described herein, each component of the CAR is contiguous and in the same reading frame with each other components of the CAR comprising backbones 1-12. In some embodiments, in the CAR comprising backbones 1-12 comprises more than one antigen specific domain, each of the antigen specific domains are contiguous and in the same reading frame as the other antigen specific domains in the same CAR.

[00228] Also provided herein are one or more polypeptides encoded by one or more nucleic acid molecules encoding backbones 13 to 22 comprising conventional CAR II and an accessory module encoding a NF- κ B stimulatory molecule (*e.g.*, vFLIP-K13, hNEMO-K277A, FKBPx2-hNEMO-K277A, FKBPx2-hNEMO-L753(251), FKBPx2-hNEMO-L600(200), FKBPx2-RIP-ID, IKK2-S177E-S181E, IKK1-S176E-S180E, MyD88-L265P,

TCL-1A or their variants) as described herein. The accessory module in backbones 13-22 can be replaced by other accessory modules encoding different molecules, including different NF- κ B activators (*e.g.*, K13-opt, hNEMO-K277A-delta-V249-K255 or hNEMO-K277L *etc.*). In some embodiments, the antigen-specific domain of the CAR comprising backbones-13-22 is specific to one, two, three or more antigens on target cells, such as cancer cells. As described herein, each component of the CAR is contiguous and in the same reading frame with each other components of the CAR comprising backbones 13-24. In some embodiments, in the CAR comprising backbones 13-24 comprises more than one antigen specific domain, each of the antigen specific domains are contiguous and in the same reading frame as the other antigen specific domains in the same CAR.

[00229] Also provided herein are one or more polypeptides encoded by one or more nucleic acid molecules encoding backbones 37 to 46 comprising Ab-TCR and an accessory module encoding a NF- κ B stimulatory molecule (*e.g.*, vFLIP-K13, hNEMO-K277A, FKBPx2-hNEMO-K277A, FKBPx2-hNEMO-L753(251), FKBPx2-hNEMO-L600(200), FKBPx2-RIP-ID, IKK2-S177E-S181E, IKK1-S176E-S180E, MyD88-L265P, TCL-1A or their variants) as described herein. The accessory module in backbones 37 to 46 can be replaced by other accessory modules encoding different molecules, including different NF- κ B activators (*e.g.*, K13-opt, hNEMO-K277A-delta-V249-K255 or hNEMO-K277L *etc.*).

[00230] Also provided herein are one or more polypeptides encoded by one or more nucleic acid molecules encoding backbones 49 to 58 comprising double chain cTCR/SIR and an accessory module encoding a NF- κ B stimulatory molecule (*e.g.*, vFLIP-K13, hNEMO-K277A, FKBPx2-hNEMO-K277A, FKBPx2-hNEMO-L753(251), FKBPx2-hNEMO-L600(200), FKBPx2-RIP-ID, IKK2-S177E-S181E, IKK1-S176E-S180E, MyD88-L265P, TCL-1A or their variants) as described herein. The accessory module in backbones 49 to 58 can be replaced by other accessory modules encoding different molecules, including different NF- κ B activators (*e.g.*, K13-opt, hNEMO-K277A-delta-V249-K255 or hNEMO-K277L *etc.*).

[00231] Also provided herein are one or more polypeptides encoded by one or more nucleic acid molecules encoding backbones 61 to 70 comprising one-and-a-half chain (OHC) cTCR/SIR and an accessory module encoding a NF- κ B stimulatory molecule (*e.g.*, vFLIP-K13, hNEMO-K277A, FKBPx2-hNEMO-K277A, FKBPx2-hNEMO-L753(251), FKBPx2-hNEMO-L600(200), FKBPx2-RIP-ID, IKK2-S177E-S181E, IKK1-S176E-S180E, MyD88-L265P, TCL-1A or their variants) as described herein. The accessory module in backbones 61 to 70 can be replaced by other accessory modules encoding different molecules, including

different selective NF- κ B activators (*e.g.*, K13-opt, hNEMO-K277A-delta-V249-K255 or hNEMO-K277L *etc.*).

[00232] In various embodiments, the polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, comprise one, two, three or more NF- κ B stimulatory molecule (*e.g.*, K13-vFLIP, K13opt, NEMO, NEMO K277A, human NEMO-K277L, human NEMO-K277A-DeltaV249-K255, or mouse NEMO K270A or their variants).

[00233] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of the backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to the thrombopoietin receptor, MPL. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to CD19. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to CD20. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to CD22. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to CD23. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6

or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to CD30. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to CD32. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to CD33. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to CD123. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to CD138. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to CD200R. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to CD276. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14,

backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to CD324. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to BCMA. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to CS1. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to ALK1. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to ROR1. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to CDH6. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to CDH16. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-

specific domain of the CARs is specific to CDH17. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to CDH19. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to EGFRviii. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to Her2. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to Her3. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to Mesothelin. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to Folate Receptor alpha. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to Folate Receptor

beta. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to CLL-1. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to CLEC5A. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to NY-ESO/MHC class I complex. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to WT1/MHC class I complex. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to WT1/MHC class I complex. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to WT1/MHC class I complex. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to AFP/MHC class I complex. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the

CARs is specific to HPV16-E7/MHC class I complex. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to gp100/MHC class I complex. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to hTERT/MHC class I complex. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to MART1/MHC class I complex. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to HTLV1-Tax/MHC class I complex. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to PR1/MHC class I complex. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to HIV1-gag/MHC class I complex. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49,

backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to HIV1-envelop gp120. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to DLL3. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to PTK7. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to TROP2. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to LAMP1. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to Tim1. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to TCR gamma-delta. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-

specific domain of the CARs is specific to TCR beta1 constant chain. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to TCR beta2 constant chain. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to GCC. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to B7H4. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to LHR. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to TSHR. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to Tn-Muc1. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to

TSLPR. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to Tissue Factor. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to SSEA-4. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-33, wherein the antigen-specific domain of the CARs is specific to SLea. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to Muc1/MHC class I complex. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to Muc16. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to NYBR-1. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to IL13Ra2. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional

CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to IL11Ra. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to L1CAM. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to EpCAM1. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to gpNMB. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to GRP78. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to GPC3. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to GRPC5D. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1,

backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to GFRA4. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to FITC. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to CD79b. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to Lym1. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to Lym2. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 4 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to CLD18A2. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 4 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to CD43 epitope expressed on leukemia cells. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 4 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37,

backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to CD179a. In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs which are part of the conventional CARs 1 to 6 or are part of backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, wherein the antigen-specific domain of the CARs is specific to Fc portion of an antibody (*i.e.* Ig Fc). An exemplary CAR with the antigen-specific domain specific to Ig Fc is represented by SEQ ID NO: 1629 and contains the extracellular domain of CD16-V158 as the antigen specific domain. In any of the foregoing, the nucleic acid molecule encoding the CAR construct further comprise a NF- κ B activator coding sequence, or alternatively, a NF- κ B activator coding sequence can be present on a second nucleic acid molecule.

[00234] The nucleic acid sequences encoding for the desired components of the non-naturally occurring immune receptors, *e.g.*, CARs, and/or a selective NF- κ B activator coding sequence described herein can be obtained using recombinant methods known in the art, such as, for example by screening libraries from cells expressing the nucleic acid molecule, by deriving the nucleic acid molecule from a vector known to include the same, or by isolating directly from cells and tissues containing the same, using standard techniques. Alternatively, the nucleic acid of interest can be produced synthetically, rather than cloned.

[00235] In some embodiments, the nucleic acid molecule encoding the non-naturally occurring immune receptors, *e.g.*, CARs, and/or accessory molecules (*e.g.*, a NF- κ B activator sequence) described herein is provided as a messenger RNA (mRNA) transcript. In another embodiment, the nucleic acid molecule encoding the non-naturally occurring immune receptors, *e.g.*, CARs, and/or accessory molecules (*e.g.*, a selective NF- κ B activator coding sequence) described herein is provided as a DNA construct.

[00236] Cloning and expression methods will be apparent to a person of skill in the art and may be as described in WO 2015/142675; Sambrook *et al.*, 2012, MOLECULAR CLONING: A LABORATORY MANUAL, volumes 1 -4, Cold Spring Harbor Press, NY; June *et al.* 2009 Nature Reviews Immunology 9.10: 704-716; WO 01/96584; WO 01/29058; U.S. Pat. No. 6,326,193, the contents of each of which are herein incorporated by reference in their entirety as though set forth herein. Physical methods for introducing polynucleotides of into host cells such as calcium phosphate transfection and the like are well known in the art and will be apparent to a person of skill in the art. In exemplary embodiments, such methods are set forth in Sambrook *et al.*, 2012, MOLECULAR CLONING: A LABORATORY

MANUAL, volumes 1 -4, Cold Spring Harbor Press, NY); U.S. Pat. Nos. 5,350,674 and 5,585,362, the contents of each of which are herein incorporated by reference in their entirety as though set forth herein. In another embodiment, a CAR vector is transduced into a cell, *e.g.*, a T cell or a NK cell, by causing transient perturbations in cell membrane using a microfluid device as described in patent application WO 2013/059343 A1 (PCT/US2012/060646) and in Ding X et al, Nat. Biomed. Eng. 1, 0039 (2017) the contents of each of which are herein incorporated by reference in their entirety as though set forth herein.

[00237] The disclosure provides a recombinant nucleic acid construct comprising a nucleic acid molecule encoding a non-naturally occurring immune receptor, *e.g.*, CAR,, wherein the nucleic acid molecule comprises a nucleic acid sequence encoding one or more antigen binding domains, wherein the nucleotide sequences encoding each of the antigen binding domains are contiguous with and in the same reading frame as the nucleic acid sequences encoding a: (i) optional hinge/linker, (ii) transmembrane domain, and (iii) optional intracellular domain, or (a) a T cell receptor constant chain. An exemplary T cell receptor constant chain that can be used in the construction of SIR includes, but is not limited to, constant chain of TCR α , TCR β 1, TCR β 2, TCR γ , TCR δ , preTCR α and variants and mutants thereof. In some embodiments, a NF- κ B activator (*e.g.*, a selective NF- κ B activator) coding sequence is on the same recombinant nucleic acid construct but upon expression is not linked to the non-naturally occurring immune receptor, *e.g.*, CAR, but is rather cleaved off (*e.g.*, via a peptide cleavable linker) or is part of its own expression cassette in the polynucleotide.

[00238] The disclosure also provides a vector or vectors comprising a nucleic acid sequence or sequences encoding a non-naturally occurring immune receptor, *e.g.*, CAR, described herein and an accessory module. In some embodiments, the accessory module encodes a NF- κ B activator, *e.g.*, a selective NF- κ B activator. In some embodiment, the selective NF- κ B activator is a non-naturally occurring NF- κ B activator. In one embodiment, the non-naturally occurring immune receptor, *e.g.*, CAR, and the accessory module, *e.g.*, an accessory module encoding a NF- κ B activator, are encoded by a single vector. In another embodiment, the non-naturally occurring immune receptor, *e.g.*, CAR, and the accessory module, *e.g.*, an accessory module encoding a NF- κ B activator, are encoded by more than one vector. In yet another embodiment, a non-naturally occurring immune receptor, *e.g.*, CAR, and the accessory module, *e.g.*, an accessory module encoding a NF- κ B activator, are each encoded by a separate vector or by separate nucleic acids. In one embodiment, the two functional polypeptide units (*e.g.*, CAR and accessory module) are encoded by a single vector or a single nucleic acid. In one embodiment, the vector or the vectors are chosen from DNA

vector(s), RNA vector(s), plasmid(s), lentivirus vector(s), adenoviral vector(s), retrovirus vector(s), baculovirus vector(s), sleeping beauty transposon vector(s), or a piggyback transposon(s). In one embodiment, the vector is a lentivirus vector or a retroviral vector. In another embodiment, the vector is a sleeping beauty transposon vector. The nucleic acid sequences of exemplary vectors are provided in SEQ ID NO: 3840-3841. The vectors pLenti-EF1 α (SEQ ID NO: 3840) and pLenti-EF1 α -DWPRE (SEQ ID NO: 3841) are empty lentiviral vectors that differ by the fact that pLenti-EF1 α -DWPRE lacks the WPRE region. The nucleic acid sequence of pCCL3-MNDU3-WPRE vector is given in SEQ ID NO: 7779. A non-naturally occurring immune receptor coding sequence of the disclosure can be cloned between the Nhe I and Sal I sites in these vectors.

[00239] A retroviral vector may also be, *e.g.*, a gammaretroviral vector. A gammaretroviral vector may include, *e.g.*, a promoter, a packaging signal (ψ), a primer binding site (PBS), one or more (*e.g.*, two) long terminal repeats (LTR), and a transgene of interest, *e.g.*, a gene encoding a non-naturally occurring immune receptor, *e.g.*, CAR. A gammaretroviral vector may lack viral structural genes such as gag, pol, and env. Exemplary gammaretroviral vectors include Murine Leukemia Virus (MLV), Spleen-Focus Forming Virus (SFFV), and Myeloproliferative Sarcoma Virus (MPSV), and vectors derived therefrom. Other gammaretroviral vectors are described, *e.g.*, in Tobias Maetzig *et al.*, "Gammaretroviral Vectors: Biology, Technology and Application" *Viruses*. 2011 Jun; 3(6): 677-713. In another embodiment, the vector comprising the nucleic acid encoding the desired non-naturally occurring immune receptors of the disclosure is an adenoviral vector (A5/35).

[00240] In some embodiments, a vector of the disclosure can further comprise a promoter. Non-limiting examples of a promoter include, for example, a MNDU3 promoter, a CMV IE gene promoter, an EF-1 α promoter, an ubiquitin C promoter, a core-promoter or a phosphoglycerate kinase (PGK) promoter. In some embodiments, the promoter is an EF-1 promoter. In some embodiments, the vector comprises a poly(A) tail. In some embodiments, the vector comprises a 3'UTR.

[00241] The disclosure also includes an RNA construct that can be directly transfected into a cell. A method for generating mRNA for use in transfection involves *in vitro* transcription (IVT) of a template with specially designed primers, followed by poly A addition, to produce a construct containing 3' and 5' untranslated sequence ("UTR") (*e.g.*, a 3' and/or 5' UTR described herein), a 5' cap (*e.g.*, a 5' cap described herein) and/or Internal Ribosome Entry Site (IRES) (*e.g.*, an IRES described herein), the nucleic acid to be

expressed, and a poly A tail, typically 50-2000 bases in length (SEQ ID NO:3855). RNA so produced can efficiently transfect different kinds of cells. In one embodiment, the template includes sequences for the non-naturally occurring immune receptor, *e.g.*, CAR, and/or the NF- κ B stimulatory molecule. In one embodiment, an RNA CAR-NF κ B vector is transduced into a cell, *e.g.*, a T cell or a NK cell, by electroporation. In another embodiment, an RNA CAR vector and/or a NF- κ B activator vector is transduced into a cell, *e.g.*, a T cell or a NK cell, by causing transient perturbations in cell membrane using a microfluid device. The different chains (or functional polypeptide units) can be also introduced in a cell using one or more than one vector a combination of different vectors or techniques. In another embodiment, a non-naturally occurring immune receptor, *e.g.*, CAR, can be introduced using a retroviral vector while the accessory module encoding a NF- κ B activator is introduced using a lentiviral vector. In another aspect, a non-naturally occurring immune receptor, *e.g.*, CAR, is introduced using a lentiviral vector while the accessory module (*e.g.*, a NF- κ B activator) is introduced using a sleeping beauty transposon. In yet another aspect, a non-naturally occurring immune receptor, *e.g.*, CAR, is introduced using a lentiviral vector while the accessory module (*e.g.*, a NF- κ B activator) is introduced using a RNA transfection. In yet another aspect, a non-naturally occurring immune receptor, *e.g.*, CAR, is produced in a cell by genetic recombination at the endogenous TCR chain loci using gene targeting techniques known in the art while the accessory module is introduced using a lentiviral or a retroviral vector.

[00242] RNA can be introduced into target cells using any of a number of different methods, for instance, commercially available methods which include, but are not limited to, electroporation (Amaza Nucleofector-II (Amaza Biosystems, Cologne, Germany)), (ECM 830 (BTX) (Harvard Instruments, Boston, Mass.) or the Gene Pulser II (BioRad, Denver, Colo.), Multiporator (Eppendorf, Hamburg Germany), cationic liposome mediated transfection using lipofection, polymer encapsulation, peptide mediated transfection, or biolistic particle delivery systems such as "gene guns" (see, for example, Nishikawa, *et al.* Hum Gene Ther., 12(8):861-70 (2001) or by causing transient perturbations in cell membranes using a microfluidic device (see, for example, patent applications WO 2013/059343 A1 and PCT/US2012/060646).

[00243] In some embodiments, the non-viral method includes the use of a transposon (also called a transposable element). In some embodiments, a transposon is a piece of DNA that can insert itself at a location in a genome, for example, a piece of DNA that is capable of self-replicating and inserting its copy into a genome, or a piece of DNA that can be spliced

out of a longer nucleic acid and inserted into another place in a genome. For example, a transposon comprises a DNA sequence made up of inverted repeats flanking genes for transposition.

[00244] Exemplary methods of nucleic acid delivery using a transposon include a Sleeping Beauty transposon system (SBTS) and a piggyBac (PB) transposon system. See, *e.g.*, Aronovich *et al.* Hum. Mol. Genet. 20.R1(2011):R14-20; Singh *et al.* Cancer Res. 15(2008):2961-2971; Huang *et al.* Mol. Ther. 16(2008):580-589; Grabundzija *et al.* Mol. Ther. 18(2010):1200-1209; Kebriaei *et al.* Blood. 122.21(2013):166; Williams. Molecular Therapy 16.9(2008): 1515-16; Bell *et al.* Nat. Protoc. 2.12(2007):3153-65; and Ding *et al.* Cell. 122.3(2005):473-83, all of which are incorporated herein by reference.

[00245] The SBTS includes two components: 1) a transposon containing a transgene and 2) a source of transposase enzyme. The transposase can transpose the transposon from a carrier plasmid (or other donor DNA) to a target DNA, such as a host cell chromosome/genome. For example, the transposase binds to the carrier plasmid/donor DNA, cuts the transposon (including transgene(s)) out of the plasmid, and inserts it into the genome of the host cell. See, *e.g.*, Aronovich *et al. supra*.

[00246] Exemplary transposons include a pT2-based transposon. See, *e.g.*, Grabundzija *et al.* Nucleic Acids Res. 41.3(2013): 1829-47; and Singh *et al.* Cancer Res. 68.8(2008): 2961-2971, all of which are incorporated herein by reference. Exemplary transposases include a Tc 1/mariner-type transposase, *e.g.*, the SB 10 transposase or the SB 11 transposase (a hyperactive transposase which can be expressed, *e.g.*, from a cytomegalovirus promoter). See, *e.g.*, Aronovich *et al.*; Kebriaei *et al.*; and Grabundzija *et al.*, all of which are incorporated herein by reference.

[00247] Use of the SBTS permits efficient integration and expression of a transgene, *e.g.*, a nucleic acid encoding a CAR and/or a NF- κ B activator described herein. Provided herein are methods of generating a cell, *e.g.*, T cell or NKT cell or stem cell or iPSC or synthetic T cell, that stably expresses a CAR and/or a NF- κ B activator described herein, *e.g.*, using a transposon system such as SBTS.

[00248] In accordance with methods described herein, in some embodiments, one or more nucleic acids, *e.g.*, plasmids, containing the SBTS components are delivered to a cell (*e.g.*, T or NKT cell or stem cell or iPSC or synthetic T cell). For example, the nucleic acid(s) are delivered by standard methods of nucleic acid (*e.g.*, plasmid DNA) delivery, *e.g.*, methods described herein, *e.g.*, electroporation, transfection, or lipofection. In some embodiments, the nucleic acid contains a transposon comprising a transgene, *e.g.*, a nucleic

acid encoding a non-naturally occurring immune receptor, *e.g.*, CAR, and/or a NF- κ B activator described herein. In some embodiments, the nucleic acid contains a transposon comprising a transgene (*e.g.*, a nucleic acid encoding a non-naturally occurring immune receptor, *e.g.*, CAR, and/or a NF- κ B activator described herein) as well as a nucleic acid sequence encoding a transposase enzyme. In other embodiments, a system with two nucleic acids is provided, *e.g.*, a dual-plasmid system, *e.g.*, where a first plasmid contains a transposon comprising a transgene, and a second plasmid contains a nucleic acid sequence encoding a transposase enzyme. For example, the first and the second nucleic acids are codelivered into a host cell.

[00249] As described above and elsewhere herein, the disclosure demonstrates that co-expression of an immune receptor (*e.g.*, a CAR, an endogenous TCR or a recombinant TCR) of the disclosure with an NF- κ B stimulatory molecule (*e.g.*, a selective NF- κ B activator, *e.g.*, a non-naturally occurring NF- κ B activating agent, *e.g.*, hNEMO-K277A) improves the functions of immune cells such as survival, expansion, proliferation, activation, persistence, cytokine production and *in vivo* activity. In some embodiments, the immune receptor is a non-naturally occurring immune receptor (*e.g.*, CAR or recombinant TCR). In some embodiments, the immune receptor is a naturally occurring immune receptor (*e.g.*, a native TCR). In one embodiment, an NF- κ B stimulatory molecule is co-expressed with a first generation, second generation, third generation CAR, TFP, AbTCR, or SIR. As mentioned above, the NF- κ B stimulatory molecule can be, but preferably is not, linked to a CAR, TCR or SIR backbone. Moreover, in certain embodiments, a CAR of the disclosure does not include a CD28 or 41BB domain, and optionally includes a CD3 domain.

[00250] In one embodiment, the disclosure demonstrates that expression of a selective NF- κ B activator improves the functions of immune cells (*e.g.*, T cells, dendritic cells, CAR-T cells or TCR-T cells *etc.*) such as survival, expansion, proliferation, activation, persistence, cytokine production and *in vivo* activity. A selective NF- κ B activator as described herein, refers to an agent that activates the NF- κ B signaling pathway selectively with no or minimal activation of the other signaling pathways. In one embodiment, a selective NF- κ B activator activates NF- κ B signaling pathway with no or minimal activation of one or more of signaling pathways selected from the group of AKT, PI3K, JNK, p38 kinase, ERK, JAK/STAT and interferon signaling pathways. A number of methods to measure the activation of the NF- κ B, AKT, PI3K, JNK, p38 kinase, ERK, JAK/STAT and interferon signaling pathways are known in the art. These assays can be used in the methods of the disclosure either singly or in combinations to identify selective activators of NF- κ B pathway.

[00251] In one embodiment, a selective NF- κ B activator induces more than 20% (*e.g.*, more than 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%) increase in NF- κ B activity as measured by Phospho-NF- κ B p65 (Ser536) antibody (Cell Signaling Technology; Danvers, MA) but less than 20% increase in the activity of the AKT pathway as measured using Phospho-Akt (Ser473) antibody (Cell Signaling Technology; Danvers, MA) when exposed to or expressed in a test human T cell as compared to a control human T cell. In some embodiments, a selective NF- κ B activator induces more than 20% (*e.g.*, more than 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%) increase in NF- κ B activity as measured by Phospho-NF- κ B p65 (Ser536) antibody (Cell Signaling Technology; Danvers, MA) but less than 20% increase in the activity of the JNK pathway as measured using Phospho-SAPK/JNK (Thr183/Tyr185) antibody (*e.g.*, clone G9; Cell Signaling Technology; Danvers, MA) when exposed to or expressed in a test human T cell as compared to a control human T cell. In some embodiments, a selective NF- κ B activator induces more than 20% (*e.g.*, more than 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%) increase in NF- κ B activity as measured by Phospho-NF- κ B p65 (Ser536) antibody (Cell Signaling Technology; Danvers, MA) but less than 20% increase in the activity of the p38 kinase pathway as measured using Phospho-p38 MAPK (Thr180/Tyr182) antibody (*e.g.*, clone D3F9; Cell Signaling Technology; Danvers, MA) when exposed to or expressed in a test human T cell as compared to a control human T cell. In some embodiments, a selective NF- κ B activator induces more than 20% (*e.g.*, more than 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%) increase in NF- κ B activity as measured by Phospho-NF- κ B p65 (Ser536) antibody (Cell Signaling Technology; Danvers, MA) but less than 20% increase in the activity of the STAT pathway as measured using Phospho-Stat1 (Tyr701) antibody (*e.g.*, Clone D4A7; Cell Signaling Technology; Danvers, MA) when exposed to or expressed in a test human T cell as compared to a control human T cell. In some embodiments, a selective NF- κ B activator induces more than 20% (*e.g.*, more than 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%) increase in NF- κ B activity as measured by Phospho-NF- κ B p65 (Ser536) antibody (Cell Signaling Technology; Danvers, MA) but less than 20% increase in the activity of the STAT pathway as measured using Phospho-Stat2 (Tyr690) antibody (Cell Signaling Technology; Danvers, MA) when exposed to or expressed in a test human T cell as compared to a control human T cell. In some embodiments, a selective NF- κ B activator induces more than 20% (*e.g.*, more than 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%) increase in NF- κ B activity as measured by Phospho-NF- κ B p65 (Ser536) antibody (Cell Signaling Technology; Danvers, MA) but less than 20% increase in the activity of the STAT pathway as measured using Phospho-Stat3 (Tyr705) antibody (*e.g.*,

Clone D3A7, Cell Signaling Technology; Danvers, MA) when exposed to or expressed in a test human T cell as compared to a control human T cell. In some embodiments, a selective NF- κ B activator induces more than 20% (*e.g.*, more than 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%) increase in NF- κ B activity as measured by Phospho-NF- κ B p65 (Ser536) antibody (Cell Signaling Technology; Danvers, MA) but less than 20% increase in the activity of the STAT pathway as measured using Phospho-Stat5 (Tyr694) antibody (*e.g.*, Clone D47E7, Cell Signaling Technology; Danvers, MA) when exposed to or expressed in a test human T cell as compared to a control human T cell. In some embodiments, a selective NF- κ B activator induces more than 20% (*e.g.*, more than 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%) increase in NF- κ B activity as measured by Phospho-NF- κ B p65 (Ser536) antibody (Cell Signaling Technology; Danvers, MA) but less than 20% increase in the activity of the ERK pathway as measured using Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (*e.g.*, Clone D13.14.4E, Cell Signaling Technology; Danvers, MA) when exposed to or expressed in a test human T cell as compared to a control human T cell.

[00252] In one embodiment, a selective NF- κ B activator induces more than 20% (*e.g.*, more than 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%) increase in NF- κ B activity as measured by Phospho-I κ B α (Ser32) antibody (*e.g.*, 14D4, Clone Cell Signaling Technology; Danvers, MA) subunit but less than 20% increase in the activity of the AKT pathway as measured using Phospho-Akt (Ser473) antibody (Cell Signaling Technology; Danvers, MA) when exposed to or expressed in a test human T cell as compared to a control human T cell. In some embodiments, a selective NF- κ B activator induces more than 20% (*e.g.*, more than 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%) increase in NF- κ B activity as measured by Phospho-I κ B α (Ser32) antibody (*e.g.*, 14D4, Clone Cell Signaling Technology; Danvers, MA) but less than 20% increase in the activity of the JNK pathway as measured using Phospho-SAPK/JNK (Thr183/Tyr185) antibody (*e.g.*, clone G9; Cell Signaling Technology; Danvers, MA) when exposed to or expressed in a test human T cell as compared to a control human T cell. In some embodiments, a selective NF- κ B activator induces more than 20% (*e.g.*, more than 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%) increase in NF- κ B activity as measured by Phospho-I κ B α (Ser32) antibody (*e.g.*, 14D4, Clone Cell Signaling Technology; Danvers, MA) but less than 20% increase in the activity of the p38 kinase pathway as measured using Phospho-p38 MAPK (Thr180/Tyr182) antibody (*e.g.*, clone D3F9; Cell Signaling Technology; Danvers, MA) when exposed to or expressed in a test human T cell as compared to a control human T cell. In some embodiments, a selective NF- κ B activator induces more than 20% (*e.g.*, more than 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%)

increase in NF- κ B activity as measured by Phospho-I κ B α (Ser32) antibody (*e.g.*, 14D4, Clone Cell Signaling Technology; Danvers, MA) but less than 20% increase in the activity of the STAT pathway as measured using Phospho-Stat1 (Tyr701) antibody (*e.g.*, Clone D4A7; Cell Signaling Technology; Danvers, MA) when exposed to or expressed in a test human T cell as compared to a control human T cell. In some embodiments, a selective NF- κ B activator induces more than 20% (*e.g.*, more than 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%) increase in NF- κ B activity as measured by Phospho-I κ B α (Ser32) antibody (*e.g.*, 14D4, Clone Cell Signaling Technology; Danvers, MA) but less than 20% increase in the activity of the STAT pathway as measured using Phospho-Stat2 (Tyr690) antibody (Cell Signaling Technology; Danvers, MA) when exposed to or expressed in a test human T cell as compared to a control human T cell. In some embodiments, a selective NF- κ B activator induces more than 20% (*e.g.*, more than 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%) increase in NF- κ B activity as measured by Phospho-I κ B α (Ser32) antibody (*e.g.*, 14D4, Clone Cell Signaling Technology; Danvers, MA) but less than 20% increase in the activity of the STAT pathway as measured using Phospho-Stat3 (Tyr705) antibody (*e.g.*, Clone D3A7, Cell Signaling Technology; Danvers, MA) when exposed to or expressed in a test human T cell as compared to a control human T cell. In some embodiments, a selective NF- κ B activator induces more than 20% (*e.g.*, more than 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%) increase in NF- κ B activity as measured by Phospho-I κ B α (Ser32) antibody (*e.g.*, 14D4, Clone Cell Signaling Technology; Danvers, MA) but less than 20% increase in the activity of the STAT pathway as measured using Phospho-Stat5 (Tyr694) antibody (*e.g.*, Clone D47E7, Cell Signaling Technology; Danvers, MA) when exposed to or expressed in a test human T cell as compared to a control human T cell. In some embodiments, a selective NF- κ B activator induces more than 20% (*e.g.*, more than 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%) increase in NF- κ B activity as measured by Phospho-I κ B α (Ser32) antibody (*e.g.*, 14D4, Clone Cell Signaling Technology; Danvers, MA) but less than 20% increase in the activity of the ERK pathway as measured using Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (*e.g.*, Clone D13.14.4E, Cell Signaling Technology; Danvers, MA) when exposed to or expressed in a test human T cell as compared to a control human T cell.

[00253] Alternate methods of measuring the activation of the NF- κ B, AKT, JNK, p38, ERK, JAK/STAT and interferon signaling pathways are known in the art and can be used to identify selective activator of the NF- κ B signaling pathway. For example, a selective NF- κ B activator induces greater increase in the NF- κ B DNA binding activity when exposed to or expressed in a target cell (*e.g.*, a T cell or 293FT cell) as compared to increase in c-Jun, c-

Fos, JunD, ATF2, STAT3, NFAT1c, ELK-1, CREB, IRF3 or IRF7 DNA binding activities. Kits to measure DNA binding activities of different transcription factors belonging to different signaling pathways are available commercially (*e.g.*, TransAM® Transcription Factor Assays; Active Motif) and can be used to identify selective activator of the NF- κ B signaling pathway.

[00254] In an embodiment, a selective NF- κ B activator induces greater increase in the ratio of increase in I κ B α phosphorylation to increase in AKT phosphorylation as compared to CD28 when both of them are expressed in human T cells or when signaling through both is activated in human T cells under comparable conditions. In an embodiment, a selective NF- κ B activator induces greater fold increase in the ratio of increase in I κ B α phosphorylation to increase in AKT phosphorylation as compared to 41BB when both of them are expressed in human T cells under comparable conditions or when signaling through both is activated in human T cells under comparable conditions. In an embodiment, a selective NF- κ B activator when co-expressed with a 1st generation CAR lacking a costimulatory domain induces greater increase in the ratio of increase in I κ B α phosphorylation to increase in AKT phosphorylation as compared to a 2nd generation CAR containing a CD28 costimulatory domain when both of them are expressed in human T cells and exposed to target antigen containing cells under comparable conditions. In an embodiment, a selective NF- κ B activator when co-expressed with a 1st generation CAR lacking a costimulatory domain (*e.g.*, a CAR represented by SEQ ID NO: 1016) induces greater increase in the ratio of increase in I κ B α phosphorylation to increase in AKT phosphorylation as compared to a 2nd generation CAR containing a 41BB costimulatory domain (*e.g.*, a CAR represented by SEQ ID NO: 1318) when both of them are expressed in human T cells and exposed to target antigen containing cells (*e.g.*, RAJI) under comparable conditions. For example, T cells expressing the CD19-directed first generation CAR co-expressing K13 (CD8SP-FMC63-(vL-vH)-Myc-z-P2A-K13-Flag-T2A-PAC; SEQ ID NO: 1016) show greater increase in the ratio of I κ B α phosphorylation to AKT phosphorylation as compared to T cells expressing the CD19-directed 2nd generation CAR with 41BB costimulatory domain (CD8SP-FMC63-(vL-vH)-Myc-BBz-T2A-PAC; SEQ ID NO: 1318) when both the CAR-T cells are exposed to RAJI cells at E:T ratio of 1:5 for between 1-24 hours. The phosphorylation of I κ B α and AKT are calculated by using methods known in the art (*e.g.*, immunoblotting or Flow cytometry) using antibodies specific to their phosphorylated forms. The increase in I κ B α phosphorylation is calculated by subtracting the I κ B α phosphorylation in control T cells lacking the expression of CAR from the I κ B α phosphorylation in CAR-T cells after exposure to RAJI cells. The increase in AKT

phosphorylation is calculated by subtracting the AKT phosphorylation in control T cells lacking the expression of CAR from the AKT phosphorylation in CAR-T cells after exposure to RAJI cells. The ratio of increase in I κ B α to increase in AKT phosphorylation is calculated by dividing the increase in I κ B α phosphorylation from the increase in AKT phosphorylation.

[00255] In an embodiment, a selective NF- κ B activator induces greater increase in the ratio of increase in p65/RelA phosphorylation to increase in AKT phosphorylation as compared to CD28 when both of them are expressed in human T cells or when signaling through both is activated in human T cells under comparable conditions. In an embodiment, a selective NF- κ B activator induces greater fold increase in the ratio of increase in p65/RelA phosphorylation to increase in AKT phosphorylation as compared to 41BB when both of them are expressed in human T cells under comparable conditions or when signaling through both is activated in human T cells under comparable conditions. In an embodiment, a selective NF- κ B activator when co-expressed with a 1st generation CAR lacking a costimulatory domain induces greater increase in the ratio of increase in p65/RelA phosphorylation to increase in AKT phosphorylation as compared to a 2nd generation CAR containing a CD28 costimulatory domain when both of them are expressed in human T cells and exposed to target antigen containing cells under comparable conditions. In an embodiment, a selective NF- κ B activator when co-expressed with a 1st generation CAR lacking a costimulatory domain (*e.g.*, a CAR represented by SEQ ID NO: 1016) induces greater increase in the ratio of increase in p65/RelA phosphorylation to increase in AKT phosphorylation as compared to a 2nd generation CAR containing a 41BB costimulatory domain (*e.g.*, a CAR represented by SEQ ID NO: 1318) when both of them are expressed in human T cells and exposed to target antigen containing cells (*e.g.*, RAJI) under comparable conditions. For example, T cells expressing the CD19-directed first generation CAR co-expressing K13 (CD8SP-FMC63-(vL-vH)-Myc-z-P2A-K13-Flag-T2A-PAC; SEQ ID NO: 1016) show greater increase in the ratio of p65/RelA phosphorylation to AKT phosphorylation as compared to T cells expressing the CD19-directed 2nd generation CAR with 41BB costimulatory domain (CD8SP-FMC63-(vL-vH)-Myc-BBz-T2A-PAC; SEQ ID NO: 1318) when both the CAR-T cells are exposed to RAJI cells at an Effector:Target (E:T) ratio of 1:5 for between 1-24 hours (*e.g.*, 1 hour, 2 hours, 4 hours, 12 hours, or 24 hours). The phosphorylation of p65/RelA and AKT are calculated by using methods known in the art (*e.g.*, immunoblotting or Flow cytometry) using antibodies specific to their phosphorylated forms. The increase in p65/RelA phosphorylation is calculated by subtracting the p65/RelA phosphorylation in control T cells lacking the expression of CAR from the p65/RelA

phosphorylation in CAR-T cells after both are exposed to RAJI cells. The increase in AKT phosphorylation is calculated by subtracting the AKT phosphorylation in control T cells lacking the expression of CAR from the AKT phosphorylation in CAR-T cells after both are exposed to RAJI cells. The ratio of increase in p65/RelA to increase in AKT phosphorylation is calculated by dividing the increase in p65/RelA phosphorylation from the increase in AKT phosphorylation.

[00256] In an embodiment, a selective NF- κ B activator when co-expressed with a 1st generation CAR lacking a costimulatory domain (*e.g.*, a CAR represented by SEQ ID NO: 1016) induces greater increase in the ratio of increase in I κ B α phosphorylation to increase in JNK, ERK, or p38 kinase phosphorylation as compared to a 2nd generation CAR containing a 41BB costimulatory domain (*e.g.*, a CAR represented by SEQ ID NO: 1318) when both of them are expressed in human T cells and exposed to target antigen containing cells (*e.g.*, RAJI) for appropriate time interval (*e.g.*, 1-24 hours) under comparable conditions. In an embodiment, a selective NF- κ B activator when co-expressed with a 1st generation CAR lacking a costimulatory domain (*e.g.*, a CAR represented by SEQ ID NO: 1016) induces greater increase in the ratio of increase in p65/RelA phosphorylation to increase in JNK, ERK, or p38 kinase phosphorylation as compared to a 2nd generation CAR containing a 41BB costimulatory domain (*e.g.*, a CAR represented by SEQ ID NO: 1318) when both of them are expressed in human T cells and exposed to target antigen containing cells (*e.g.*, RAJI) for appropriate time interval (*e.g.*, 1-24 hours) under comparable conditions.

[00257] In one embodiment, a NF- κ B activator, including a selective NF- κ B activator, is a non-naturally occurring agent and is expressed in the cell exogenously. In one embodiment, the selective NF- κ B activator is of viral origin, *i.e.*, it is encoded by a virus or is derived from a virally encoded protein or has a domain of more than 10 amino acid residues (*e.g.*, more than 15 amino acid residues, 20 amino acid residues, 30 amino acid residues or 50 amino acid residues) with more than 80% (*e.g.*, more than 85%, 90%, 95%, or 99%) identity to one or more viral proteins. An exemplary selective NF- κ B activator of viral origin is vFLIP K13 (SEQ ID NO:) which is derived from Kaposi's sarcoma associated herpesvirus. In another embodiment, the selective NF- κ B activator is of mammalian or cellular origin. Exemplary selective NF- κ B activators of mammalian origin are human NEMO-K277A mutant, human NEMO-K277-deltaV249-K255 mutant, mouse NEMO-K270A mutant, IKK2-S177E-S181E and IKK1-S176E-S180E. In another embodiment, the selective NF- κ B activator is of human origin; *i.e.* it has a domain of more than 10 amino acid residues (*e.g.*, more than 15 amino acid residues, 20 amino acid residues, 30 amino acid residues or 50

amino acid residues) with more than 80% (*e.g.*, more than 85%, 90%, 95%, or 99%) identity to one or more human proteins. In some embodiments, a selective NF- κ B activator is composed of two or more fusion proteins (*e.g.*, FKBP $\times$ 2-NEMO). In some embodiments, the two or more fusion partners of a selective NF- κ B activator are each derived from human proteins or have more than 80% identity to the human proteins.

[00258] In some embodiments, the selective NF- κ B activator is encoded by the wild-type nucleic acid sequence while in other embodiments the selective NF- κ B activator is encoded by codon-optimized nucleic acid sequence or a mutant sequence. In an exemplary embodiment, vFLIP K13 is encoded by human codon optimized nucleic acid sequence, *e.g.*, K13-opt (SEQ ID NO: 7768).

[00259] In some embodiments, the immune cells express a single selective NF- κ B activator while in other embodiments the immune cells express more than one selective NF- κ B activator (*e.g.*, NEMO-K277A plus K13-opt or IKK2-S177E-S181E plus IKK1-S176E-S180E).

[00260] In some embodiments, the selective NF- κ B activator is expressed in an immune cell in a constitutive manner. In other embodiments, the selective NF- κ B activator is expressed in an immune cell in an inducible manner. In an exemplary embodiment, inducible expression of a selective NF- κ B activator can be achieved through the use of an inducible promoter. Examples of inducible promoters include, but are not limited to a metallothionine inducible promoter, a glucocorticoid inducible promoter, a progesterone inducible promoter, and a tetracycline inducible promoter. RheoSwitch[®] system represents another transcriptional regulator platform for controlling the expression of a protein.

[00261] Methods to control the activity of proteins are known in the art and can be used to control the activity of the NF- κ B activator, including selective NF- κ B activator. In an exemplary embodiment, this involves the expression in the target cell, such as a T cell or an NK cell, of a NEMO or a NEMO mutant fused to a dimerization domain or a switch domain. In an exemplary embodiment, the switch domain comprises of one or more copies of a FKBP12 domain or an FKBP12v36 domain. In some embodiments, the switch domain is attached to the carboxy-terminus of the NF- κ B activator (*e.g.*, NEMO) while in other embodiments the switch domain is attached to the amino-terminus of the NF- κ B activator (*e.g.*, NEMO). Exposure of target cells expressing such a fusion protein to a suitable dimerizer (*e.g.*, Rimiducid) results in oligomerization of NEMO, which in turn leads to NF- κ B activation. In an alternate embodiment, the activity of the selective NF- κ B activators can be also controlled by fusing them to the ligand binding domain of a mutated estrogen receptor

as has been described (Matta H *et al.*, Journal of Biological Chemistry, 282, 34, 2007). The mutated estrogen receptor does not bind to the physiological ligand estrogen but binds with very high affinity to the synthetic ligand 4-OHT (4-hydroxytamoxifen) and regulates the activity of the fusion partner (*e.g.*, NF- κ B activator, *e.g.*, vFLIP K13 or NEMO) in a 4-OHT-dependent fashion.

[00262] In some embodiments, the selective NF- κ B activator is expressed in the immune cells by alteration in its genomic copy using gene editing techniques known in the art. In an exemplary embodiment, a gene editing system (*e.g.*, TALON, Zn finger nuclease or CRISP/Cas9) is used to convert one or both alleles of human NEMO to human NEMO-K277A mutant form. In another exemplary embodiment, a gene editing system is used to convert one or both alleles of human NEMO to human NEMO-K277A-delta-V249-K255 mutant form. The sequence of human NEMO gene targeting constructs that can be used to induce K277A and K277A-delta-V249-K255 mutations are provided in SEQ ID NO: 7771 and 7772, respectively. These sequences can be cloned in a suitable vector (*e.g.*, integration defective lentiviral vector, AAV vector or adenoviral vector). Examples of genomic target sequences for human NEMO for which CRISP/Cas9 gRNAs comprising complementary targeting sequences can be generated are provided in SEQ ID NO: 7759-7762. The gRNA sequences are cloned into the pX330-U6-Chimeric_BB-CBh-hSpCas9 vector (Addgene). Alternatively, the gRNA sequences can be cloned in the pLenti-CRISPR-v2 vector available from Addgene (Plasmid #52961) and following the instructions provided by the distributor. Introduction of the NEMO targeting construct and gRNA encoding constructs into the T cells is carried out essentially as described previously (Knipping F *et al.*, Molecular Therapy: Methods & Clinical Development, Vol 4, 2017).

[00263] In another or further embodiment of any of the foregoing embodiments described herein, the immune effector cells that express an accessory module encoding a selective NF- κ B activator (*e.g.*, hNEMO-K277A, hNEMO-K277A-deltaV249-K555, mNEMO-K270A, K13-opt, IKK2-S177E-S181E, or IKK1-S176E-S180E) show improved *in vitro* activity (*e.g.* target antigen induced IL2 production, proliferation, expansion, and delay in terminal differentiation, delay in senescence *etc.*) against a target antigen expressing cell as compared to a corresponding immune effector cell lacking the accessory module when compared under similar conditions. NF- κ B activation in the immune effector cells is measured by using techniques known in the art including, but not limited to, measurement of phosphorylated I κ B α , phosphorylated p65, total I κ B α , p65 nuclear translocation, upregulation of NF- κ B responsive genes, electrophoretic mobility shift assay (EMSA) and NF- κ B-based

reporter assay *etc.* In some embodiments, selective NF- κ B activation is determined by measuring fold increase in activation of NF- κ B in the immune effector cells over the fold increase in activation of AKT pathway. In some embodiments, immune effector cells that express an accessory module encoding a selective NF- κ B activator (*e.g.*, K13-opt (human codon optimized K13) or hNEMO-K277A) show higher *in vitro* activity (*e.g.* target antigen induced IL2 production, proliferation, expansion, and delay in terminal differentiation, and delay in senescence) towards target antigen expressing cells as compared to the corresponding immune effector cells that lack the expression of an accessory module encoding a selective NF- κ B activator (*e.g.*, K13-opt or hNEMO-K277A) when both are tested under similar experimental conditions. In an exemplary embodiment, CD19-CAR-expressing immune effector cells that express an accessory module encoding a selective NF- κ B activator (*e.g.*, K13-opt (human codon optimized K13) or hNEMO-K277A) show higher *in vitro* activity (*e.g.* target antigen induced IL2 production, proliferation, expansion, and delay in terminal differentiation, and delay in senescence) towards Nalm6 cells as compared to the corresponding CD19-CAR-expressing effector cells that do not express an accessory module encoding a selective NF- κ B activator (*e.g.*, K13-opt or hNEMO-K277A) when both are tested under similar experimental conditions. In some embodiments, the *in vitro* activity (*e.g.* target antigen induced IL2 production, proliferation, expansion, and delay in terminal differentiation, and delay in senescence) of the immune effector cells that express an accessory module encoding a selective NF- κ B activator against the target antigen-expressing cells (*i.e.* target cells) is at least 5%, 10%, 20%, 30%, 40%, 50% or 100% more than the *in vitro* activity of a corresponding immune effector cells that do not express an accessory module encoding a selective NF- κ B activator. In some embodiments, the *in vitro* activity (*e.g.* target antigen induced IL2 production, proliferation, expansion, and delay in terminal differentiation, and delay in senescence) of the immune effector cells that express a selective NF- κ B activator (*e.g.*, hNEMO-K277A) against the target antigen-expressing cells (*i.e.* target cells) is at least 1.25-fold, 1.5-fold, 2-fold, 5-fold or 10-fold more than the *in vitro* activity of a corresponding immune effector cells that lack the expression of the selective NF- κ B activator. In an embodiment, the immune effector T cells (*e.g.*, CD19-CAR-T cells) that express a selective NF- κ B activator produce at least 5%, 10%, 20%, 30%, 40%, 50% or 100% more IL2 when exposed to a target antigen expressing cell (*e.g.*, Nalm-6 cells) as compared to the control immune effector T cells (*e.g.*, CD19-CAR-T cells) that lack the expression of the selective NF- κ B activator. In an embodiment, the immune effector T cells (*e.g.*, CD19-CAR-T cells) that express a selective NF- κ B activator show at least 5%, 10%,

20%, 30%, 40%, 50% or 100% more proliferation when exposed to a target antigen expressing cell (*e.g.*, Nalm-6 cells) as compared to the control immune effector T cells (*e.g.*, CAR-T cells) that lack the expression of the selective NF- κ B activator. In an embodiment, the immune effector T cells (*e.g.*, CD19-CAR-T cells) that express a selective NF- κ B activator show at least 5%, 10%, 20%, 30%, 40%, 50% or 100% less markers of exhaustion when exposed to a target antigen expressing cell (*e.g.*, Nalm-6 cells) as compared to the control immune effector T cells (*e.g.*, CAR-T cells) that lack the expression of the selective NF- κ B activator. In an embodiment, the immune effector T cells (*e.g.*, CD19-CAR-T cells) that express a selective NF- κ B activator show at least 5%, 10%, 20%, 30%, 40%, 50% or 100% less markers of terminal differentiation when exposed to a target antigen expressing cell (*e.g.*, Nalm-6 cells) as compared to the control immune effector T cells (*e.g.*, CAR-T cells) that lack the expression of the selective NF- κ B activator. In an embodiment, the immune effector T cells (*e.g.*, CD19-CAR-T cells) that express a selective NF- κ B activator show at least 5%, 10%, 20%, 30%, 40%, 50% or 100% more cytotoxicity when serially exposed to target antigen expressing cells (*e.g.*, Nalm-6 cells) over a period of 3-4 weeks as compared to the control immune effector T cells (*e.g.*, CAR-T cells) that lack the expression of the selective NF- κ B activator. In some embodiments, the immune effector cell that express an accessory module encoding a selective NF- κ B activator is a T cell (*e.g.*, a CD8 T cell, a CD4 T cell, a CAR-T cell, a TIL, a TREG cell, an NKT cell), a NK cell (*e.g.*, a CAR-NK cell), a macrophage (*e.g.*, a CAR-expressing macrophage), an antigen presenting cell (*e.g.*, a dendritic cell), a stem cell, an induced pluripotent stem cell (iPSC) or a stem cell that can give rise to an immune effector cell.

[00264] In another or further embodiment of any of the foregoing embodiments described herein, the immune effector cells that express an accessory module encoding a selective NF- κ B activator, show higher *in vivo* activity (*e.g.* *in vivo* expansion, *in vivo* persistence, tumor reduction, reduction in bioluminescence value obtained from a luciferase expressing tumor or animal survival) against a target antigen expressing cell as compared to control immune effector cells that do not express the accessory module encoding a selective NF- κ B activator when both are tested under similar conditions. NF- κ B activation in the immune effector cells is measured by using techniques known in the art including, but not limited to, measurement of phosphorylated I κ B α , total I κ B α , p65 nuclear translocation, upregulation of NF- κ B responsive genes, electrophoretic mobility shift assay (EMSA) and NF- κ B-based reporter assay *etc.* In some embodiments, selective NF- κ B activation is determined by measuring fold increase in activation of NF- κ B in the immune effector cells

over the fold increase in activation of AKT pathway. For example, in some embodiments, CD19-CAR-expressing immune effector cells that express an accessory module encoding a selective NF- κ B activator (*e.g.*, K13-opt (human codon optimized K13) or hNEMO-K277A) show higher *in vivo* activity (*e.g.* *in vivo* expansion, *in vivo* persistence, tumor reduction, reduction in bioluminescence value obtained from a FLuc expressing tumor or animal survival) towards Nalm6-FLuc cells in an NSG mouse xenograft model as compared to the corresponding CD19-CAR-expressing effector cells that lack an accessory module encoding a selective NF- κ B activator (*e.g.*, K13-opt (human codon optimized K13) or hNEMO-K277A) when tested under similar experimental conditions. In some embodiments, the *in vivo* activity (*e.g.* *in vivo* expansion, *in vivo* persistence, tumor reduction, reduction in bioluminescence value obtained from a FLuc expressing tumor or animal survival) of the immune effector cells (*e.g.*, CD19-CAR-T cells) that express an accessory module encoding a selective NF- κ B activator against the target antigen-expressing cells (*e.g.*, Nalm-6) in a NSG mouse xenograft model is at least 5, 10, 20, 30, 40, 50% or 100% more than the *in vivo* activity of a corresponding immune effector cells that lack an accessory module encoding a selective NF- κ B activator. In some embodiments, the *in vivo* activity (*e.g.* *in vivo* expansion, *in vivo* persistence, tumor reduction, reduction in bioluminescence value obtained from a FLuc expressing tumor or animal survival) of the immune effector cells (*e.g.*, CD19-CAR-T cells) that encode an accessory module encoding a selective NF- κ B activator against the target antigen-expressing cells (*e.g.*, Nalm6) in a NSG mouse xenograft model is at least 1.25-fold, 1.5-fold, 2-fold, 5-fold or 10-fold more than the *in vivo* activity of a corresponding immune effector cells that lack the expression of an accessory module encoding a selective NF- κ B activator. In some embodiments, the immune effector cell expressing an accessory module encoding a selective NF- κ B activator is a T cell (*e.g.*, a CD8 T cell, a CD4 T cell, a CAR-T cell, a TIL, a TREG cell, an NKT cell), a NK cell (*e.g.*, a CAR-NK cell), a macrophage (*e.g.*, a CAR-expressing macrophage), an antigen presenting cell (*e.g.*, a dendritic cell), a stem cell, an induced pluripotent stem cell (iPSC) or a stem cell that can give rise to an immune effector cell.

[00265] In another or further embodiment of any of the foregoing embodiments described herein, the immune effector cells expressing the accessory module, *e.g.*, hNEMO-K277A, hNEMO-K277A-deltaV249-K555, mNEMO-K270A, K13-opt, IKK2-S177E-S181E, IKK1-S176E-S180E, or MYD88-L265P show higher *in vivo* activity (*e.g.* *in vivo* expansion, *in vivo* persistence, tumor reduction, reduction in bioluminescence value obtained from a FLuc expressing tumor or animal survival) against a target antigen expressing cell as

compared to a corresponding immune effector cell lacking the accessory module when compared under similar conditions. For example, in some embodiments, CD19-CAR-expressing immune effector cells that also co-expresses hNEMO-K277A, hNEMO-K277A-deltaV249-K555, mNEMO-K270A, K13-opt, IKK2-S177E-S181E, IKK1-S176E-S180E, or MYD88-L265P show higher *in vivo* activity (*e.g.* *in vivo* expansion, *in vivo* persistence, tumor reduction, reduction in bioluminescence value obtained from a FLuc expressing tumor or animal survival) towards Nalm6-FLuc cells in an NSG mouse xenograft model as compared to the corresponding CD19-CAR-expressing effector cells that lack hNEMO-K277A expression when tested under similar experimental conditions. In some embodiments, the *in vivo* activity (*e.g.* *in vivo* expansion, *in vivo* persistence, tumor reduction, reduction in bioluminescence value obtained from a FLuc expressing tumor or animal survival) of the immune effector cells expressing the accessory module described herein (*e.g.*, hNEMO-K277A, hNEMO-K277A-deltaV249-K555, mNEMO-K270A, K13-opt, IKK2-S177E-S181E, IKK1-S176E-S180E, or MYD88-L265P) against the target antigen-expressing cells (*i.e.* target cells) in a NSG mouse xenograft model is at least 5, 10, 20, 30, 40, 50% or 100% more than the *in vivo* activity of a corresponding immune effector cell that lacks the expression of the accessory module. In some embodiments, the *in vivo* activity (*e.g.* *in vivo* expansion, *in vivo* persistence, tumor reduction, reduction in bioluminescence value obtained from a FLuc expressing tumor or animal survival) of the immune effector cells expressing the accessory module described herein (*e.g.*, hNEMO-K277A, hNEMO-K277A-deltaV249-K555, mNEMO-K270A, K13-opt, IKK2-S177E-S181E, IKK1-S176E-S180E, or MYD88-L265P) against the target antigen-expressing cells (*i.e.* target cells) in a NSG mouse xenograft model is at least 1.25-fold, 1.5-fold, 2-fold, 5-fold or 10-fold more than the *in vivo* activity of a corresponding immune effector cell that lacks the expression of the accessory module. In some embodiments, the accessory module-expressing effector cell is a T cell (*e.g.*, a CD8 T cell, a CD4 T cell, a CAR-T cell, a TIL, a TREG cell, an NKT cell), a NK cell (*e.g.*, a CAR-NK cell), a macrophage (*e.g.*, a CAR-expressing macrophage), an antigen presenting cell (*e.g.*, a dendritic cell), an induced pluripotent stem cell (iPSC) or a stem cell that can give rise to an immune effector cell.

[00266] The disclosure further provides that expression of a selective NF- κ B activator can be used to improve the cytokine secretion, antigen presentation and immune response generated by antigen presenting cells, including dendritic cells. The disclosure further provides a method of improving the efficacy of vaccine, including cancer vaccines, by expression of a selective NF- κ B activator in the antigen presenting cells *ex vivo* or *in vivo*. In

one embodiment, the use of selective NF- κ B activators increase cytokine production (*e.g.*, TNF α) by antigen presenting cells (*e.g.*, dendritic cells) by more than at least 15%.

[00267] The disclosure further provides that an accessory module encoding CMV-141 (SEQ ID NO: 7770) can be expressed in the immune effector cells, *e.g.*, T cells, *e.g.*, CAR-T cells or TCR-T cells, to delay their exhaustion and improve their long term persistence. The CMV-141 can be expressed in immune effector cells in an inducible or constitutive manner.

[00268] In some embodiments, cells, *e.g.*, T or NKT or stem cells or iPSC or synthetic T cell, are generated that express a non-naturally occurring immune receptor, *e.g.*, CAR, and/or an NF- κ B stimulatory molecule described herein by using a combination of gene insertion using the SBTS and genetic editing using a nuclease (*e.g.*, Zinc finger nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), the CRISPR/Cas system, or engineered meganuclease reengineered homing endonucleases).

[00269] In another embodiment, the disclosure provides a method of making a cell (*e.g.*, an immune effector cell or population thereof) comprising introducing into (*e.g.*, transducing) a cell, *e.g.*, a T cell, a NKT cell or a stem cell or a iPSC or a synthetic T cell described herein, with a vector comprising a nucleic acid encoding a non-naturally occurring immune receptor, *e.g.*, CAR, and/or an NF- κ B stimulatory molecule.

[00270] In various embodiments, the cells for modifications with a non-natural immune receptor and/or NF- κ B stimulatory molecule described herein, including T cells or NK cells may be obtained from a subject desiring therapy. T cells can be obtained from a number of sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. T cells could be tissue resident gamma-delta T cells, which can be cultured and expanded *in vitro* prior to expression of the non-naturally occurring immune receptor, *e.g.*, CAR, and/or NF- κ B stimulatory molecule.

[00271] In one embodiment, the disclosure provides a number of chimeric antigen receptors (CAR) comprising an antigen binding domain (*e.g.*, antibody or antibody fragment, TCR or TCR fragment) engineered for specific binding to a disease-associated antigen, *e.g.*, a tumor antigen described herein. In one embodiment, the disclosure provides an immune effector cell (*e.g.*, T cell, NK cell) engineered to express a non-naturally occurring immune receptor (*e.g.*, a CAR or a recombinant TCR) and/or NF- κ B stimulatory molecule, wherein the engineered immune effector cell exhibits a therapeutic property. In one embodiment, the disclosure provides an immune effector cell (*e.g.*, T cell, NK cell) engineered to express a non-naturally occurring immune receptor (*e.g.*, a CAR or a recombinant TCR) and/or NF- κ B

stimulatory molecule, wherein the engineered immune effector cell exhibits anticancer or anti-infection (*e.g.*, anti-HIV-1) property. In some embodiments, the NF- κ B stimulatory molecule may be expressed in a T cell (*e.g.* a Tumor infiltrating lymphocyte or TIL) with its endogenous TCR, wherein the the engineered immune effector cell exhibits anticancer or anti-infection (*e.g.*, anti-HIV-1) property. In one embodiment, a cell is transformed with the non-naturally occurring immune receptor (*e.g.*, a CAR or a recombinant TCR) and an NF- κ B stimulatory molecule and the non-naturally occurring immune receptor is expressed on the cell surface. In some embodiments, the cell (*e.g.*, T cell, NK cell) is transduced with a viral vector encoding a non-naturally occurring immune receptor (*e.g.*, a CAR or a recombinant TCR) and/or NF- κ B stimulatory molecule. In some embodiments, the viral vector is a retroviral vector. In some embodiments, the viral vector is a lentiviral vector. In some such embodiments, the cell may stably express the non-naturally occurring immune receptor (*e.g.*, a CAR or a recombinant TCR) and/or NF- κ B stimulatory molecule. In another embodiment, the cell (*e.g.*, T cell, NK cell) is transfected with a nucleic acid, *e.g.*, mRNA, cDNA, DNA, encoding a non-naturally occurring immune receptor (*e.g.*, a CAR or a recombinant TCR) and/or NF- κ B stimulatory molecule. In some such embodiments, the cell may transiently express the non-naturally occurring immune receptor (*e.g.*, a CAR or a recombinant TCR) and/or NF- κ B stimulatory molecule. In some embodiments, the NF- κ B stimulatory molecule may be expressed in a T cell (*e.g.* a Tumor infiltrating lymphocyte or TIL) with its endogenous TCR.

[00272] The disclosure provides immune effector cells (*e.g.*, T cells, NK cells) that are engineered to contain one or more non-naturally occurring immune receptors (*e.g.*, CARs/TCRs) and/or NF- κ B stimulatory molecules that direct the immune effector cells to diseased cells or disease-associated cells, such as cancer cells. This is achieved through an antigen binding domain on the immune receptor that is specific for a cancer associated antigen. There are two classes of cancer associated antigens (tumor antigens) that can be targeted by the CARs of the disclosure: (1) cancer associated antigens that are expressed on the surface of cancer cells; and (2) cancer associated antigens that itself is intracellular, however, a fragment of such antigen (peptide) is presented on the surface of the cancer cells by MHC (major histocompatibility complex). The disclosure also provides immune effector cells (*e.g.*, T cells, NK cells) that contain endogenous TCRs and/or engineered to express one or more NF- κ B stimulatory molecules that direct the immune effector cells to diseased cells or disease-associated cells, such as cancer cells.

[00273] Furthermore, the disclosure provides CARs, TCRs and CAR/TCR-expressing cells that also express an NF- κ B stimulatory molecule and their use in medicaments or methods for treating, among other diseases, cancer or any malignancy or autoimmune diseases or infectious disease or degenerative disease or allergic disease involving cells or tissues which express a tumor antigen or disease associated antigen as described herein.

[00274] In one embodiment, the disclosure provides an immune effector cell (*e.g.*, T cell, NK cell) engineered to express a non-naturally occurring immune receptor, *e.g.*, CAR and/or TCR, and an NF- κ B stimulatory molecule, wherein the engineered immune effector cell exhibits an anti-disease property, such as antitumor property. One type of antigen is a cancer associated antigen (*i.e.*, tumor antigen) described herein. In one aspect, the antigen binding domain of the non-naturally occurring immune receptor, *e.g.*, CAR, comprises a partially humanized antibody fragment. In one embodiment, the antigen binding domain of the non-naturally occurring immune receptor, *e.g.*, CAR, comprises a partially humanized scFv. Accordingly, the disclosure provides non-naturally occurring immune receptors, *e.g.*, CARs, that comprises a humanized antigen binding domain and is engineered into a cell, *e.g.*, a T cell or a NK cell, wherein the cell also expresses an NF- κ B stimulatory molecule and methods of their use for adoptive therapy.

[00275] In one embodiment, the disclosure provides an immune effector cell (*e.g.*, T cell, NK cell) with its endogenous immune receptor (*e.g.* a TCR) that is engineered to express an NF- κ B stimulatory molecule, wherein the engineered immune effector cell exhibits an anti-disease property, such as antitumor property or anti-HIV-1 property.

[00276] Further provided herein are genetically engineered cells, comprising the polynucleotides and/or the non-naturally occurring immune receptors described herein. In some embodiments, the cell is a T-lymphocyte (T-cell). In some embodiment the cell is a naïve T cells, a central memory T cells, an effector memory T cell, a regulatory T cell (Treg) or a combination thereof. In some embodiments, the cell is a natural killer (NK) cell, a hematopoietic stem cell (HSC), an embryonic stem cell, or a pluripotent stem cell. Genetically engineered cells which may comprise and express the non-naturally occurring immune receptors (*e.g.*, CARs and/or TCRs) of the disclosure in combination with an NF- κ B stimulatory molecule, include, but are not limited to, T-lymphocytes (T-cells), naïve T cells (TN), memory T cells (for example, central memory T cells (TCM), effector memory cells (TEM)), natural killer cells, hematopoietic stem cells and/or pluripotent embryonic/induced stem cells capable of giving rise to therapeutically relevant progeny. In an embodiment, the genetically engineered cells are autologous cells. In an embodiment, the genetically

engineered cells are allogeneic cells. By way of example, individual T-cells of the invention may be CD4⁺/CD8⁻, CD4⁻/CD8⁺, CD4⁻/CD8⁻ or CD4⁺/CD8⁺. The T-cells may be a mixed population of CD4⁺/CD8⁻ and CD4⁻/CD8⁺ cells or a population of a single clone. CD4⁺ T-cells of the invention may produce IL-2, IFN γ , TNF α and other T-cell effector cytokines when co-cultured *in vitro* with cells expressing the target antigens (for example CD20⁺ and/or CD19⁺ tumor cells). CD8⁺ T-cells of the invention may lyse antigen-specific target cells when co-cultured *in vitro* with the target cells. In some embodiments, T cells may be any one or more of CD45RA⁺ CD62L⁺ naïve cells, CD45RO⁺ CD62L⁺ central memory cells, CD62L⁻ effector memory cells or a combination thereof (Berger *et al.*, Adoptive transfer of virus-specific and tumor-specific T cell immunity. *Curr Opin Immunol* 2009 21(2)224-232). Genetically modified cells may be produced by stably transfecting cells with DNA encoding the non-naturally occurring immune receptors (*e.g.*, CARs and/or TCRs) and/or NF- κ B stimulatory molecule of the disclosure. The transfected cells demonstrating presence of a single integrated un-rearranged vector and expression of the non-naturally occurring immune receptors (*e.g.*, CARs and/or TCRs) and/or NF- κ B stimulatory molecule may be expanded *ex vivo*. In one embodiment, the cells selected for *ex vivo* expansion are CD8⁺ and demonstrates the capacity to specifically recognize and lyse antigen-specific target cells.

[00277] Stimulation of the T-cells by an antigen under proper conditions results in proliferation (expansion) of the cells and/or production of IL-2. The cells comprising the non-naturally occurring immune receptors (*e.g.*, CARs and/or TCRs) and/or NF- κ B stimulatory molecule of the disclosure will expand in number in response to the binding of one or more antigens to the antigen-specific targeting regions of the non-naturally occurring immune receptors (*e.g.*, CARs and/or TCRs). The disclosure also provides a method of making and expanding cells expressing a non-naturally occurring immune receptor (*e.g.*, CAR and/or TCR). The method comprises transfecting or transducing the cells with the vector(s) expressing the non-naturally occurring immune receptor (*e.g.*, CAR and/or TCR) and/or NF- κ B stimulatory molecule and stimulating the cells with cells expressing the target antigens, recombinant target antigens, or an antibody to the receptor to cause the cells to proliferate, so as to make and expand T-cells. In an embodiment, the cells may be any one or more of T-lymphocytes (T-cells), natural killer (NK) cells, hematopoietic stem cells (HSCs) or pluripotent embryonic/induced stem cells capable of giving rise to therapeutically relevant progeny. In an embodiment, the NF- κ B stimulatory molecule can be expressed in the cells

(*e.g.*, T cells, NK cells, or stem cell that can give rise to immune cells) without the introduction of the non-naturally occurring immune receptors (*e.g.*, CARs and/or TCRs).

[00278] Immune effector cells such as T cells and NK cells comprising non-naturally occurring immune receptors (*e.g.*, CARs and/or TCRs) and/or NF- κ B stimulatory molecule as described herein may 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; 6,867,041; and U.S. Patent Application Publication No. 20060121005.

[00279] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding conventional CARs 1 to 6 or conventional CARs 1 to 6 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-13, backbone-14, backbone-37, backbone-38, backbone-49, backbone-50, backbone-60 or backbone-61, and an NF- κ B stimulatory molecule wherein the antigen-specific domain of the CARs is specific to MPL, CD19, CD20, BCMA, CD22, CD30, CD33, CD123, CD138, CLL1, TCR-beta 1 constant chain, TCR-beta2 constant chain, TCRgamma/delta, mesothelin, IL13Ra2, ALK, PTK7, DLL3, TROP2, Tim1, LAMP1, CS1, Lym1, Lym2, TSHR, NY-ESO-1/MHC class I complex, WT1/MHC class I complex, Ras/ MHC class I complex, AFP/MHC class I complex, HPV-E6/MHC class I complex, HPV-E7/MHC class I complex, CD179a, CLD18A2, CD43 epitope, or HIV1 env protein gp120.

[00280] In one embodiment, the cell is a T cell and the T cell is deficient in one or more of endogenous T cell receptor chains. T cells stably lacking expression of a functional TCR according to the disclosure may be produced using a variety of approaches such as use of Zn finger nucleases (ZFN), CRISP/Cas9 and shRNA targeting the endogenous T cell receptor chains. A non-limiting exemplary method relating to shRNAs is described in US 2012/0321667A1, which is incorporated herein by reference. Another non-limiting exemplary method relating to eliminating endogenous TCR expression using ZFNs targeting constant regions of α and β chains of TCRs is described in Torikai H *et al.* (Blood, 119(24), June, 14 2012).

[00281] A T cell lacking a functional endogenous TCR can be, *e.g.*, engineered such that it does not express any functional endogenous TCR on its surface, engineered such that it does not express one or more subunits (*e.g.* constant chains of endogenous TCR α , TCR β 1, TCR β 2, TCR γ , TCR δ or pre-TCR α) that comprise a functional endogenous TCR or engineered such that it produces very little functional endogenous TCR on its surface. Alternatively, the T cell can express a substantially impaired endogenous TCR, *e.g.*, by

expression of mutated or truncated forms of one or more of the subunits of the TCR. The term "substantially impaired TCR" means that this TCR will not elicit an adverse immune reaction in a host. In yet a further alternative a non-naturally occurring immune receptor (*e.g.*, CAR and/or TCR) and/or NF- κ B stimulatory molecule can be cloned into a TCR loci in at T cell genome and thus the non-naturally occurring immune receptors (*e.g.*, CARs and/or TCRs) and/or NF- κ B stimulatory molecule would be under the control of the endogenous T cell expression system.

[00282] The disclosure demonstrates that in contrast to the situation with the 1st or the 2nd generation CAR constructs, TFPs based on CD3 ϵ , CD3 γ , and CD3 δ chains (designated as CD3 $\epsilon/\gamma/\delta$ TFPs) have poor expression and activity when expressed in $\alpha\beta$ T cells that lack or have impaired functional endogenous or native TCR α chain polypeptide on their surface. For example, it is observed that CD3 $\epsilon/\gamma/\delta$ TFPs have impaired expression and activity (*e.g.*, T cell activation, proliferation, cytokine production and cytotoxicity *etc.*) in $\alpha\beta$ T cell in which the endogenous TRAC genomic locus has been disrupted by the TFP expression cassette. The disclosure provides a solution to this problem by re-expressing a TCR α constant chain (TRAC chain) polypeptide or a fragment thereof in T cells in which the expression of native full length TCR α chain polypeptide has been reduced or eliminated. In an embodiment, the re-expressed TCR α constant chain polypeptide or TCR α constant chain fragment allows the reconstitution of a functional CD3 $\epsilon/\gamma/\delta$ TFP-TCR-CD3 signaling complex in a $\alpha\beta$ T cell in which the expression of the native TCR α constant chain is impaired, reduced or eliminated. In an embodiment, the re-expressed TCR α constant chain or TCR α constant chain fragment improves by more than 15% (*e.g.*, more than 20%, 50%, 75%, or 100% *etc.*) target antigen-induced cytokine (*e.g.*, IL2, TNF α , IFN γ) production, proliferation and/or cytotoxic activity of CD3 $\epsilon/\gamma/\delta$ TFP-expressing $\alpha\beta$ T cell in which the expression of the native TCR α constant chain is impaired, reduced or eliminated. In an embodiment, the re-expressed TCR α constant chain or TCR α constant chain fragment allows enhanced expression of the CD3 $\epsilon/\gamma/\delta$ TFP in a $\alpha\beta$ T cell in which the expression of the native TCR α constant chain is impaired, reduced or eliminated. In an embodiment, the re-expressed TCR α constant chain or TCR α constant chain fragment allows more than 15% (*e.g.*, more than 20%, 50%, 75%, or 100% *etc.*) increase in expression of the CD3 $\epsilon/\gamma/\delta$ TFP in a $\alpha\beta$ T cell in which the expression of the native TCR α constant chain is impaired, reduced or eliminated. In an exemplary embodiment, the expression and signaling activity of CD3 $\epsilon/\gamma/\delta$ TFPs can be restored in $\alpha\beta$ T cells in which the expression of native TCR α chain is reduced or eliminated by introducing into such T cells a nucleic acid construct encoding an exogenous TCR α constant chain (TRAC chain) (*e.g.*, SEQ

ID NO: 1010). In a preferred embodiment, the nucleotide sequence encoding the exogenous TRAC chain is codon optimized and differs from the endogenous or native TCR α constant chain in its nucleotide sequence. In an alternate embodiment, the nucleotide sequence encoding the exogenous TRAC chain is codon optimized and carries one or more amino acid substitutions that are known to enhance the expression of human TCR α constant chain (see **Table 3**). In an exemplary embodiment, the exogenous TRAC chains that can be used to allow re-expression and/or activity of TFP-TCR-CD3 complex in $\alpha\beta$ T cells in which the expression of endogenous TCR α gene has been down-regulated or eliminated have sequence as shown in SEQ ID NO: 3886 to 3894 or have sequences which encode for polypeptides with greater than 80% homology to the polypeptides encoded by sequences shown in SEQ ID NO: 3886 to 3894. To enable its cell surface expression, the nucleotide sequence encoding the exogenous TCR α constant chain (TRAC) is operationally linked to nucleotide sequence encoding a signal peptide. In an embodiment, additional non-naturally occurring sequences (*e.g.*, linkers or antigen binding domain) may be optionally added to the sequence encoding the TRAC chain as long as they do not interfere with its ability to recruit other components of the TCR-CD3 signaling complex and/or CD3 $\epsilon/\gamma/\delta$ TFP. In an embodiment, the exogenous TCR α constant chain polypeptide is not operationally linked to the native V α sequence (*i.e.* antigen binding domain) present in the T cell in which it is expressed. In an embodiment, the expression of exogenous TCR α constant chain polypeptide does not allow the $\alpha\beta$ T cell to regain its native antigen recognition specificity and/or affinity, *e.g.*, to recognize the MHC-peptide antigen complex which was recognized by the T cell with its endogenous TCR α chain. In an exemplary embodiment, the accessory module encoding an exogenous TCR α constant chain can be expressed in $\alpha\beta$ T cells either by itself (SEQ ID NO: 1010) using an appropriate method (*e.g.*, lentiviral mediated gene transfer) or it can be co-expressed with the TFP expression cassettes using a single vector (*e.g.* a lentiviral vector). Alternate methods of delivery and expression of two or more genes or RNAs are known in the art and described in this disclosure and can be used in the alternate embodiments of the invention. The nucleotide sequence of exemplary constructs coexpressing a TCR α constant chain with CD3 $\epsilon/\gamma/\delta$ TFP constructs targeting MPL are shown in SEQ ID NOs: 3538, 3540, and 3542. In the exemplary construct CD8SP-MPL-Hu-161-2-(vL-vH)-CD3 ϵ -ECDTMCP-opt2-F-P2A-IgSP-[TRAC-opt2] (SEQ ID NO: 3538), the first cassette encodes a CD3 ϵ -TFP comprising a CD8 signal peptide followed by a humanized scFV targeting the human MPL protein and extracellular, transmembrane and cytosolic domain of CD3 ϵ . This TFP encoding cassette is followed in frame by a linker encoding Furine-SGSG-P2A and a cassette encoding a signal peptide

(IgSP) and a codon optimized nucleotide sequence encoding TRAC. In an exemplary embodiment, the entire cassette can be expressed in $\alpha\beta$ T cells lacking endogenous TCR α chain using a lentiviral vector.

[00283] In an alternate embodiment, the expression of TCR α constant chain polypeptide can be restored in $\alpha\beta$ T cells that lack or have impaired functional endogenous or native TCR α chain polypeptide on their surface by using the endogenous TCR α constant chain gene. In an exemplary embodiment, the expression of TCR α constant chain polypeptide can be restored in $\alpha\beta$ T cells that lack or have impaired functional endogenous or native TCR α chain polypeptide on their surface by functionally linking in frame a nucleic acid sequence encoding a signal peptide to at least one copy of the endogenous TCR α constant chain gene using techniques of gene editing known in the art. In an exemplary embodiment, the nucleic acid sequence encoding a signal peptide is operationally linked in frame to the first exon of at least one of the endogenous TCR α constant chain genes so as to allow the expression of a TCR α constant chain polypeptide on the surface of the T cells. In an embodiment, the expression cassette encoding the signal peptide and TCR α constant chain gene is under the transcriptional regulatory control of the endogenous TCR α promoter. In an embodiment, the expression cassette encoding the signal peptide and TCR α constant chain gene shares the 3' untranslated sequence and regulatory control of the endogenous TCR α gene. In an alternate embodiment, the expression cassette encoding the signal peptide and TCR α constant chain gene is under an exogenous promoter (*e.g.*, EF1 α or CMV promoter).

[00284] In an exemplary embodiment, expression of TCR α constant chain polypeptide can be restored in $\alpha\beta$ T cells in which the endogenous or the native TCR α chain gene has been disrupted by targeted integration of a cassette encoding a TFP by designing the targeting cassette such that TFP cassette is followed in frame by a 2A cleavable linker, a signal peptide (*e.g.*, a CD8 signal peptide or an IgH signal peptide) and the first exon of the TCR α constant chain (TRAC) (**Fig 5C**). An exemplary such targeting construct is represented by SEQ ID NO: 3860. In this embodiment, the TCR α constant chain is expressed from the endogenous TCR α constant chain (TRAC) gene whose cell surface expression is facilitated by the signal peptide present in the targeting cassette. In this embodiment, the TCR α constant chain is expressed under the regulatory control of the TCR α gene promoter and TCR α 3' untranslated region. An alternate exemplary targeting construct is represented by SEQ ID NO: 3859 and can be used in alternate embodiments of the disclosure to disrupt the endogenous TCR α chain by targeting integration of a cassette encoding a TFP while simultaneously allowing re-expression of a TCR α constant chain from an expression cassette

encoding a signal peptide followed by a codon optimized TCR α constant chain cDNA and a polyA sequence (**Fig. 5B**).

[00285] The disclosure also demonstrates that in contrast to the situation with the 1st or the 2nd generation CAR constructs, CD3 ϵ / γ / δ TFPs lose their activity when expressed in $\alpha\beta$ T cells (*i.e.* T cells expressing TCR α and TCR β chains) that lack or have impaired functional endogenous or native TCR β 1 and TCR β 2 chain polypeptides on their surface. For example, the disclosure provides that CD3 ϵ / γ / δ TFPs have impaired expression and activity (*e.g.*, T cell activation, proliferation, cytokine production and cytotoxicity *etc.*) in $\alpha\beta$ T cells in which the endogenous TCR β 1 and TCR β 2 genomic loci have been disrupted. The disclosure provides a solution to this problem by re-expressing TCR β 1 or TCR β 2 constant chain polypeptides or a fragment thereof in T cells in which the expression of native full length TCR β 1 and TCR β 2 chain polypeptides have been reduced or eliminated. In an embodiment, the re-expressed TCR β 1/ β 2 constant chain polypeptide or TCR β 1/ β 2 constant chain fragment allows the reconstitution of a functional TFP-TCR-CD3 signaling complex in a T cell, *e.g.*, a $\alpha\beta$ T cell, in which the expression of the native TCR β 1 and/or β 2 constant chain is impaired, reduced or eliminated. In an embodiment, the re-expressed TCR β 1/ β 2 constant chain or TCR β 1/ β 2 constant chain fragment improves by more than 15% (*e.g.*, more than 20%, 50%, 75%, or 100% *etc.*) target antigen-induced cytokine (*e.g.*, IL2, TNF α , IFN γ) production, proliferation and/or cytotoxic activity of TFP-expressing $\alpha\beta$ T cell in which the expression of the native TCR β 1 and/or β 2 constant chain is impaired, reduced or eliminated. In an embodiment, the re-expressed TCR β 1/ β 2 constant chain or TCR β 1/ β 2 constant chain fragment allows enhanced expression of the CD3 ϵ / γ / δ TFP in a $\alpha\beta$ T cell in which the expression of the native TCR β 1 and/or TCR β 2 constant chains is impaired, reduced or eliminated. In an embodiment, the re-expressed TCR β 1/ β 2 constant chain or TCR β 1/ β 2 constant chain fragment allows more than 15% (*e.g.*, more than 20%, 50%, 75%, or 100% *etc.*) increase in expression of the CD3 ϵ / γ / δ TFP in a $\alpha\beta$ T cell in which the expression of the native TCR β 1 and/or TCR β 2 constant chain is impaired, reduced or eliminated. In an exemplary embodiment, the expression and signaling activity of CD3 ϵ / γ / δ TFPs can be restored in T cells in which the expression of native TCR β 1 and/or TCR β 2 chain is reduced or eliminated by introducing into such T cells a nucleic acid construct encoding an exogenous TCR β 1/ β 2 constant chain (TRBC chain) (*e.g.*, SEQ ID NO: 1011). In a preferred embodiment, the nucleotide sequence encoding the exogenous TCR β 1/ β 2 constant chain is codon optimized and differs from the endogenous or native TCR β 1 and TCR β 2 constant chains in its nucleotide sequence. In an alternate embodiment, the nucleotide sequence encoding the exogenous TCR β 1/ β 2 constant

chain is codon optimized and carries one or more amino acid substitutions that are known to enhance the expression of human TCR β 1/ β 2 constant chain (see **Table 4a, 4b**). In an exemplary embodiment, the exogenous TCR β 1/ β 2 chains that can be used to allow re-expression and/or activity of TFP-TCR-CD3 complex in T cells in which the expression of endogenous TCR β 1 and β 2 chains has been down-regulated or eliminated have sequence as shown in SEQ ID NO: 3895 to 3900 or have sequences which encode for polypeptides with greater than 80% homology to the polypeptides encoded by sequences shown in SEQ ID NO: 3895 to 3900. To enable its cell surface expression, the nucleotide sequence encoding the exogenous TCR β 1/ β 2 constant chain (TRBC) is operationally linked to nucleotide sequence encoding a signal peptide. In an embodiment, additional non-naturally occurring sequences (*e.g.*, linkers or antigen binding domain) may be optionally added to the sequence encoding the TCR β 1/ β 2 constant chain (TRBC) as long as they do not interfere with its ability to recruit other components of the TCR-CD3 signaling complex and/or TFP. In an embodiment, the exogenous TCR β 1/ β 2 constant chain polypeptide is not operationally linked to the native V β sequence (*i.e.* antigen binding domain) present in the T cell in which it is expressed. In an embodiment, the expression of exogenous TCR β 1/ β 2 constant chain polypeptide does not allow the T cell to regain its native antigen recognition specificity and/or affinity, *e.g.*, to recognize the MHC-peptide antigen complex which was recognized by the T cell with its endogenous TCR β 1/ β 2 chain. In an exemplary embodiment, the accessory module encoding the exogenous TCR β 1/ β 2 constant chain can be expressed in T cells either by itself (*e.g.*, SEQ ID NO: 1011) using an appropriate method (*e.g.*, lentiviral mediated gene transfer) or it can be co-expressed with the CD3 ϵ / γ / δ TFP expression cassettes using a single vector (*e.g.* a lentiviral vector). Alternate methods of delivery and expression of two or more genes or RNAs are known in the art and described in this disclosure and can be used in the alternate embodiments of the disclosure. The nucleotide sequence of exemplary constructs coexpressing a TCR β constant chain with TFP constructs targeting MPL are shown in SEQ ID NOs: 3537, 3539, and 3541. In the exemplary construct CD8SP-MPL-Hu-161-2-(vL-vH)-CD3 ϵ -ECDTMCP-opt2-F-P2A-IgSP-[TRBC-opt2] (SEQ ID NO: 3537), the first cassette encodes a TFP comprising a CD8 signal peptide followed by a humanized scFV targeting the human MPL protein and extracellular, transmembrane and cytosolic domain of CD3 ϵ . This TFP encoding cassette is followed in frame by a linker encoding Furine-SGSG-P2A and a cassette encoding a signal peptide (IgSP) and a codon optimized nucleotide sequence encoding a TCR β constant chain (TRBC). In an exemplary embodiment, the entire cassette can be expressed in T cells lacking endogenous TCR β chain using a lentiviral vector.

[00286] In an alternate embodiment, the expression of TCR β 1 or β 2 constant chain polypeptide can be restored in $\alpha\beta$ T cells that lack or have impaired functional endogenous or native TCR α chain polypeptide on their surface by using the endogenous TCR β 1 or β 2 constant chain gene. In an exemplary embodiment, the expression of TCR β 1/ β 2 constant chain polypeptide and the co-expressed TFP can be restored in $\alpha\beta$ T cells that lack or have impaired functional endogenous or native TCR β chain polypeptide on their surface by operationally linking in frame a nucleic acid sequence encoding a signal peptide to at least one copy of the endogenous TCR β 1 or TCR β 2 constant chain gene using techniques of gene editing known in the art. In an exemplary embodiment, the nucleic acid sequence encoding a signal peptide is operationally linked in frame to the first exon of at least one of the endogenous TCR β 1 or TCR β 2 constant chain genes so as to allow the expression of a TCR β 1/ β 2 constant chain polypeptide and the coexpressed TFP on the surface of the T cells. In an embodiment, the expression cassette encoding the signal peptide and TCR β 1/ β 2 constant chain is under the transcriptional regulatory control of the endogenous TCR β 1/ β 2 promoter. In an embodiment, the expression cassette encoding the signal peptide and TCR β 1/ β 2 constant chain is under the 3' untranslated sequence and regulatory control of the endogenous TCR β 1/ β 2 gene. In an alternate embodiment, the expression cassette encoding the signal peptide and TCR β 1/ β 2 constant chain is under an exogenous promoter (*e.g.*, EF1 α or CMV promoter).

[00287] In an exemplary embodiment, expression of TCR β 1/ β 2 constant chain polypeptide can be restored in $\alpha\beta$ T cells in which the endogenous or the native TCR β 1 and TCR β 2 chain genes have been disrupted by targeted integration of cassettes encoding a TFP by designing the targeting cassette such that TFP cassette is followed in frame by a 2A cleavable linker, a signal peptide (*e.g.*, a CD8 signal peptide or an IgH signal peptide) and the first exon of the TCR β 1/ β 2 constant chain (TRBC).

[00288] It has been observed that directing the CAR cassette to the TRAC locus result in approximately 95% T cells becoming TCR negative. Such TCR-negative T cells could be used in an allogeneic setting as they are less likely to cause graft vs host disease (GVHD). However, re-expression of TRAC chain in T cells in which the TRAC locus has been targeted by a TFP cassette would potentially lead to expression of the full length TCR β chain including the V β region. Such T cells, even though lacking the MHC recognition provided by V α region, would be potentially able to recognize allo-antigens presented by MHC complex through their TCR β chains and therefore potentially cause GVHD. In alternate embodiments of the disclosure, both TCR α and TCR β 1 or β 2 chains are re-expressed in CD3 ϵ / γ / δ TFP-

expressing $\alpha\beta$ T cells in which the expression of endogenous TCR α and TCR β 1 and TCR β 2 chains have been down-regulated or eliminated.

[00289] In the above example, an exogenous TRAC or TRBC is coexpressed with a TFP-expressing construct to restore the expression and/or activity of CD3 $\epsilon/\gamma/\delta$ TFP in $\alpha\beta$ T cells in which the expression of endogenous TCR α and/or TCR β chains have been down-regulated or eliminated by, for example, targeting of their genomic loci. In an alternate embodiment of the invention, expression of exogenous TCR α and/or TCR β 1/ β 2 constant chains is used to restore TCR/CD3 complex expression in any $\alpha\beta$ T cell, including a wild-type $\alpha\beta$ T cell or an $\alpha\beta$ T cell expressing a chimeric antigen receptor, a chimeric T cell receptor (cTCR), an AbTCR, or a synthetic immune receptor. Finally, a similar approach can be used to restore CAR/TFP and/or TCR/CD3 expression in γ/δ T cells in which the expression of endogenous TCR γ and/or TCR δ chains have been down-regulated or eliminated. Exemplary constant chains of TCR γ (TRGC) and TCR δ (TRDC) that can be expressed in γ/δ T cells in which the expression of endogenous TCR γ and/or TCR δ chains have been down-regulated or eliminated are represented by SEQ ID NO: 3912 and 3913.

[00290] The disclosure also provides that the expression and activity of CD3 $\epsilon/\gamma/\delta$ TFP can be restored in T cells with impaired or lack of expression the native TCR $\alpha/\beta/\gamma$ or δ chains by re-expressing fragments or variants of the constant chains of TCR $\alpha/\beta/\gamma$ or δ . The fragments/variants of constant chains of TCR $\alpha/\beta/\gamma$ and δ that can be used to restore the expression of CD3 $\epsilon/\gamma/\delta$ TFP in cells lacking the native TCR $\alpha/\beta/\gamma$ or δ chains are provided in SEQ ID Nos: 15141-15144 (**Table 6D**). The expression cassettes encoding these chains with a IgH signal peptide are listed in SEQ ID Nos: 15145-15148 (**Table 7**).

[00291] The disclosure further provides that the expression and activity of CD3 $\epsilon/\gamma/\delta$ TFP can be restored in T cells with impaired or lack of expression native TCR $\alpha/\beta/\gamma$ or δ chains by coexpression of a SIR or an Ab-TCR comprising the missing TCR $\alpha/\beta/\gamma$ or δ constant chains. Thus, in a $\alpha\beta$ T cells with impaired or lack of expression of the native TCR α chain, the expression and activity of a CD3 $\epsilon/\gamma/\delta$ TFP can be rescued by expression of a SIR comprising a TCR α constant chain. In an exemplary embodiment, in a $\alpha\beta$ T cells with impaired or lack of expression of the native TCR α chain, the expression and activity of a CD3 $\epsilon/\gamma/\delta$ TFP (e.g., a TFP encoded by SEQ ID NOs: 8708-8714) can be rescued by expression of a SIR (e.g., a SIR represented by SEQ ID NO: 9668, 9669, or 9684 *etc.*) comprising a TCR α constant chain. In another exemplary embodiment, in a $\alpha\beta$ T cells with impaired or lack of expression of the native TCR α chain, the expression and activity of a CD3 $\epsilon/\gamma/\delta$ TFP (e.g., a TFP encoded by SEQ ID NOs: 8708-8714) can be rescued by

expression of a Ab-TCR (*e.g.*, a Ab-TCR represented by SEQ ID NO: 9677 or 9678 *etc.*) comprising a portion of TCR α constant chain. The disclosure provides that for combination therapies with allogeneic T cells involving two CARs, a CD3 α / γ / δ TFP is preferably combined with a SIR and/or a Ab-TCR which incorporate the TCR constant chain or TCR constant chain fragment whose expression is reduced or missing in the allogeneic T cells.

[00292] The disclosure further provides that in a $\alpha\beta$ T cells with impaired or lack of expression of the native TCR β chains, the expression and activity of a CD3 ϵ / γ / δ TFP can be rescued by expression of a SIR comprising a TCR β constant chain. In an exemplary embodiment, in a $\alpha\beta$ T cells with impaired or lack of expression of the native TCR β 1/ β 2 chains, the expression and activity of a CD3 ϵ / γ / δ TFP (*e.g.*, a TFP encoded by SEQ ID NOs: 8708-8714) can be rescued by expression of a SIR (*e.g.*, a SIR represented by SEQ ID NO: 9668, 9669, or 9684 *etc.*) comprising a TCR β constant chain. In another exemplary embodiment, in a $\alpha\beta$ T cells with impaired or lack of expression of the native TCR α chain, the expression and activity of a CD3 ϵ / γ / δ TFP (*e.g.*, a TFP encoded by SEQ ID NOs: 8708-8714) can be rescued by expression of a Ab-TCR (*e.g.*, a Ab-TCR represented by SEQ ID NO: 9677 or 9678 *etc.*) comprising a portion of TCR β constant chain.

[00293] The disclosure further provides that in a $\gamma\delta$ T cells with impaired or lack of expression of the native TCR γ chain, the expression and activity of a CD3 ϵ / γ / δ TFP can be rescued by expression of a SIR comprising a TCR γ constant chain. In an exemplary embodiment, in a $\gamma\delta$ T cells with impaired or lack of expression of the native TCR γ chain, the expression and activity of a CD3 ϵ / γ / δ TFP (*e.g.*, a TFP encoded by SEQ ID NOs: 8708-8714) can be rescued by expression of a SIR (*e.g.*, a SIR represented by SEQ ID NO: 9689) comprising a TCR γ constant chain. In another exemplary embodiment, in a $\gamma\delta$ T cells with impaired or lack of expression of the native TCR γ chain, the expression and activity of a CD3 ϵ / γ / δ TFP (*e.g.*, a TFP encoded by SEQ ID NOs: 8708-8714) can be rescued by expression of a Ab-TCR (*e.g.*, a Ab-TCR represented by SEQ ID NO: 9676) comprising a portion of TCR γ constant chain.

[00294] The disclosure further provides that in a $\gamma\delta$ T cells with impaired or lack of expression of the native TCR δ chain, the expression and activity of a CD3 ϵ / γ / δ TFP can be rescued by expression of a SIR comprising a TCR δ constant chain. In an exemplary embodiment, in a $\gamma\delta$ T cells with impaired or lack of expression of the native TCR δ chain, the expression and activity of a CD3 ϵ / γ / δ TFP (*e.g.*, a TFP encoded by SEQ ID NOs: 8708-8714) can be rescued by expression of a SIR (*e.g.*, a SIR represented by SEQ ID NO: 9689) comprising a TCR δ constant chain. In another exemplary embodiment, in a $\gamma\delta$ T cells with

impaired or lack of expression of the native TCR δ chain, the expression and activity of a CD3 ϵ / γ / δ TFP (e.g., a TFP encoded by SEQ ID NOs: 8708-8714) can be rescued by expression of a Ab-TCR (e.g., a Ab-TCR represented by SEQ ID NO: 9676) comprising a portion of TCR δ constant chain.

[00295] The disclosure also provides methods and constructs that allow a next generation CAR (e.g., SIR and AbTCR), cTCR, and TCR to be expressed under the physiological regulatory mechanisms afforded by endogenous TCR genes. The disclosure also provides methods and constructs that allow a next generation CAR (e.g., SIR and AbTCR), cTCR, and TCR to be expressed under the promoter and 3' untranslated regulatory mechanisms afforded by endogenous TCR genes. In one embodiment, the disclosure provides methods so that an expression cassette encoding a SIR/cTCR/Ab-TCR/TCR is targeted to the endogenous TCR α , TCR β 1/ β 2, TCR γ or TCR δ gene locus. In an embodiment, the SIR/cTCR/Ab-TCR/TCR is targeted to the endogenous TCR α gene locus (TRAC) so that the TCR α constant chain of the SIR/cTCR/Ab-TCR/TCR is expressed wholly or in part from the endogenous native TCR α constant chain gene. In an embodiment, the SIR/cTCR/Ab-TCR/TCR is targeted to the endogenous TCR α gene locus (TRAC) so that the TCR α constant chain of the SIR/cTCR/Ab-TCR/TCR is encoded completely or in part by at least one of the exons of the endogenous TCR α constant chain gene. In an embodiment, the SIR/cTCR/Ab-TCR/TCR is targeted to the endogenous TCR α gene locus (TRAC) so that the TCR α constant chain of the SIR/cTCR/Ab-TCR/TCR shares completely or in part the 3' untranslated region and polyadenylation sequence of the native/endogenous TCR α constant chain gene.

[00296] In an embodiment, the SIR/cTCR/Ab-TCR/TCR is targeted to the endogenous TCR β gene locus (TRBC) so that the TCR β constant chain of the SIR/cTCR/Ab-TCR/TCR is expressed wholly or in part from the endogenous native TCR β 1 or TCR β 2 constant chain gene. In an embodiment, the SIR/cTCR/Ab-TCR/TCR is targeted to the endogenous TCR β 1/ β 2 gene locus (TRBC) so that the TCR β constant chain of the SIR/cTCR/Ab-TCR/TCR is encoded completely or in part by at least one of the exons of the endogenous TCR β constant chain gene. In an embodiment, the SIR/cTCR/Ab-TCR/TCR is targeted to the endogenous TCR β 1/ β 2 gene locus (TRBC) so that the TCR β constant chain of the SIR/cTCR/Ab-TCR/TCR shares completely or in part the 3' untranslated region and polyadenylation sequence of the native/endogenous TCR β constant chain gene.

[00297] In an embodiment, the SIR/cTCR/Ab-TCR/TCR is targeted to the endogenous TCR γ gene locus (TRGC) so that the TCR γ constant chain of the SIR/cTCR/Ab-TCR/TCR is expressed wholly or in part from the endogenous native TCR γ constant chain gene. In an

embodiment, the SIR/cTCR/Ab-TCR/TCR is targeted to the endogenous TCR γ gene locus (TRGC) so that the TCR γ constant chain of the SIR/cTCR/Ab-TCR/TCR is encoded completely or in part by at least one of the exons of the endogenous TCR γ constant chain gene. In an embodiment, the SIR/cTCR/Ab-TCR/TCR is targeted to the endogenous TCR γ gene locus (TRGC) so that the TCR γ constant chain of the SIR/cTCR/Ab-TCR/TCR shares completely or in part the 3' untranslated region and polyadenylation sequence of the native/endogenous TCR γ constant chain gene.

[00298] In an embodiment, the SIR/cTCR/Ab-TCR/TCR is targeted to the endogenous TCR δ gene locus (TRDC) so that the TCR δ constant chain of the SIR/cTCR/Ab-TCR/TCR is expressed wholly or in part from the endogenous native TCR δ constant chain gene. In an embodiment, the SIR/cTCR/Ab-TCR/TCR is targeted to the endogenous TCR δ gene locus (TRGC) so that the TCR δ constant chain of the SIR/cTCR/Ab-TCR/TCR is encoded completely or in part by at least one of the exons of the endogenous TCR δ constant chain gene. In an embodiment, the SIR/cTCR/Ab-TCR/TCR is targeted to the endogenous TCR δ gene locus (TRDC) so that the TCR δ constant chain of the SIR/cTCR/Ab-TCR/TCR shares completely or in part the 3' untranslated region and polyadenylation sequence of the native/endogenous TCR δ constant chain gene.

[00299] T cells or natural killer (NK) or stem cells, can be obtained from a subject. The term "subject" is intended to include living organisms in which an immune response can be elicited (*e.g.*, mammals). Examples of subjects include humans, monkeys, chimpanzees, dogs, cats, mice, rats, and transgenic species thereof. T cells can be obtained from a number of sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. T cells could be tissue resident gamma-delta T cells, which can be cultured and expanded *in vitro* prior to expression of the CAR/TCR and/or an NF- κ B stimulatory molecule.

[00300] In certain embodiments of the disclosure, immune effector cells, *e.g.*, T cells, can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as Ficoll™ separation. In one preferred aspect, cells from the circulating blood of an individual are obtained by apheresis. The apheresis product usually contains lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets. In one aspect, the cells collected by apheresis may be washed to remove the plasma fraction and, optionally, to place the cells in an appropriate buffer or media for subsequent processing steps. In one embodiment, the cells

are washed with phosphate buffered saline (PBS). In an alternative embodiment, the wash solution lacks calcium and may lack magnesium or may lack many if not all divalent cations.

[00301] Initial activation steps in the absence of calcium can lead to magnified activation. As those of ordinary skill in the art would readily appreciate a washing step may be accomplished by methods known to those in the art, such as by using a semi -automated "flow-through" centrifuge (for example, the Cobe 2991 cell processor, the Baxter CytoMate, or the Haemonetics Cell Saver 5) according to the manufacturer's instructions. After washing, the cells may be resuspended in a variety of biocompatible buffers, such as, for example, Ca-free, Mg-free PBS, PlasmaLyte A, or other saline solution with or without buffer. Alternatively, the undesirable components of the apheresis sample may be removed and the cells directly resuspended in culture media.

[00302] It is recognized that the methods of the application can utilize culture media conditions comprising 5% or less, for example 2%, human AB serum, and employ known culture media conditions and compositions, for example those described in Smith *et al.*, "Ex vivo expansion of human T cells for adoptive immunotherapy using the novel Xeno-free CTS Immune Cell Serum Replacement" *Clinical & Translational Immunology* (2015) 4, e31; doi: 10.1038/cti.2014.31.

[00303] In one aspect, T cells are isolated from peripheral blood lymphocytes by lysing the red blood cells and depleting the monocytes, for example, by counterflow centrifugal elutriation or centrifugation through a PERCOLL™ gradient.

[00304] In one embodiment, the disclosure provides methods of treating or preventing a disease by providing to the subject in need thereof immune effector cells (*e.g.*, T cells) or stem cells that can give rise to immune effector cells that are engineered to express an X-CAR or a X-TCR and an NF-κB stimulatory molecule, wherein X represents a disease associated antigen as described herein, and wherein the disease causing or disease-associated cells express said X antigen. **Table 9** provides a list of different antigens and the exemplary diseases that can be prevented, inhibited or treated using immune effector cells expressing CARs targeting these antigens.

[00305] In another embodiment, the disclosure provides methods of treating or preventing a cancer, infection, autoimmune or allergic diseases by providing to the subject in need thereof immune effector cells (*e.g.*, T cells) or stem cells that can give rise to immune effector cells that are engineered to express a non-naturally occurring immune receptor (*e.g.*, CAR and/or TCR) of the disclosure and/or an NF-κB stimulatory molecule. In one embodiment, the NF-κB stimulatory molecule is a selective NF-κB activator. In one

embodiment, the NF- κ B activator, *e.g.*, a selective NF- κ B activator, is a non-viral NF- κ B activator. In one embodiment, the NF- κ B activator, *e.g.*, a selective NF- κ B activator, is not a transmembrane protein and is expressed in the cytosol or is preferentially present in the cytosol. In one embodiment, the NF- κ B activator, *e.g.*, a selective NF- κ B activator, is constitutively active. In one embodiment, the NF- κ B activator, *e.g.*, a selective NF- κ B activator, is not constitutively active. In one embodiment, the selective NF- κ B activator is activated by the administration of an inducer (*e.g.*, a dimerizer). In one embodiment, the selective NF- κ B activator is ν FLIP K13, NEMO-K277A or its derivatives. The CAR/NF- κ B stimulatory molecule-expressing immune effector cells are administered to the patient. In one aspect the disease associated cell is a cancer cell, an infected cell (*e.g.*, HIV-1 infected cell), or a plasma cell or a B cell or a T cell.

[00306] In another embodiment, the disclosure provides methods of treating or preventing a cancer, infection, autoimmune or allergic diseases by providing to the subject in need thereof immune effector cells (*e.g.*, T cells) or stem cells that can give rise to immune effector cells that are engineered to express a naturally occurring immune receptor (*e.g.*, a native TCR) and an NF- κ B stimulatory molecule. In one embodiment, the NF- κ B stimulatory molecule is a selective NF- κ B activator. In one embodiment, the NF- κ B activator, *e.g.*, a selective NF- κ B activator, is a non-viral NF- κ B activator. In one embodiment, the NF- κ B activator, *e.g.*, a selective NF- κ B activator, is not a transmembrane protein and is expressed in the cytosol or is preferentially present in the cytosol. In one embodiment, the NF- κ B activator, *e.g.*, a selective NF- κ B activator, is constitutively active. In one embodiment, the NF- κ B activator, *e.g.*, a selective NF- κ B activator, is not constitutively active. In one embodiment, the selective NF- κ B activator is activated by the administration of an inducer (*e.g.*, a dimerizer). In one embodiment, the selective NF- κ B activator is ν FLIP K13, NEMO-K277A or its derivatives. The native TCR and NF- κ B stimulatory molecule-expressing immune effector cells are administered to the patient. In one aspect the disease associated cell is a cancer cell, an infected cell (*e.g.*, HIV-1 infected cell), or a plasma cell or a B cell or a T cell.

[00307] In another embodiment, the disclosure provides methods of treating or preventing a cancer, infection, autoimmune or allergic diseases by providing to the subject in need thereof immune effector cells (*e.g.*, T cells) or stem cells that can give rise to immune effector cells that are engineered to express a naturally occurring (*e.g.*, native TCR) or a non-naturally occurring immune receptor (*e.g.*, CAR and/or recombinant TCR) of the disclosure

and/or an NF- κ B stimulatory molecule. In some embodiments, the activity of CAR-T or TCR-T cells may be controlled using a water soluble salt of Dasatinib.

[00308] In another aspect, a method of treating a subject, *e.g.*, reducing or ameliorating a hyperproliferative disorder or condition (*e.g.*, a cancer), *e.g.*, solid tumor, a soft tissue tumor, a blood cancer, or a metastatic lesion, in a subject is provided.

[00309] In yet another embodiment, the disclosure pertains to a method of treating a disease in a subject. The method comprises administering to the subject a cell expressing a naturally occurring and/or a non-naturally occurring immune receptor (*e.g.*, CAR and/or recombinant TCR) of the disclosure and/or an NF- κ B stimulatory molecule of the disclosure such that the disease is treated in the subject. In one aspect the method comprises administering to the subject a cell expressing its endogenous (or native) TCR and an NF- κ B stimulatory molecule of the disclosure such that the disease is treated in the subject. In one aspect, the disease associated with expression of a disease associate antigen as described herein is an infectious disease. In one aspect the infectious disease is disease associated with infection by HIV1, HIV2, HTLV1, Epstein Barr virus (EBV), cytomegalovirus (CMV), adenovirus, adeno-associated virus, BK virus, Human Herpesvirus 6, Human Herpesvirus 8, influenza A virus, , influenza B virus parainfluenza virus, avian flu virus, MERS and SARS coronaviruses, Crimean Congo Hemorrhagic fever virus, rhino virus, enterovirus, Dengue virus, West Nile virus, Ebola virus, Marburg virus, Lassa fever virus, zika virus, RSV, measles virus, mumps virus, rhino virus, varicella virus, herpes simplex virus 1 and 2, varicella zoster virus, HIV-1, HTLV1, Hepatitis virus, enterovirus, hepatitis B virus, Hepatitis C virus, Nipah and Rift valley fever viruses, Japanese encephalitis virus, mycobacterium tuberculosis, atypical mycobacteria species, Pneumocystis jirovecii, toxoplasmosis, rickettsia, nocardia, aspergillus, mucor, or candida.

[00310] In some embodiments, the non-naturally occurring immune receptor (*e.g.*, CAR and/or TCR) specifically binds an HIV antigen. In some embodiments, the HIV antigen is an HIV-1 antigen. In some embodiments, the HIV antigen is an HIV envelope protein or a portion thereof. In some embodiments, the HIV antigen is gp120 or a portion thereof. In some embodiments the HIV antigen is the CD4 binding site on gp120. In some embodiments, the HIV antigen is the CD4-induced binding site on gp120. In some embodiments, the HIV antigen is the N-glycan on gp120. In some embodiments, the HIV antigen is the V2 of gp120. In some embodiments, the HIV antigen is the membrane proximal region on gp41.

[00311] The disclosure includes a type of cellular therapy where immune effector cells (*e.g.*, T cells or stem cells that give rise to T cells) are genetically modified to express a CAR

or TCR of the disclosure and/or an NF- κ B stimulatory molecule and the CAR-expressing T cell or stem cell is infused to a recipient in need thereof. The disclosure also includes a type of cellular therapy where immune effector cells (*e.g.*, T cells or stem cells that give rise to T cells) are genetically modified to express a NF- κ B stimulatory molecule and such cells are infused to a recipient in need thereof. The infused cells are able to kill disease associated cells (*e.g.*, tumor cells or virally infected cells) in the recipient. Unlike antibody therapies, the NF- κ B activator modified immune effector cells (*e.g.*, T cells, stem cells) are able to replicate *in vivo* resulting in long-term persistence that can lead to sustained tumor control. In various aspects, the NF- κ B activator modified immune effector cells (*e.g.*, T cells or stem cells that can give rise to T cells) administered to the patient, or their progeny, persist in the patient for at least four months, five months, six months, seven months, eight months, nine months, ten months, eleven months, twelve months, thirteen months, fourteen month, fifteen months, sixteen months, seventeen months, eighteen months, nineteen months, twenty months, twenty-one months, twenty-two months, twentythree months, two years, three years, four years, or five years after administration of the T cell or stem cells to the patient.

[00312] The disclosure also includes a type of cellular therapy where stem cells (*e.g.*, hematopoietic stem cell or lymphoid stem cells or embryonic stem cells, or induced pluripotent stem cells) that are capable of giving rise to immune effector cells (*e.g.*, T cells) are modified to express a non-naturally occurring immune receptor (*e.g.*, CAR and/or TCR) of the disclosure and/or an NF- κ B stimulatory molecule and are administered to a recipient in need thereof. The administered stem cells give rise to immune effector cells (*e.g.*, T cells) after transplantation into the recipient, which (*i.e.* the immune effector cells) are able to kill disease associated cells in the recipient. Thus, in various aspects, the immune effector cells (*e.g.*, T cells) that are produced in the patient after administration of CAR/NF κ B-activator-expressing stem cells, persist in the patient for at least one week, 2 weeks, 3 weeks, one month, two months, three months, four months, five months, six months, seven months, eight months, nine months, ten months, eleven months, twelve months, thirteen months, fourteen month, fifteen months, sixteen months, seventeen months, eighteen months, nineteen months, twenty months, twenty-one months, twenty-two months, twenty-three months, two years, three years, four years, five years, ten years or twenty years after administration of the T cell or stem cells to the patient. The disclosure also includes a type of cellular therapy where stem cells that are capable of giving rise to immune effector cells (*e.g.*, T cells) are modified to express a non-naturally occurring immune receptor (*e.g.*, CAR and/or TCR) of the disclosure and/or an NF- κ B stimulatory molecule and are differentiated *in vitro* to

generate immune effector cells that are infused to a recipient in need thereof. The infused immune effector cells (*e.g.*, T cells) after infusion into the recipient are able to kill disease associated cells in the recipient. Thus, in various aspects, the immune effector cells (*e.g.*, T cells) that are administered to the patient persist in the patient for at least 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, one week, 2 weeks, 3 weeks, one month, two months, three months, four months, five months, six months, seven months, eight months, nine months, ten months, eleven months, twelve months, thirteen months, fourteen month, fifteen months, sixteen months, seventeen months, eighteen months, nineteen months, twenty months, twenty-one months, twenty-two months, twentythree months, two years, three years, four years, five years, ten years or twenty years.

[00313] The disclosure includes a type of cellular therapy where immune effector cells (*e.g.*, T cells or stem cells that give rise to T cells) are genetically modified to express CARs targeting two or more different antigens in the same cell and such T cell or stem cell is infused to a recipient in need thereof. In an embodiment, at least one of the CARs targets an antigen expressed on the hematopoietic cells. In an embodiment, at least one of the CARs targets an antigen selected from the group of CD19, CD20, CD22, BCMA, CS1, CD138, Lym1, Lym2, CD33 and CD123. In an embodiment, at least one of the CARs targets an antigen expressed on the hematopoietic cells and at least one other CARs targets and antigen expressed on solid tumors. In an embodiment, at least one of the CARs targets an antigen selected from the group of CD19, CD20, CD22, BCMA, CS1, CD138, Lym1, Lym2, CD33 or CD123 and at least one other CAR targets an antigen selected from the group of Mesothelin, Her2, Folate Receptor 1, ROR1, IL13Ra2, AFP, WT1, Ras, NY-ESO-1, DLL3, CD70 and PTK7. In an embodiment, at least one of the CARs is a SIR. In an embodiment, at least one of the CARs is an Ab-TCR. In an embodiment, at least one of the CARs is a SIR and the other CAR is a CD3 ϵ / γ / δ TFP. In an embodiment, at least one of the CARs is a Ab-TCR and the other CAR is a CD3 ϵ / γ / δ TFP. In an embodiment, the cells have impaired expression of at least one of the native TCR chains. The disclosure also includes a type of cellular therapy where immune effector cells (*e.g.*, T cells or stem cells that give rise to T cells) are genetically modified to express CARs targeting two different antigens and an NF- κ B stimulatory molecule and such cells are infused to a recipient in need thereof. In embodiment, the cells are autologous while in other embodiments the cells are allogenic. The infused cells are able to kill disease associated cells (*e.g.*, tumor cells or virally infected cells) in the recipient.

[00314] With respect to ex vivo immunization, at least one of the following occurs *in vitro* prior to administering the cell into a mammal: i) expansion of the cells, ii) introducing a nucleic acid encoding a non-naturally occurring immune receptor (*e.g.*, CAR and/or TCR) of the disclosure and/or an NF- κ B stimulatory molecule to the cells or iii) cryopreservation of the cells.

[00315] Ex vivo procedures are well known in the art and are discussed more fully below. Briefly, cells are isolated from a mammal (*e.g.*, a human) and genetically modified (*i.e.*, transduced or transfected *in vitro*) with a one or more vectors that express a non-naturally occurring immune receptor (*e.g.*, CAR and/or TCR) of the disclosure and/or an NF- κ B stimulatory molecule disclosed herein. The non-naturally occurring immune receptor (*e.g.*, CAR and/or TCR) and NF- κ B activator-modified cell can be administered to a mammalian recipient to provide a therapeutic benefit. The mammalian recipient may be a human and the non-naturally occurring immune receptor (*e.g.*, CAR and/or TCR) and NF- κ B activator-modified cell can be autologous with respect to the recipient. Alternatively, the cells can be allogeneic, syngeneic or xenogeneic with respect to the recipient.

[00316] The procedure for ex vivo expansion of hematopoietic stem and progenitor cells is described in U.S. Pat. No. 5,199,942, incorporated herein by reference, can be applied to the cells of the present invention. Other suitable methods are known in the art, therefore the present invention is not limited to any particular method of ex vivo expansion of the cells. Briefly, ex vivo culture and expansion of immune effector cells (*e.g.*, T cells) comprises: (1) collecting CD34+ hematopoietic stem and progenitor cells from a mammal from peripheral blood harvest or bone marrow explants; and (2) expanding such cells ex vivo. In addition to the cellular growth factors described in U.S. Pat. No. 5,199,942, other factors such as flt3-L, IL-1, IL-3 and c-kit ligand, can be used for culturing and expansion of the cells.

[00317] Generally, 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 certain aspects, the cells of the disclosure are used in the treatment of patients at risk for developing diseases, disorders and conditions associated with expression of a disease associate antigen as described herein. Thus, the disclosure provides methods for the treatment or prevention of diseases, disorders and conditions associated with expression of a disease associate antigen as described herein comprising administering to a subject in need thereof, a therapeutically effective amount of the CAR/TCR/NF- κ B stimulatory molecule-modified immune effector cells (*e.g.*, T cells) or stem cells that are capable of generating immune effector cells of the disclosure.

[00318] In one aspect the CAR/TCR/NF- κ B stimulatory molecule-expressing cells of the disclosures may be used to treat a proliferative disease such as a cancer or malignancy or is a precancerous condition such as a myelodysplasia, a myelodysplastic syndrome or a preleukemia. Further a disease associated with a cancer associate antigen as described herein expression include, but not limited to, *e.g.*, atypical and/or non-classical cancers, malignancies, precancerous conditions or proliferative diseases expressing a cancer associated antigen as described herein. Noncancer related indications associated with expression of a disease associate antigen as described herein include, but are not limited to, *e.g.*, autoimmune disease, (*e.g.*, lupus), inflammatory disorders (allergy and asthma), infectious conditions (*e.g.*, HIV1, CMV, EBV, influenza) and transplantation.

[00319] The CAR/TCR/NF- κ B stimulatory molecule-modified immune effector cells (*e.g.*, T cells) of the disclosure may be administered either alone, or as a pharmaceutical composition in combination with diluents and/or with other components such as IL-2 or other cytokines or cell populations.

[00320] Hematological cancer or blood cancer conditions are the types of cancer such as leukemia, lymphoma, and malignant lymphoproliferative conditions that affect blood, bone marrow and the lymphatic system.

[00321] Leukemia can be classified as acute leukemia and chronic leukemia. Acute leukemia can be further classified as acute myelogenous leukemia (AML) and acute lymphoid leukemia (ALL). Chronic leukemia includes chronic myelogenous leukemia (CML) and chronic lymphoid leukemia (CLL). Other related conditions include myelodysplastic syndromes (MDS, formerly known as "preleukemia") which are a diverse collection of hematological conditions united by ineffective production (or dysplasia) of myeloid blood cells and risk of transformation to AML.

[00322] Lymphoma is a group of blood cell tumors that develop from lymphocytes. Exemplary lymphomas include non-Hodgkin lymphoma and Hodgkin lymphoma.

[00323] The disclosure provides for compositions and methods for treating and preventing cancer. In one aspect, the cancer is a hematologic cancer or blood cancer including but is not limited to hematological cancer is a leukemia or a lymphoma. In one aspect, the CAR/TCR/NF κ B-expressing cells of the disclosure may be used to treat cancers and malignancies such as, but not limited to, *e.g.*, acute leukemias including but not limited to, *e.g.*, B-cell acute lymphoid leukemia ("BALL"), T-cell acute lymphoid leukemia ("TALL"), acute lymphoid leukemia (ALL); one or more chronic leukemias including but not limited to, *e.g.*, chronic myelogenous leukemia (CML), chronic lymphocytic leukemia

(CLL); additional hematologic cancers or hematologic conditions including, but not limited to, *e.g.*, B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma, Follicular lymphoma, Hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, Marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, and "preleukemia" which are a diverse collection of hematological conditions united by ineffective production (or dysplasia) of myeloid blood cells, and the like. Further a disease associated with a cancer associate antigen as described herein expression includes, but not limited to, *e.g.*, atypical and/or non-classical cancers, malignancies, precancerous conditions or proliferative diseases expressing a cancer associate antigen as described herein.

[00324] The disclosure provides a method of administering to a subject an effective amount of a cell, *e.g.*, an immune effector cell, or a population thereof, each cell comprising a non-naturally occurring immune receptor (*e.g.*, CAR and/or TCR) and/or NF- κ B stimulatory molecule, optionally in combination with an agent that increases the efficacy and/or safety of the immune cell. In various embodiments, the agent that increases the efficacy and/or safety of the immune cell is selected from the group consisting of (i) a protein phosphatase inhibitor; (ii) a kinase inhibitor; (iii) a cytokine; (iv) an inhibitor of an immune inhibitory molecule; (v) an agent that decreases the level or activity of a T_{REG} cell; (vi) an agent that increase the proliferation and/or persistence of a CAR/NF- κ B stimulatory molecule-modified cells; (vii) a chemokine; (viii) an agent that increases the expression of CARs/TCRs; (ix) an agent that allows regulation of the expression or activity of a CAR; (x) an agent that allows control over the survival and/or persistence of the modified cells; (xi) an agent that controls the side effects of the modified cells; (xii) a Brd4 inhibitor; (xiii) an agent that delivers a therapeutic (*e.g.* sHVEM) or prophylactic agent to the site of the disease; (xiv) an agent that increases the expression of the target antigen against which the CAR is directed; (xv) an adenosine A2a receptor antagonist; and (xvi) any combination of (i)-(xv).

[00325] In some embodiments, the disease to be treated or prevented is a hematologic cancer. In further embodiments, the hematologic cancer is leukemia. Non-limiting examples of acute leukemias include B-cell acute lymphoid leukemia ("BALL"), T-cell acute lymphoid leukemia ("TALL"), acute lymphoid leukemia (ALL); one or more chronic leukemias including but not limited to chronic myelogenous leukemia (CML), chronic

lymphocytic leukemia (CLL); additional hematologic cancers or hematologic conditions including, but not limited to B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma, follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, Marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, nonHodgkin lymphoma, Hodgkin lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, and "preleukemia" which are a diverse collection of hematological conditions united by ineffective production (or dysplasia) of myeloid blood cells, and to disease associated with expression of a tumor antigen described herein include, but not limited to, atypical and/or non-classical cancers, malignancies, precancerous conditions or proliferative diseases expressing a tumor antigen as described herein; and any combination thereof. In another embodiment, the disease associated with a tumor antigen described herein is a solid tumor.

[00326] In some embodiments, the tumor antigen associated with the disease is selected from: CD5; CD19; CD123; CD22; CD30; CD171; CS-1 (also referred to as CD2 subset 1, CRACC, SLAMF7, CD319, and 19A24); C-type lectin-like molecule-1 (CLL-1 or CLECL1); CD33; epidermal growth factor receptor variant III (EGFRviii); ganglioside G2 (GD2); ganglioside GD3 (aNeu5Ac(2-8)aNeu5Ac(2-3)bDGalp(1-4)bDGlc(1-1)Cer); TNF receptor family member B cell maturation (BCMA); Tn antigen ((Tn Ag) or (GalNAc α -Ser/Thr)); prostate-specific membrane antigen (PSMA); Receptor tyrosine kinase-like orphan receptor 1 (ROR1); FmsLike Tyrosine Kinase 3 (FLT3); Tumor-associated glycoprotein 72 (TAG72); CD38; CD44v6; a glycosylated CD43 epitope expressed on acute leukemia or lymphoma but not on hematopoietic progenitors, a glycosylated CD43 epitope expressed on non-hematopoietic cancers, Carcinoembryonic antigen (CEA); Epithelial cell adhesion molecule (EPCAM); B7H3 (CD276); KIT (CD117); Interleukin-13 receptor subunit alpha-2 (IL-13Ra2 or CD213A2); Mesothelin; Interleukin 11 receptor alpha (IL-11Ra); prostate stem cell antigen (PSCA); Protease Serine 21 (Testisin or PRSS21); vascular endothelial growth factor receptor 2 (VEGFR2); Lewis(Y) antigen; CD24; Platelet-derived growth factor receptor beta (PDGFR-beta); Stage-specific embryonic antigen-4 (SSEA-4); CD20; Folate receptor alpha; Receptor tyrosine-protein kinase ERBB2 (Her2/neu); Mucin 1, cell surface associated (MUC1); epidermal growth factor receptor (EGFR); neural cell adhesion molecule (NCAM); Prostase; prostatic acid phosphatase (PAP); elongation factor 2 mutated (ELF2M); Ephrin B2; fibroblast activation protein alpha (FAP); insulin-like growth factor 1 receptor

(IGF-I receptor), carbonic anhydrase IX (CAIX); Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2); glycoprotein 100 (gpl00); oncogene fusion protein consisting of breakpoint cluster region (BCR) and Abelson murine leukemia viral oncogene homolog 1 (Abl) (bcr-abl); tyrosinase; ephrin type-A receptor 2 (EphA2); Fucosyl GM1; sialyl Lewis adhesion molecule (sLe); ganglioside GM3 (aNeu5Ac(2-3)bDCIalp(1-4)bDGlc(1-1)Cer); transglutaminase 5 (TGS5); high molecular weight-melanomaassociated antigen (HMWMAA); o-acetyl-GD2 ganglioside (OAcGD2); tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related (TEM7R); claudin 6 (CLDN6); thyroid stimulating hormone receptor (TSHR); G protein coupled receptor class C group 5, member D (GPRC5D); chromosome X open reading frame 61 (CXORF61); CD97; CD179a; anaplastic lymphoma kinase (ALK); Polysialic acid; placenta-specific 1 (PLAC1); hexasaccharide portion of globoH glycosphingolipid (GloboH); mammary gland differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3 (ADRB3); pannexin 3 (PANX3); G protein-coupled receptor 20 (GPR20); lymphocyte antigen 6 complex, locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame Protein (TARP); Wilms tumor protein (WT1); Cancer/testis antigen 1 (NY-ESO-1); Cancer/testis antigen 2 (LAGE-1a); Melanoma-associated antigen 1 (MAGE-A1); ETS translocation-variant gene 6, located on chromosome 12p (ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, Member 1A (XAGE1); angiopoietin-binding cell surface receptor 2 (Tie 2); melanoma cancer testis antigen-1 (MAD-CT-1); melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; tumor protein p53 (p53); p53 mutant; prostein; surviving; telomerase; prostate carcinoma tumor antigen-1 (PCT A-1 or Galectin 8), melanoma antigen recognized by T cells 1 (MelanA or MART1); Rat sarcoma (Ras) mutant; human Telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoints; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease, serine 2 (TMPRSS2) ETS fusion gene); N-Acetyl glucosaminyl-transferase V (NA17); paired box protein Pax-3 (PAX3); Androgen receptor; Cyclin B1; v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Tyrosinase-related protein 2 (TRP-2); Cytochrome P450 1B 1 (CYP1B 1); CCCTC-Binding Factor (Zinc Finger Protein)-Like (BORIS or Brother of the Regulator of Imprinted Sites), Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3); Paired box protein Pax-5 (PAX5); proacrosin binding protein sp32 (OY-TES1); lymphocyte-specific protein tyrosine kinase (LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint 2 (SSX2); Receptor for Advanced Glycation End

products (RAGE-1); renal ubiquitous 1 (RU1); renal ubiquitous 2 (RU2); legumain; human papilloma virus E6 (HPV E6); human papilloma virus E7 (HPV E7); intestinal carboxyl esterase; heat shock protein 70-2 mutated (mut hsp70-2); CD79a; CD79b; CD72; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR or CD89); Leukocyte immunoglobulin-like receptor subfamily A member 2 (LILRA2); CD300 molecule-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2); lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); and immunoglobulin lambda-like polypeptide 1 (IGLL1), MPL, , Biotin, c-MYC epitope Tag, CD34, LAMP1 TROP2, GFRalpha4, CDH17, CDH6, NYBR1, CDH19, CD200R, Slea (CA19.9; Sialyl Lewis Antigen) Fucosyl-GM1, PTK7, gpNMB, CDH1-CD324, DLL3, CD276/B7H3, IL11Ra, IL13Ra2, CD179b-IGL11, ALK TCRgamma-delta, NKG2D, CD32 (FCGR2A), CSPG4-HMW-MAA, Tim1-/HVCRI, CSF2RA (GM-CSFR-alpha), TGFbetaR2, VEGFR2/KDR, Lews Ag, TCR-beta1 chain, TCR-beta2 chain, TCR-gamma chain, TCR-delta chain, FITC, Leutenizing hormone receptor (LHR), Follicle stimulating hormone receptor (FSHR), Chorionic Gonadotropin Hormone receptor (CGHR), CCR4, SLAMF6, SLAMF4, HIV1 envelope glycoprotein, HTLV1-Tax, CMV pp65, EBV-EBNA3c, influenza A hemagglutinin (HA), GAD, PDL1, Guanylyl cyclase C (GCC), KSHV-K8.1 protein, KSHV-gH protein, auto-antibody to desmoglein 3 (Dsg3), autoantibody to desmoglein 1 (Dsg1), HLA, HLA-A, HLA-A2, HLA-B, HLA-C, HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, HLA-DR, HLA-G, IGE, CD99, RAS G12V, Tissue Factor 1 (TF1), AFP, GPRC5D, claudin18.2 (CLD18A2 OR CLDN18A.2)), P-glycoprotein, STEAP1, LIV1, NECTIN-4, CRIPTO, GPA33, BST1/CD157, low conductance chloride channel, and antigen recognized by TNT antibody.

[00327] In some embodiments, the disease to be treated is an infectious disease including, but not limited to, infection by HIV1, HIV2, HTLV1, Epstein Barr virus (EBV), cytomegalovirus (CMV), adenovirus, adeno-associated virus, BK virus, Human Herpesvirus 6, Human Herpesvirus 8 influenza virus, parainfluenza virus, avian flu virus, MERS and SARS coronaviruses, Crimean Congo Hemorrhagic fever virus, rhino virus, enterovirus, Dengue virus, West Nile virus, Ebola virus, Marburg virus, Lassa fever virus, zika virus, RSV, measles virus, mumps virus, rhino virus, varicella virus, herpes simplex virus 1 and 2, varicella zoster virus, HIV-1, HTLV1, Hepatitis virus, enterovirus, hepatitis B virus, Hepatitis C virus, Nipah and Rift valley fever viruses, Japanese encephalitis virus, mycobacterium tuberculosis, atypical mycobacteria species, Pneumocystis jirovecii,

toxoplasmosis, rickettsia, nocardia, aspergillus, mucor, or candida. In such diseases, the target antigen associated with the disease is selected from: HIV1 envelope glycoprotein, HIV1-gag, HTLV1-Tax, CMV pp65, EBV-EBNA3c, influenza A hemagglutinin (HA) and GAD.

[00328] The disease to be treated or prevented by the methods and compositions of the disclosure can be an immune or degenerative disease, *e.g.*, diabetes mellitus, multiple sclerosis, rheumatoid arthritis, pemphigus vulgaris, ankylosing spondylitis, Hashimoto's thyroiditis, SLE, sarcoidosis, scleroderma, mixed connective tissue disease, graft versus host disease or Alzheimer's disease. In such embodiments, the target antigen associated with the disease is an autoantibody.

[00329] Further non-limiting examples of diseases associated with expression of a target antigen include any one of the following cancers or related conditions: colon cancer, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, cancer of the small intestine, cancer of the esophagus, melanoma, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, solid tumors of childhood, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers, combinations of said cancers, and metastatic lesions of said cancers.

[00330] In certain embodiments of the methods or uses described herein, the CAR/TCR-expressing cell comprising a non-naturally occurring immune receptor (*e.g.*, CAR and/or TCR) and/or NF- κ B stimulatory molecule is administered in combination with an agent that increases the efficacy of the immune effector cell, *e.g.*, one or more of a protein phosphatase inhibitor, a kinase inhibitor, a cytokine, a chemokine, a scFV fragment, a bispecific antibody, an inhibitor of an immune inhibitory molecule; a cellular signaling protein, a viral signaling protein, or an agent that decreases the level or activity of a T_{REG} cell. Non-limiting examples of protein phosphatase inhibitors include a SHP-1 inhibitor and/or an

SHP-2 inhibitor. Non-limiting examples of kinase inhibitors include a CDK4 inhibitor, a CDK4/6 inhibitor (*e.g.*, palbociclib), a BTK inhibitor (*e.g.*, ibrutinib or RN-486), an mTOR inhibitor (*e.g.*, rapamycin or everolimus (RAD001)), an MNK inhibitor, or a dual P13K/mTOR inhibitor. In one embodiment, the BTK inhibitor does not reduce or inhibit the kinase activity of interleukin-2-inducible kinase (ITK). Non limiting examples of an A2a receptor antagonist include Vipadenant. In some embodiments, the agent that inhibits the immune inhibitory molecule may be one or more of an antibody or antibody fragment, an inhibitory nucleic acid, a clustered regularly interspaced short palindromic repeats (CRISPR), a transcription-activator like effector nuclease (TALEN), or a zinc finger endonuclease (ZFN) that inhibits the expression of the inhibitory molecule. In other embodiments of the methods or uses described herein, the agent that decreases the level or activity of the TREG cells is chosen from cyclophosphamide, antiGITR antibody, CD25-depletion, or a combination thereof. In certain embodiments of the methods or uses described herein, the immune inhibitory molecule is selected from the group consisting of PD1, PD-L1, CTLA-4, TIM-3, LAG-3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, TGFR beta, CEACAM-1, CEACAM-3, and CEACAM-5. In other embodiments, the cytokine is chosen from IL2, IL-7, IL-15 or IL-21, or any combination thereof. In other embodiments, the immune effector cell comprising the CAR/TCR and/or NF- κ B stimulating molecule and a second, *e.g.*, any of the combination therapies disclosed herein (*e.g.*, the agent that that increases the efficacy of the immune effector cell) are administered substantially simultaneously or sequentially. In one embodiment the cytokine is administered to the subject simultaneously (*e.g.*, administered on the same day) with or shortly after administration (*e.g.*, administered 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 7 days after administration) of the cell or population of cells comprising a CAR/TCR and/or NF- κ B stimulatory molecule. In other embodiments, the cytokine is administered to the subject after a prolonged period of time (*e.g.*, at least 2 weeks, 3 weeks, 4 weeks, 6 weeks, 8 weeks, 10 weeks, or more) after administration of the cell or population of cells, or after assessment of the subject's response to the cell.

[00331] In other embodiments, the cells expressing a non-naturally occurring immune receptor (*e.g.*, CAR and/or TCR) and/or NF- κ B stimulatory molecule are administered in combination with an agent that ameliorates one or more side effects associated with administration of a cell expressing a CAR/TCR and/or NF- κ B stimulatory molecule. Side effects associated with the CAR/TCR and/or NF- κ B stimulatory molecule)-expressing cell can be chosen from cytokine release syndrome (CRS), hemophagocytic lymphohistiocytosis (HLH) or neurological complications. Examples of such agents include steroids (*e.g.*

prednisone, dexamethasone), IL6R antagonists (*e.g.*, tocilizumab), IL1R antagonists (*e.g.*, anakinra), src kinase inhibitors (*e.g.*, dasatinib or a water soluble salt of dasatinib), a kinase inhibitor (*e.g.*, Ibrutinib), calcineurin inhibitors (*e.g.*, tacrolimus or cyclosporine A) or chemotherapy drugs (*e.g.*, cyclophosphamide, methotrexate or vincristine).

[00332] In one embodiment, the cells expressing a non-naturally occurring immune receptor (*e.g.*, CAR and/or TCR) and/or NF- κ B stimulatory molecule are administered in combination with a low, immune enhancing dose of an mTOR inhibitor. While not wishing to be bound by theory, it is believed that treatment with a low, immune enhancing, dose (*e.g.*, a dose does not completely suppress the immune system but is sufficient to improve immune function) is accompanied by a reduction in PD-1 positive T cells or an increase in PD-1 negative cells. PD-1 positive T cells, but not PD-1 negative T cells, can be exhausted by engagement with cells which express a PD-1 ligand, *e.g.*, PD-L1 or PD-L2.

[00333] Pharmaceutical compositions of the disclosure may comprise a non-naturally occurring immune receptor (*e.g.*, CAR and/or TCR) and/or NF- κ B stimulatory molecule expressing cell, *e.g.*, a plurality of CAR/TCR and/or NF- κ B stimulatory molecule-expressing cells, as described herein, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (*e.g.*, aluminum hydroxide); and preservatives. Compositions of the disclosure can be formulated for intravenous administration. The composition may further comprise a secondary active agent (*e.g.*, an anticancer, antiviral or antibiotic agent).

[00334] Pharmaceutical compositions of the disclosure may be administered in a manner appropriate to the disease to be treated (or prevented). The quantity and frequency of administration will be determined by such factors as the condition of the patient, and the type and severity of the patient's disease. When "an immunologically effective amount," "an anti-tumor effective amount," "a tumor-inhibiting effective amount," or "therapeutic amount" or "anti-infective" is indicated, the amount of the compositions of the disclosure to be administered 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) as the case may be. It can generally be stated that a pharmaceutical composition comprising the immune effector cells (*e.g.*, T cells, NK cells) described herein may be administered at a dosage of 10^4 to 10^9 cells/kg body weight, in some instances 10^5 to 10^6

cells/kg body weight, including all integer values within those ranges. T cell compositions may also be administered multiple times at these dosages. The cells can be administered by using infusion techniques that are commonly known in immunotherapy (see, *e.g.*, Rosenberg *et al.*, New Eng. J. of Med. 319:1676, 1988).

[00335] In certain aspects, it may be desired to administer activated immune effector cells (*e.g.*, T cells, NK cells) to a subject and then subsequently redraw blood (or have an apheresis performed), activate immune effector cells (*e.g.*, T cells, NK cells) therefrom according to the disclosure, and reinfuse the patient with these activated and expanded immune effector cells (*e.g.*, T cells, NK cells). This process can be carried out multiple times every few weeks. In certain aspects, immune effector cells (*e.g.*, T cells, NK cells) can be activated from blood draws of from 10cc to 400cc. In certain aspects, immune effector cells (*e.g.*, T cells, NK cells) are activated from blood draws of 20cc, 30cc, 40cc, 50cc, 60cc, 70cc, 80cc, 90cc, or 100cc.

[00336] In some embodiments, subjects may undergo leukapheresis, wherein leukocytes are collected, enriched, or depleted *ex vivo* to select and/or isolate the cells of interest, *e.g.*, T cells. These T cell isolates may be expanded by methods known in the art and treated and/or transformed such that one or more constructs of the disclosure may be introduced, thereby creating a CAR-T or TCR-T cell of the disclosure coexpressing an accessory module encoding a NF- κ B activator. Subjects in need thereof may subsequently undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. In certain aspects, following or concurrent with the transplant, subjects receive an infusion of the expanded CAR-T cells or TCR-T cells of the disclosure that optionally coexpress an accessory module encoding a NF- κ B activator. In an additional aspect, expanded cells are administered before or following surgery.

[00337] Kits to practice the disclosure are also provided. For example, kits for treating a cancer in a subject, or making a cell that expresses a non-naturally occurring immune receptor (*e.g.*, CAR and/or TCR) and/or NF- κ B stimulatory molecule disclosed herein. The kits may include at least one nucleic acid molecule or vector encoding a non-naturally occurring immune receptor (*e.g.*, CAR and/or TCR) and/or NF- κ B stimulatory molecule along with a method to introduce the nucleic acid into the immune effector cells. The kit may include a virus comprising a nucleic acid encoding a non-naturally occurring immune receptor (*e.g.*, CAR and/or TCR) and/or NF- κ B stimulatory molecule and chemicals, such as polybrene, to enhance the virus transduction. The kit may contain components for isolation of T cells for expressing a non-naturally occurring immune receptor (*e.g.*, CAR and/or TCR).

Alternatively, the kit may contain immune effector cells (*e.g.*, T cells or NK cells) or stem cells expressing a non-naturally occurring immune receptor (*e.g.*, CAR and/or TCR) and/or NF- κ B stimulatory molecule. More than one of the disclosed non-naturally occurring immune receptor (*e.g.*, CAR and/or TCR) and/or NF- κ B stimulatory molecules can be included in the kit. The kit can include a container and a label or package insert on or associated with the container.

[00338] Suitable containers include, for example, bottles, vials, syringes, *etc.* The containers may be formed from a variety of materials such as glass or plastic. The container typically holds a composition including one or more of the nucleic acid molecules, viruses, vectors, T cells *etc.* In several embodiments the container may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). A label or package insert indicates that the composition is used for treating the particular condition. The label or package insert typically will further include instructions for use of a disclosed components, for example, in a method of treating or preventing a tumor or of making a CAR-T cell. The package insert typically includes instructions customarily included in commercial packages of therapeutic products that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products. The instructional materials may be written, in an electronic form (such as a computer diskette or compact disk) or may be visual (such as video files). The kits may also include additional components to facilitate the particular application for which the kit is designed. Thus, for example, the kit may additionally contain means for measuring the expression of a CAR and/or NF- κ B stimulatory molecule on or in T cells or of determining the number or percentage of T cells that express the CAR and/or NF- κ B stimulatory molecule or of determining the functionality of cells. The kits may additionally include buffers and other reagents routinely used for the practice of a particular method. Such kits and appropriate contents are well known to those of skill in the art.

[00339] The disclosure is further described by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the disclosure should in no way be construed as being limited to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

EXAMPLES

[00340] Cell lines engineered to express luciferases (*e.g.*, GLuc or NLuc) for measuring cytotoxicity of different constructs targeting different cell surface and intracellular antigens are provided in **Table A**. Cell lines used in this experiments, target antigens on the cells lines and their growth media are shown in the following **Table A**. Cells were cultured at 37°C, in a 5% CO₂ humidified incubator. The cell lines were obtained from ATCC, NIH AIDS reagent program or were available in the laboratory.

[00341] **Table A:**

Cell line	Culture Conditions	Exemplary CAR Target Antigens Expressed
BC-1	RPMI, 20% FCS	BCMA, GPRC, CD138
BC-3	RPMI, 20% FCS	BCMA, GPRC, CD138
BCBL-1	RPMI, 20% FCS	GPRC, CD138
JSC-1	RPMI, 20% FCS	GPRC, CD138
MM1S	RPMI, 10% FCS	CD38, GPRC, CD44, CD200R
U266	RPMI, 10% FCS	BCMA, WT1/HLA-A2+, CS1, CLL1, CD138, c-MET, IL6R, CD179b, NY-ESO/HLA-A2, NYBR, LAMP1
L363	RPMI, 10% FCS	BCMA, GPRC, WT1/HLA-A2+, CS1, CLL1, CD138, NY-ESO/HLA-A2, NYBR, LAMP1
K562	RPMI, 10% FCS	CD33, IL1Ra, TnAg
BV173	RPMI, 10% FCS	CD123, CD179b, IL1Ra, WT1/HLA-A2+, CXCR4, FLT3, CD179a
Nalm6	RPMI, 10% FCS	CD19, CD20, CD22, CD179b, CD179a
HL60	RPMI, 10% FCS	CD33, CD34, CLL1, IL6R, CD32, CD179
U937	RPMI, 10% FCS	CD4, CLL1
RS:411	RPMI, 20% FCS	CD19, Folate Receptor beta (FRbeta), TGFbeta, CD179b, NKG2DNKG2D, FLT3, CD179a
MV:411	RPMI, 10% FCS	FLT3, CD123, FRbeta
Raji	RPMI, 10% FCS	CD19, CD20, CD22, BCMA, CD38, CD70, CD79, Folate Receptor beta, CLL1
HEL-92.1.7 (HEL)	RPMI, 10% FCS	MPL, CD33, CD32, CD200R
Jurkat	RPMI, 10% FCS	TnAg, TSLRP, TSHR, CD4, CD38
Daudi	RPMI, 10% FCS	BCMA, FRbeta
REC-1	RPMI, 10% FCS	NKG2DNKG2D, ROR1
KG-1	RPMI, 20% FCS	CD33, CD34, CD123, TSLRP
CEM	RPMI, 10% FCS	CD5, CD43
U937	RPMI, 10% FCS	CD4, CLL1
LAMA5	RPMI, 10% FCS	WT1/HLA-A2
A549	DMEM, 10% FCS	ROR1, CD22, TIM1, CDH17
HT29	DMEM, 10% FCS	EGFR, SLEA, c-MET
Molm-13	RPMI, 20% FCS	FLT3, IL6R, LAMP1, TSLRP, CD4, CSF2RA, CXCR4, IL6R, CSF2RA, GPC3
A431	DMEM, 10% FCS	EGFR, Folate Receptor Alpha, Her3
P19	DMEM, 10% FCS	SSEA
THP-1	RPMI, 10% FCS	CD32, CD33, CXCR4, CD123, CD44, IL6R, Folate Receptor beta, CD70, LAMP1, FLT3, CSF2RA

Cell line	Culture Conditions	Exemplary CAR Target Antigens Expressed
U87MG	DMEM, 10% FCS	CD276, gpNMB, IL13RA2
LoVo	DMEM, 10% FCS	Tissue Factor, CDH17, EGFR
SKOV-3	DMEM, 10% FCS	Folate Receptor alpha (FR1), FSHR, Her2, Her3, LHR, MSLN, TIM1, EPCAM
NCI-H1993	DMEM, 10% FCS	EGFR
Kasumi-1	RPMI, 20% FCS	CLEC5A, PR1/HLA-A2, TGFbeta,
Jeko-1	RPMI, 20% FCS	BCMA, ROR1
PC-3	DMEM, 10% FCS	CGH, TROP2, PSCA, PSMA. EPCAM, FSHR, CLDN18A2 (CLDN18.2)
HeLa	DMEM, 10% FCS	EGFR, FR1, MSLN, TSHR
LnCap	DMEM, 10% FCS	EGFR, FSHR, PSCA, PSMA, CD22, Her3, CD22, LHR, CLDN18A2 (CLDN18.2)
OVCAR-3	DMEM, 10% FCS	B7H4, CDH6, DLL3, FR1, FSH, LHR, MSLN, PTK7, TnAg, TSHR, L1CAM
MEL-624	DMEM, 10% FCS	CDH19, GD2, GD3, gp100/HLA-A2, gpNMB, HMWMAA, NYESO/HLA-A2, MART1/HLA-A2
LS174-T	DMEM, 10% FCS	CEA
MEL-526	DMEM, 10% FCS	GD2
MDA-MB231	DMEM, 10% FCS	CD324, Muc1
L1236	RPMI, 20% FCS	CD30, CD23, PDL1
L428	RPMI, 20% FCS	CD30, CD123, CCR4, PDL1
L540	RPMI, 20% FCS	CD30, CCR4, PDL1
Molt-16	RPMI, 20% FCS	IL1ra, NKG2DNKG2D
CEM	RPMI, 10% FCS	CD5
MG-63	DMEM, 10% FCS	IL13RA2
Karpas-299	RPMI, 20% FCS	Alk, GPRC, PDL1
MCF7	DMEM, 10% FCS	B7D4, CD276, TROP2, Her3, Muc1, LewisY, LHR
AA-2	RPMI, 10% FCS	HIV1 env glycoprotein (gp120)
HL2/3	DMEM, 10% FCS	HIV1 env glycoprotein (gp120)
TF228.1.16	DMEM, 10% FCS	HIV1 env glycoprotein (gp120), CCR4
TT	DMEM, 10% FCS	TGF-Beta, TSHR, GFRalpha4
DMS79	RPMI, 10% FCS	Fucosyl-GM1, Slea (CA19.9; Sialyl Lewis Antigen)
LAN-5	DMEM, 10% FCS	ALK, DLL3, GFRalpha4, FUCOSYL-GM1
PEER1	RPMI, 10% FCS	TSHR
SK-MEL-37	DMEM, 10% FCS	DLL3, GD2
F9	DMEM, 10% FCS	SSEA
HepG2	DMEM, 10% FBS	GPC3, AFP/HLA-A2

[00342] Jurkat cell line (clone E6-1) engineered with a NFAT-dependent EGFP (or GFP) reporter gene was a gift from Dr. Arthur Weiss at University of California San Francisco and have been described to study CAR-signaling ((Wu, CY *et al.*, Science 350:293-302,2015). Jurkat cells were maintained in RPMI-1640 medium supplemented with 10% FBS, penicillin and streptomycin.

[00343] *Generation of lentiviral vectors encoding chimeric antigen receptors against MPL*

[00344] The pLENTI-Blast vector was derived from pLenti6v5gw_lacZ vector (Invitrogen; ThermoFisher Scientific) by removal of the LacZ gene. pLenti-MP2 was a gift from Pantelis Tsoulfas (Addgene plasmid # 36097) and was used to generate pLenti-EF1 α or pLenti-EF1 α [SEQ ID NO:3837] lentiviral vector by replacement of the CMV promoter with human EF1 α promoter using standard molecular biology techniques. pLenti-EF1 α -DWPRE [SEQ ID NO:3838] was derived from the pLENTI-EF1 α vector by deletion of WPRE sequence. An internal Sac II fragment was deleted from the EF1 α promoter to generate EF1 α (EF1 α)-D-SACII-Promoter (SEQ ID NO: 3842). The psPAX2 vector was a gift from Didier Trono (Addgene plasmid # 12260). The pLP/VSVG envelope plasmid and 293FT cells were obtained from Invitrogen (ThermoFisher Scientific). The retroviral transfer vector MSCVneo, MSCVhygro, and MSCVpac and the packaging vector pKAT were obtained from Dr. Robert Illaria's laboratory. phRGTK Renilla Luciferase plasmid was from Promega.

[00345] The generation of Chimeric antigen receptor containing vectors with BBz, CD28z and z-K13 backbones, the generation and use of GGS-NLuc fusion proteins, and the generation and use of luciferase (*e.g.*, GLuc) reporter cell lines for measurement of cellular cytotoxicity using the Matador assays have been described (PCT/US2017/024843, PCT/US2017/025602 and PCT/US2017/052344).

[00346] ***Lentivirus and retrovirus vectors***

[00347] Lentiviruses were generated by calcium phosphate based transfection in 293FT cells essentially as described previously (Matta H et al, Cancer biology and therapy. 2(2):206-10. 2003). 293FT cells were grown in DMEM with 10% FCS 4 mM L-Glutamine, 0.1 mM MEM Non-Essential Amino Acids, and 1 mM MEM Sodium Pyruvate (hereby referred to as DMEM-10). For generation of lentivirus, 293FT cells were plated in 10 ml of DMEM-10 medium without antibiotics in a 10 cm tissue culture plate so that they will be approximately 80 confluent on the day of transfection. The following day, the cells were transfected by calcium phosphate transfection method using 10 μ g of lentiviral expression plasmid encoding different genes, 7.5 μ g of PSPAX2 plasmid and 2 μ g of PLP/VSVG plasmid. Approximately 15-16 hours post-transfection, 9 ml of media was removed and replaced with 5 ml of fresh media. Approximately, 48 hours post- transfection, 5 ml of supernatant was collected (first collection) and replaced with fresh 5 ml media. Approximately 72 hrs post-transfection, all media was collected (second collection, usually around 6 ml). The collected supernatants were pooled and centrifuged at 1000 rpm for 1 minute to remove any cell debris and non-adherent cells. The cell-free supernatant was

filtered through 0.45 µm syringe filter. In some cases, the supernatant was further concentrated by ultra-centrifugation at 18500 rpm for 2 hours at 4°C. The viral pellet was re-suspended in 1/10 of the initial volume in XVIVO medium. The virus was either used fresh to infect the target cells or stored frozen in aliquots at -80°C.

[00348] *Infection of T cells and PBMC*

[00349] Buffy coat cells were obtained from healthy de-identified adult donors from the Blood Bank at Children Hospital of Los Angeles and used to isolate peripheral blood mononuclear cells (PBMC) by Ficoll-Hypaque gradient centrifugation. PBMC were either used as such or used to isolate T cells using CD3 magnetic microbeads (Miltenyi Biotech) and following the manufacturer's instructions. PBMC or isolated T cells were re-suspended in XVIVO medium (Lonza) supplanted with 10 ng/ml CD3 antibody, 10ng/ml CD28 antibody and 100 IU recombinant human-IL2. Cells were cultured at 37°C, in a 5% CO₂ humidified incubator. Cells were activated in the above medium for 1 day prior to infection with lentiviral vectors. In general, primary cells (*e.g.* T cells) were infected in the morning using spin-infection (1800 rpm for 90 minutes at 37°C with 300µl of concentrated virus that had been re-suspended in XVIVO medium in the presence of 8 µg/ml of Polybrene® (Sigma, Catalog no. H9268). The media was changed in the evening and the infection was repeated for two more days for a total of 3 infections. After the 3rd infection, the cells were pelleted and resuspended in fresh XVIVO media containing 10ng/ml CD3 antibody, 10ng/ml CD28 antibody and 100 IU recombinant human-IL2 and supplemented with respective antibiotics (if indicated) and place in the cell culture flask for selection, unless indicated otherwise. Cells were cultured in the above medium for 10-15 days in case no drug selection was used and for 20-30 days in case drug-selection was used. In cases, where cells were infected with a lentivirus expressing EGFP, they were expanded without drug-selection or flow-sorted to enrich for EGFP-expressing cells. For infection of cancer cell lines, approximately 500,000 cells were infected with 2 ml of the un-concentrated viral supernatant in a total volume of 3 ml with Polybrene® (Sigma, Catalog no. H9268). Then next morning, the cells were pelleted and resuspended in the media with respective antibiotics and place in the cell culture flask for selection.

[00350] Essentially a similar procedure as described above for lentivirus vector production was used for generation of retroviral vectors with the exception that 293FT cells were generally transfected in 10 cm tissue culture plates in 10 ml of DMEM-10 medium using 10 µg of retroviral construct, 4µg of pKAT and 2µg of VSVG plasmid. The virus

collection and infection of target cells was carried out essentially as described above for lentiviral vectors.

[00351] ***Antibodies and drugs***

[00352] Blinatumomab was obtained from Amgen. Digitonin was purchased from Sigma (Cat. no D141) and a stock solution of 100mg/ml was made in DMSO. A diluted stock of 1 mg/ml was made in PBS. Final concentration of digitonin used for cell lysis was 30µg/ml unless indicated otherwise.

[00353] ***ELISA***

[00354] Human IL2, IFN γ , IL6 and TNF α were measured in the cell culture supernatant of CAR-expressing Jurkat-NFAT-GFP effector cells or T cells that had been co-cultured with the specific target cell lines for 24 to 96 hours using commercially available ELISA kits from R&D systems (Minneapolis, MN) and BD Biosciences and following the recommendations of the manufacturer.

[00355] ***FACS analysis for detecting expression of CAR***

[00356] Mouse Anti-Human c-Myc APC-conjugated Monoclonal Antibody (Catalog # IC3696A) was from R&D Systems (Minneapolis, MN). Biotinylated protein L was purchased from GeneScript (Piscataway, NJ), reconstituted in phosphate buffered saline (PBS) at 1 mg/ml and stored at 4°C. Streptavidin-APC (SA1005) was purchased from ThermoFisher Scientific.

[00357] For detection of CARs using Myc staining, 1×10^6 cells were harvested and washed three times with 3 ml of ice-cold $1 \times$ PBS containing 4% bovine serum albumin (BSA) wash buffer. After wash, cells were resuspended in 0.1 ml of the ice-cold wash buffer containing 10 µl of APC-conjugated Myc antibody and incubated in dark for 1 hour followed by two washings with ice cold wash buffer.

[00358] For detection of CARs using Protein L staining, 1×10^6 cells were harvested and washed three times with 3 ml of ice-cold $1 \times$ PBS containing 4% bovine serum albumin (BSA) wash buffer. After wash, cells were resuspended in 0.1 ml of the ice-cold wash buffer containing 1 µg of protein L at 4°C for 1 hour. Cells were washed three times with ice-cold wash buffer, and then incubated (in the dark) with 10µl of APC-conjugated streptavidin in 0.1 ml of the wash buffer for 30 minutes followed by two washings with ice cold wash buffer. FACS was done using FACSVerse analyzer from BD Biosciences.

[00359] ***Cell death assay***

[00360] To measure cell death, a novel assay based on ectopic cytosolic expression of Gluc, NLuc and other luciferases was utilized as described in PCT/US2017/052344 “A Non-

Radioactive Cytotoxicity Assay". The method involves expression of a reporter in a target cells in a manner so that it is preferentially retained within the healthy cells but is either released from dead and dying cells or whose activity can be preferentially measured in dead and dying cells. The preferred reporter for this assay are 1) non-secreted forms of luciferases from the copepods, such as *Gaussia princeps*, 2) engineered luciferase reporters from deep sea shrimp, such as NanoLuc. The sequence of several such exemplary reporter vectors is provided in SEQ ID NO: 3845 to SEQ ID NO: 3851. The above vectors were used to generate retrovirus and lentiviruses which in turn were used to generate polyclonal population of several target cell lines stably expressing GLuc, NLuc, or TurboLuc following selection with appropriate antibiotics. Unless indicated otherwise, the target cells stably expressing the different luciferases were plated in triplicate in a 384 well plate in the media used for growing the target cells. Target cells which grow in suspension were generally plated at a concentration of $2-3 \times 10^4$ per well, while target cells which grow as adherent monolayers were plated at a concentration of $1-2 \times 10^4$ per well. Unless indicated otherwise, the target cells were cocultured with the genetically modified T cells (*i.e.* those expressing CAR) at an Effector: Target (E:T) ratio varying from 1: 1 to 10:1 for 4 hours to 96 hours. In the case target cells grow as adherent cells (*e.g.*, HeLa cells), they were allowed to attach to the bottom of the wells overnight before the T cells were added. T cells mediated induction of lysis of target cells was assayed by increase of luciferase activity as measured by BioTek synergy plate reader by directly injecting $0.5 \times$ CTZ assay buffer containing native coelenterazine (Nanolight) as described below.

[00361] CTZ assay

[00362] A 100X stock solution of native coelenterazine (CTZ; Nanolight, cat # 303) was made by dissolving 1mg of lyophilized CTZ powder in 1.1 ml of 100% Methanol supplemented with 30 μ l of 6N HCl to avoid oxidation of CTZ with time. To make CTZ assay buffer, the 100X stock solution of CTZ was diluted to 0.5X concentration in PBS. Unless indicated otherwise, a total volume of 15 μ l of the CTZ assay buffer (as prepared above) was added to each well of a 384-well white plate (Greiner, 384 well white plate cat # 781075) containing cells expressing the non-secretory form of the luciferase in approximately 50-60 μ l volume of medium and plates were read for luminescence using BioTek synergyH4 plate reader. For 96 well plates, cells were plated in 200 μ l of media and approximately 50 μ l of 0.5X CTZ assay buffer was added. Unless indicated otherwise, the 0.5X CTZ assay buffer was used for assaying the activity of GLuc, TurboLuc16, and MLuc7. The CTZ assay buffer (diluted to 0.125X concentration) was also used for measurement of NLuc activity in some

experiments. In general, unless indicated otherwise, the volume of 0.5X CTZ assay buffer added was approximately 1/4th of the volume of the liquid in the well containing the cells, although the assay also worked when the 0.5X CTZ assay was added to the media containing the cells in 1:1 volume. Gluc activity in wells containing media alone (Med) and in wells in which target cells were incubated with T cells that were not infected with any CAR construct (T-UI) were used as controls, where indicated.

[00363] *Assay to detect the expression of antigens on target cells and to determine the antigen binding activity of various of antigen binding moieties used in the construction of the CARs and BiTes*

[00364] The expression of antigens on target cells was determined by bioinformatics approaches in combination with immunostaining with antigen specific antibodies or a highly sensitive antigen detection assay as described in PCT/US2017/025602 and incorporated herein in its entirety by reference. This assay involves the fusion of a GLuc or NLuc reporter fragment to the antigen binding domain of an antibody, a scFv, a vHH or any other antigen binding fragment or any receptor and ligand. The resulting fusion protein is incubated with the target cells expressing the test antigen and the binding of the fusion protein is determined by addition of coelenterazine or other suitable substrate of the luciferase reporter.

[00365] *Generation of a Diverse Pool of CAR T cells*

[00366] The above assays were used to screen the different antigen binding modules (e.g. scFv, vHH, receptors, ligands) used in the construction of the CARs of this invention and the antigen binding modules that were found to show specific binding activity were selected for construction of the CARs. Furthermore, some of the scFV fragments were also selected based on their known activity in the literature or in our laboratory.

[00367] It is possible that different CARs or subset of CARs are optimally suited for different disease conditions depending on various factors including, but not limited to, the prevalence and level of expression of the target antigen on disease causing and disease-associated cells, disease burden and rate of progression of the disease. Different CARs may be optimally suited even for a single disease condition in different patients depending on their efficacy and toxicity profile and the condition of the patient. The disclosure provides a solution to the significant technical and logistical hurdles to generating a diverse adoptive immune response.

[00368] Normal TCR diversity is produced by gene rearrangement. Rigorous positive and negative selection processes in the thymus ensure that only T cells expressing the $\alpha\beta$ TCR that are restricted to recognizing self-peptides/MHC within a low affinity range can

populate the periphery. Thus, the thymic environment allows the generation of a pool of $\alpha\beta$ T cells that are self-restricted, but not self-reactive.

[00369] Generating a diverse pool of CAR-T cells from different antigen binding domains is limited by the technical and financial hurdles of generating and testing multiple antigen binding domains. More importantly, as each of the antigen binding domains (*e.g.*, vL and vH fragments of an antibody) has a potential of binding other antigens and causing off-target toxicity, a diverse pool of CARs based only on a plurality of antigen binding domains potentially has an increased risk of toxicity. Therefore, the potential diversity of such a pool would have to be limited to reduce off-target toxicity. The current disclosure overcomes this problem by generating a diverse pool of CARs from a single or a few antigen binding domains by attaching them to different variants of TCR chains, signaling domains and backbones. The diversity of the CARs pool is further increased by the use of different linkers. The diversity of T cells expressing the pool can be further increased by use of different accessory modules described in the disclosure.

[00370] This diverse pool of CARs can be used to provide a diverse immune response against disease causing or disease associated cells expressing the said antigen. Alternatively, the diverse pool of CARs can be optionally DNA barcoded using techniques known in the art and subsequently used to select a single or a subgroup of CARs with optimal biological and clinical characteristics. These characteristics may include but are not limited to, performance in the *in vitro* biological assays (*e.g.*, cytotoxicity, cytokine secretion, binding affinity, cell surface expression, off-target effects, T cell proliferation, expression of exhaustion markers and terminal differentiation *etc.*), performance in the *in vivo* assays (*e.g.*, survival, tumor reduction, T cell persistence, T cell expansion *etc.*) and clinical experience (*e.g.*, disease remission, relapse rate, toxicities, *etc.*). The CARs of the disclosure can be used singly or in combination with other CARs and other natural and synthetic immune receptors known in the art to generate a diverse pool of immune effector cells for the prevention and treatment of various disease conditions caused by or associated with cells expressing their target antigens.

[00371] **Use of *in vitro* and *vivo* selection to select CARs with desired properties.** A pool of CARs targeting CD19 (SEQ ID NO: 1594-1608, 1016-1026, 1900-1910) are targeted to the TRAC locus in T cells using TRAC gRNA and techniques known in the art. The targeting vector also carry DNA barcodes located downstream of the stop codon of the CAR inserts. T cells can be derived from peripheral blood. In an alternate embodiment, T cells are derived from a single clone of iPSC or hematopoietic stem cells using techniques known in the art. T cells expressing the pool of CARs are co-cultured with RAJI cells *in vitro* for 1 to

21 days. Aliquots of the CAR-T cell pools are collected before the culture with the target cells and on different days after co-culture. Samples are subjected to next generation sequencing to determine the relative frequency of different CARs following exposure to the target cells. Bioinformatics analyses is used to determine the CARs that are associated with better proliferative response following co-culture with the target cells. Essentially a similar approach is used to determine the CARs that confer higher proliferative potential on T cells *in vivo* and/or persist long term *in vivo* and/or are present at higher frequency when normalized for their frequency in the starting T cell population in surviving animals as compared to animals that succumb to tumor challenge. In alternate embodiment of the disclosure, essentially a similar approach is used on human clinical samples to identify CARs that are associated with different properties and/or outcomes including but not limited to better long term survival, lower incidence of cytokine release syndrome, lower neurotoxicity and/or higher long term persistence. Such CARs can be subsequently used, either singly or in various combinations, to develop different CARs subpools, containing CARs targeting the same or different antigen binding domains, with diverse properties for the treatment of different disease conditions and different patients. In other enablements, the CAR-T cells are exposed to their target cell line and then sorted into different sets based on the degree of intracellular IFN γ as determined by flow cytometry. The frequency of different CARs in the low vs high IFN γ population is determined by next generation sequencing and normalized to their frequency in the control CAR-T cell population, *i.e.*, CAR-T cells that have not been exposed to the target cell line or are exposed to a cell line that does not express the target of CARs. From this analysis, CARs that are associated with different levels of IFN γ production can be determined. A similar approach is used to screen for and select CARs with any or a combination of desired properties or attributes including but not limited to, lower expression of exhaustion markers, lower expression of markers of terminal differentiation and/or higher expression of markers of cytotoxicity.

[00372] ***Use of NEMO-mutants to provide costimulation***

[00373] The mouse NEMO-K270A (SEQ ID NO: 992) is known to activate NF- κ B constitutively. To demonstrate the ability of this mutant to provide costimulation to T cells, CD3⁺ve T cells were cultured in X-VIVO medium (Lonza) supplanted with 10 ng/ml soluble anti-CD3, 10 ng/ml soluble anti-CD28 and 100 IU recombinant human-IL2. Cells were cultured at 37°C, in a 5% CO₂ humidified incubator, and after 1 day infected with a lentiviral vector (pLENTI-EGFP-Blasticidin) expressing EGFP and lentiviral vectors expressing mouse NEMO-K270A mutants (pLENTI-mNEMO-K270A-FLAG-Blasticidin and pLENTI-

mNEMO-K270A-HA-Blasticidin), or mouse NEMO-wt (pLENTI-mNEMO-FLAG-Blasticidin). The sequences of mNEMO-K270A and mNEMO-wt are provided in SEQ ID NOs: 992 and 991, respectively. Approximately 1 day post-infection, cells were selected with blasticidin and cell numbers calculated periodically. T cells infected with lentiviruses encoding the mouse NEMO-K270A mutants (pLENTI-mNEMO-K270A-FLAG-Blasticidin and pLENTI-mNEMO-K270A-HA-Blasticidin) were shown to proliferate more vigorously as compared to T cells infected with lentiviruses encoding EGFP or mouse NEMO-wt (pLENTI-mNEMO-FLAG-Blasticidin).

[00374] The human NEMO is longer than mouse NEMO and human NEMO-K277A (hNEMO-K277A; SEQ ID NO: 979) mutant corresponds to mouse NEMO-K270A (mNEMO-K270A) mutant. To test whether hNEMO-K277A mutant also activates NF- κ B, expression vector (pCDNA3) encoding this mutant were generated. In addition, expression constructs encoding several other mutants of hNEMO in which Lys (K) at amino acid residue 277 was replaced by different amino acid residues (*e.g.*, K277Q, K277T, K277I, K277N, K277S, K277M, K277G, K277R were generated). The different constructs were transfected in 293FT cells along with an NF- κ B-Luciferase reporeter construct and a RSV-LacZ (normalization control) reporter construct and tested for their ability to activate NF- κ B using assay described previously. **Fig. 3** shows strong activation of NF- κ B by mNEMO-K270A, hNEMO-K277A and weak activation by hNEMO-K277I and hNEMO-K277G mutant. In a similar experiment, the hNEMO-K277L and hNEMO-K277A-DeltaV249-K255 mutants also showed NF- κ B activation when transfected into 293FT cells. The hNEMO-K277A-DeltaV249-K255 mutant lacks the aminoacid residues V249-K255 of human NEMO and also carries the K277A mutation. These results suggest that constitutive active mutants of NEMO can be rapidly generated and identified by mutating mouse NEMO K270 residue and human NEMO K277 residue. A similar approach can be used to generate mutants at other NEMO residues that have the ability to activate NF- κ B.

[00375] FMC63 based CD19 CAR CAR construct were generated that coexpressed wither full length hNEMO-K277A or hNEMO-L753 mutant (encoding amino acids 1-251) in fusion with an N-terminal FKBPx2 dimerizer domain. The constructs were transfected into 293FT cells along with an NF- κ B-Luciferase reporeter construct and a RSV-LacZ reporter construct. Approximatley 8 hours, post-transfection, cells were left untreated or treated with AP20187 (100 nM). After approximately 72 hours, cell lysates were prepared and analyzed for NF- κ B luciferase and LacZ activities as described previously. NF- κ B-Luc activity was normalized for LacZ activity to control for difference in transfection efficiency. Results

showed that treatment with AP20187 led to increase in NF- κ B activity in 293FT cells transfected with CAR encoding constructs co-expressing both FKBPx2-hNEMO-K277A (SEQ ID NO: 1006) and FKBPx2-hNEMO-L753 (SEQ ID NO: 1007) mutants. These results demonstrate the ability to activate NF- κ B in an inducible manner in a CAR or TCR or chimeric TCR construct by coexpression of full length NEMO or its deletion mutants in fusion with a dimerizer domain followed by addition of a dimerizer.

[00376] J-N-G cells are infected with CD19-directed FMC63 based 1st generation CARs coexpressing FKBPx2-hNEMO-K277A or FKBPx2-hNEMO-L753. Cells are cocultured with RAJI target cells in the absence and presence of AP20187 compound and shown to induce EGFP expression, demonstrating that FKBPx2-hNEMO-K277A or FKBPx2-hNEMO-L753 can be co-expressed with a CAR without interfering with its activity.

[00377] In addition to NEMO, a number of other cellular proteins are known to activate NF- κ B constitutively and can be used in alternate embodiment of the invention to provide costimulation to T cells for the purpose of adoptive cellular therapies. Exemplary proteins include TCL-1A (SEQ ID NO: 1005) and constitutive active mutants of IKK α /IKK1 (IKK1-S176E-S180E; SEQ ID NO: 1004), IKK β /IKK2 (IKK2-S177E-S181E; SEQ ID NO: 1002) and MYD88-L265P (SEQ ID NO: 1000). In an embodiment, these proteins are expressed without a dimerizer domain to provide constitutive costimulation to T cells for the purpose of adoptive cellular therapy. These proteins can be expressed in the T cells using any vector (*e.g.*, lentiviral, retroviral, AAV or sleeping beauty transposon vectors) or non-vector (DNA or RNA transfection) method of gene delivery known in the art. Alternatively, these proteins can be expressed by alteration of their genomic copies using techniques of gene altering (*e.g.*, Cas9, Talons, Zn finger nucleases) known in the art. In an exemplary embodiment, one or more genomic copies of hNEMO are mutated to hNEMO-K277A using homologous recombination in T cells using techniques known in the art.

[00378] The expression of these costimulatory proteins can be controlled by expressing them using inducible promoters known in the art, such as Tet-inducible promoter or RheoGene system. In an embodiment, hNEMO-K277A mutant and hNEMO-K277A-DeltaV249-K255 are cloned in the pSLIK-Tet-On vector (Gopalakrishnan et al, Clinical Cancer Res; 19(18), 2013) and the resulting virus is used to infect T cells. Treatment of T cells with doxycycline is shown to induce hNEMO-K277A and hNEMO-K277A-DeltaV249-K255 expression and NF- κ B activity. NF- κ B activity is measured by AlexaFluor-conjugated Phospho-I κ B α antibody and flow cytometry.

[00379] In alternate embodiments of the invention, other NF- κ B activating proteins or their signaling domains (*e.g.*, IKK1, IKK2, RIP, *etc.*) are expressed as fusion with a dimerizer domain to provide costimulation to T cells in an inducible manner. The use of these constitutive or inducible NF- κ B activating proteins of the invention is not limited to providing costimulation to T cells as they can be used to provide costimulation to other immune cells (*e.g.*, NK cells, dendritic cells, antigen presenting cells *etc.*) where NF- κ B activation is known to enhance their function. As NF- κ B is known to protect against apoptosis and promote cell survival, these constitutive and inducible NF- κ B activating proteins can be also used in cell engineering to enhance the survival of cells used in biological products manufacturing. In an exemplary embodiment, hybridoma cells are engineered to express hNEMO-K277A, hNEMO-K277A-DeltaV249-K255 (SEQ ID NO: 7769), K13, IKK α /IKK1 (IKK1-SS/EE; SEQ ID NO: 1004), IKK β /IKK2 (IKK2-S177E-S181E; SEQ ID NO: 1002) or MYD88-L265P (SEQ ID NO: 1000) constitutively to enhance their proliferation and ability to grow at high cell density.

[00380] **NF- κ B activators for T cell adoptive cell therapy.**

[00381] Buffy coat cells are obtained from healthy de-identified adult donors from a Blood Bank and used to isolate peripheral blood mononuclear cells (PBMC) by Ficoll-Hypaque gradient centrifugation. T cells are isolated using CD3 microbeads (Miltenyi), cultured in XVIVO 15 medium supplemented with CD3/CD28 Dynabeads and 50 IU/ml of recombinant IL2. Alternatively, T cells are re-suspended in XVIVO medium (Lonza) supplanted with 10 ng/ml CD3 antibody, 10 ng/ml CD28 antibody and 100 IU recombinant human-IL2.

[00382] Next day, T cells are infected with CD19-targeted CARs (including next generation CARs)-encoding lentiviral vectors in the pCCL3-MND3 backbone. The nucleic acid sequences of the CARs are shown in SEQ ID NO: 1016-1029, 1318-1331, 1594-1604, 1900-1913, 2206-2219, 2512-2525, 2818-2831, 3124-3127, 3324-3327. In addition, T cells are infected with CAR constructs corresponding to the above constructs but which lacked the hNEMO-K277A module. For each infection, 18 million T cells are infected with 500 μ l of concentrated viruses encoding the different CAR constructs and 8 μ g/ml Polybrene by spinfection at 2800 rpm, 32°C for 90 min in 6-well plates. The plates are incubated at 37°C for 6 hours. The cells are collected, centrifuged to remove virus and Polybrene, resuspended in fresh culture medium and cultured overnight at 37°C.

[00383] Next day, spinfection is repeated and cells are transferred to T-75 cell culture flasks with XVIVO 15 medium supplemented with CD3/CD28 Dynabeads, 50IU/ml IL2 and 5% FBS.

[00384] After 4 days of expansion, CAR –T cells are checked for CAR expression using Protein L staining, CD19-binding, cytokine production (IL2, IFN γ , TNF α) and cytotoxicity (Matador assay).

[00385] After 10 days of expansion, the CAR/SIR-T cells are used for in vivo experiment. For this purpose, NSG mice are injected with 10⁶ Nalm-6-Luc cells via tail vein injection. Two days later, 3x 10⁶ CAR/SIR-T cells injected. Mice are imaged weekly by bioluminescence imaging following administration of D-Luciferin and followed for survival.

[00386] It is noted that T cells expressing the first generation CARs along with hNEMO-K277A (SEQ ID NO: 1594-1604) show superior IL2 production as measured by ELISA when exposed to RAJI cells as compared to T cells expressing 2nd generation CARs (SEQ ID NO: 1594-1604) with a BBz costimulatory domain. In addition, T cells expressing the first generation CARs along with hNEMO-K277A (SEQ ID NO: 1594-1604) show less signs of exhaustion as measured by cell proliferation, cytokine (IL2, IFN γ , TNF α) production, expression of exhaustion markers (e.g., PD1) and cytotoxicity (Matador cytotoxicity assay) when cocultured with RAJI cells over 3 weeks period as compared to T cells expressing 2nd generation CARs (SEQ ID NO: 1594-1604) with a BBz costimulatory domain. Finally, T cells expressing the first generation CARs along with hNEMO-K277A (SEQ ID NO: 1594-1604) show superior in vivo activity when administered to NSG mice xenografted with NALM-6-Luc cells as determined by T cells expansion and persistence in vivo, reduction in tumor growth and improved survival. The T cells expressing the first generation CARs along with hNEMO-K277A (Backbone 2; SEQ ID NO: 1594-1604) in general show weaker production of cytokines (e.g., IL2, IFN γ and TNF α) when exposed to RAJI cells as compared to T cells expressing first generation CARs and coexpressing vFLIP K13 (i.e., Backbone 1; SEQ ID NO: 1016-1029).

[00387] T cells expressing the CAR constructs corresponding to SEQ ID NO: 1900-1913, 2206-2219, 2512-2525, 2818-2831, 3124-3127, 3324-3327 which express hNEMO-K277A show superior in vitro and in vivo activity as compared to the T cells expressing similar constructs but which lack the hNEMO-K277A module. Co-expression of hNEMO-K277A module is also shown to improve the in vitro and in vivo performance of T cells expressing a SIR (SEQ ID NO: 9683) targeting CD20. These results demonstrate that the coexpression of hNEMO-K277A accessory module enhances the in vitro (e.g., proliferation,

cytokine production, delay of exhaustion) and in vivo activity (e.g., improved expansion of T cells and anti-tumor activity) of not only the first generation CAR constructs but also of TFP, Ab-TCR and SIRs.

[00388] A difference is also noted among the different constructs containing the same backbone but having different antigen binding domains. Thus, among the first generation CAR constructs coexpressing hNEMO-K277A (i.e. Backbone 2) constructs containing the antigen binding domain derived from 4G7 (e.g., SEQ ID NO:1599), huBly3 (e.g., SEQ ID NO: 1604), and huSJ25C1 (e.g., SEQ ID NO: 1605) scFV are generally weaker as compared to constructs containing the antigen binding domain derived from scFv based on FMC63 (e.g., SEQ ID NO: 1594), hu-FMC63-11 (e.g., SEQ ID NO: 1595), huFMC63-11-N203Q (e.g., SEQ ID NO: 1596), Bu12 (e.g., SEQ ID NO: 1597), CD19-MOR0028 (e.g., SEQ ID NO: 1602) and CD19-hu-mROO5 (e.g., SEQ ID NO: 1607). A similar trend is observed in the in vitro and in vivo activity of CARs on other backbones based on the nature of their antigen binding domains.

[00389] In the preceding experiments, the hNEMO-K277A module is co-expressed with the CAR module in the T cells using a single vector. The experiment is repeated in which the two modules are expressed using two separate lentiviral vectors. The SEQ ID of nucleic acid construct encoding an exemplary CD20 CAR lacking a hNEMO-K277A module is presented in SEQ ID: 9668. T cells are coinfecting with the two lentiviral vectors at multiplicity of infection of 5 and the ratio of the two vectors (i.e. CAR:hNEMO-K277A) is varied from 1:1 to 1:10. The T cells are expanded and tested in the in vitro and in vivo assays. Co-expression of hNEMO-K277A along with a CAR construct is shown to improve the in vitro and in vivo performance of CAR-T cells as determined by assays for IL2 production, cell proliferation, lack of exhaustion, in vivo expansion and anti-tumor activity.

[00390] In an alternate embodiment, homologous recombination using gene editing techniques known in the art (e.g., CRISP/Cas9, TALON, Zn finger nucleases etc.) is used to induce the K277A mutation in one or both copies of the endogenous human NEMO gene in T cells. The resulting T cells carrying the hNEMO-K277A mutation are then used for adoptive cellular therapy, including to express the CAR constructs targeting CD19 and TCR constructs targeting NY-ESO-1. The T cells carrying the hNEMO-K277A mutations are shown to show enhanced proliferation, cytokine production, expansion, long term persistence in vivo and anti-tumor activity as compared to control T cells lacking the hNEMO-K277A mutation.

[00391] The experiments described in the preceding paragraphs are repeated by using CAR constructs in which the hNEMO-K277A accessory module is replaced by accessory

modules encoding FKBPx2-hNEMO, FKBPx2-hNEMO-K277A (SEQ ID NO: 1006), FKBPx2-hNEMO-L753(251) (SEQ ID NO: 1007), FKBPx2-hNEMO-L600(200) (SEQ ID NO: 1008), IKK2-delta-SCD-FKBPv36x2 (SEQ ID NO: 7782), IKK1-delta-SCD-FKBPv36x2 (SEQ ID NO: 7781) and FKBPx2-RIP-ID (SEQ ID NO: 1009). T cells expressing the CAR and these accessory modules are tested using in vitro assays in the absence and presence of the dimerizer AP20187 (100nM). Addition of AP20187 is shown to induce the proliferation and cytokine production by CAR-T cells expressing the FKBPx2-hNEMO, FKBPx2-hNEMO-K277A (SEQ ID NO: 1006), FKBPx2-hNEMO-L753(251) (SEQ ID NO: 1007), IKK2-delta-SCD-FKBPv36x2 (SEQ ID NO: 7782), IKK1-delta-SCD-FKBPv36x2 (SEQ ID NO: 7781), FKBPx2-hNEMO-L600(200) (SEQ ID NO: 1008) and FKBPx2-RIP-ID (SEQ ID NO: 1009) modules when exposed to target antigen (i.e., CD19) expressing RAJI cells. In an in vivo experiment, NSG mice (n=12 per group) are xenografted with 2 million RAJI-Luc cells by tail vein injection and 3 days later administered 5 million T cells expressing a CD19-CAR and coexpressing the FKBPx2-hNEMO, FKBPx2-hNEMO-K277A (SEQ ID NO: 1006), FKBPx2-hNEMO-L753(251) (SEQ ID NO: 1007), FKBPx2-hNEMO-L600(200) (SEQ ID NO: 1008) and FKBPx2-RIP-ID (SEQ ID NO: 1009) modules. Half the mice in each group (n =6) are administered 40µg of AP20187 every day for 10 days by intraperitoneal injection as described previously (Chinnery et al, J Immunol 2009; 182:2738-2744). Administration of AP20187 is shown to promote the expansion of CAR-T cells. In an alternate embodiment, the experiment is repeated using constructs in which both the FKBP domains carry the FKBP12V36 mutation which bind to the lipid-permeable dimerizing ligand, Rimiducid, at high affinity. Dimerization of the fusion proteins is brought by administration of rimiducid. For in vitro experiments, Rimiducid is used at final concentration of 10-100nM. For in vivo studies in NSG mice, Rimiducid is administered weekly by intraperitoneal (i.p) injection at 5 mg/kg.

[00392] The experiments described in the preceding paragraphs are repeated by using constructs in which the hNEMO-K277A accessory module is replaced by accessory modules encoding hNEMO-K277A-DeltaV249-K255, IKK2-S177E-S181E, IKK1-S176E-S180E, MYD88-L265P, TCL-1A, and MTCP-1. The CAR-T cells expressing the hNEMO-K277A-DeltaV249-K255, IKK2-S177E-S181E, IKK1-S176E-S180E, MYD88-L265P accessory modules are shown to demonstrate increased cytokine production, proliferation, in vivo expansion and anti-tumor activity as compared to CAR-T cells lacking the accessory module. The CAR-T cells expressing the TCL-1A and MTCP-1 accessory module are shown to have increased proliferative response.

[00393] Use of human NEMO-K277A, human NEMO-K277A-deltaV249-K255, mouse NEMO-K270A and IKK2-S177E-S181E in vaccination

[00394] Lentiviral vectors are generated expressing human NEMO-K277A, human NEMO-K277A-deltaV249-K255, mouse NEMO-K270A and IKK2-S177E-S181E. Lentiviral vectors are also generating expressing chicken ovalbumin amin acid residues 242–353 and the C terminus of the major histocompatibility complex (MHC) class II invariant chain (Ii-OVA) as described in Rowe HM et al, Molecular Therapy, 13, 2, 2006. Finally, lentiviral vectors are generated coexpressing a cassette encoding human NEMO-K277A, human NEMO-K277A-deltaV249-K255, mouse NEMO-K270A or IKK2-S177E-S181E with a cassette encoding Ii-OVA where the two cassettes are separated by a 2A cleavage sequence.

[00395] *Transduction of DCs and flow cytometry.* Murine bone marrow-derived Dendritic cells (DCs) are prepared as previously described. Immature DCs are transduced on day 4 at an MOI of 20 with lentiviral vectors as described (Rowe HM et al, Molecular Therapy, 13, 2, 2006) and fed every 4 days with fresh medium containing granulocyte-macrophage colony-stimulating factor (50 ng/ml; from Peprotech). On day 5 posttransduction, DCs are harvested, washed, and blocked for Fc receptors before surface staining for maturation markers with the following biotin-conjugated Abs: anti-CD11c, anti-CD86, and anti-I-Ab (MHC class II) (all from BD Pharmingen); anti-CD40 (from Serotec); and anti-CD80, anti-ICAM-1, and anti-Kb (MHC class I) (all from eBioscience). A hamster isotype control Ab (biotin conjugated) is purchased from BD Pharmingen. Abs are then labeled with streptavidin RPE Cy-5 2o reagent (DakoCytomation) before flow cytometry. Lipopolysaccharide (LPS) (50 ng/ml) (Sigma) is added to untransduced DCs and left overnight as a positive control for maturation. Zymosan A (10 µg/ml) treatment (for 30 min at 37°C) is used as a control for ERK activation.

[00396] *ELISA.* Culture supernatants are harvested from DCs plated at 5×10^5 cells per well (in 1.5 ml). IL-12p70 and tumor necrosis factor alpha (TNF-α) are detected by sandwich enzyme-linked immunosorbent assay (ELISA), using kits from eBioscience according to the manufacturer's guidelines.

[00397] *DC purification from lymph nodes.* C57/BL6 mice (Harlan) are injected subcutaneously (s.c.) at the base of the tail with 1×10^8 infectious units (i.u.) lentivector. Six days later, lymph nodes (para-aortical and inguinal) are harvested (cells from mice in each group were pooled), incubated with collagenase CLS-4 (Worthington), and mashed to obtain single-cell suspensions. Fc receptors are blocked before CD11c-positive cells are selected using MACS beads (Miltenyi Biotec).

[00398] *Pentamer staining.* One million splenocytes per sample are incubated with 10 μ l of phycoerythrin-conjugated SIINFEKL/Kb pentamer or tetramer (Proimmune) for 12 min at room temperature. The cells are then washed and incubated on ice with biotin-conjugated anti-CD8 (Serotec) for 15 min before being washed and incubated with streptavidin-allophycocyanin (eBioscience) for 15 min. Samples are washed and acquired on a BD LSR machine using Cell-Quest software (BD Biosciences).

[00399] *Intracellular cytokine staining.* Splenocytes are incubated overnight with or without OVA257-264 peptide. Monensin solution (eBiosciences; final concentration, 2 μ M) is added and left for 3 h before surface staining cells for CD8. The cells are then fixed and permeabilized using a Cytofix/Cytoperm kit from BD Biosciences. An allophycocyanin-conjugated anti-gamma interferon (anti-IFN- γ) Ab (BD Pharmingen) is then added and left for 30 min before the cells are washed and samples are run on a BD LSR machine.

[00400] *ELISPOT assay.* Enzyme-linked immunospot (ELISPOT) plates (Millipore) are coated overnight at 4°C with purified anti-IFN- γ (BD Pharmingen). Ex vivo ELISPOT assays is performed with serial dilutions of total splenocytes in triplicate with or without class I OVA257-264 peptide (Proimmune). Plates are cultured overnight and developed according to the manufacturer's directions. Spots are counted using an AID ELISPOT counter and software.

[00401] *Tumor therapy.* EG7.OVA tumor cells are grown in RPMI plus 0.4 mg/ml G418 (Invitrogen). C57BL/6 mice are challenged with 2×10^6 tumor cells injected s.c. into the flank and then vaccinated. Animals are killed once they had a tumor that reached a diameter of >15 mm.

[00402] Mouse BM-derived Dendritic cells (DCs) are infected with the lentivectors encoding human NEMO-K277A, human NEMO-K277A-deltaV249-K255, mouse NEMO-K270A and IKK2-S177E-S181E either alone or in combination with Ii-OVA. Expression of human NEMO-K277A, human NEMO-K277A-deltaV249-K255, mouse NEMO-K270A and IKK2-S177E-S181E is shown to result in nuclear translocation of p65 (RelA) in the nuclei of the DCs at a level similar to that in the LPS-treated DCs but not in the untreated or control vector DCs, in which the level of cytoplasmic p65 is higher. Increased nuclear NF- κ B binding activity is also detected in human NEMO-K277A-, human NEMO-K277A-deltaV249-K255-, mouse NEMO-K270A- and IKK2-S177E-S181E-transduced DCs but there is no affect on the activation of the MAPK pathway as determined by nuclear API binding activity.

[00403] After transduction of BM-derived DCs with human NEMO-K277A-, human NEMO-K277A-deltaV249-K255-, mouse NEMO-K270A- and IKK2-S177E-S181E, maturation markers on the transduced or nontransduced cells are analyzed. CD86, CD40, ICAM-1, and CD80 are upregulated on human NEMO-K277A-, human NEMO-K277A-deltaV249-K255-, mouse NEMO-K270A- and IKK2-S177E-S181E -expressing DCs compared to transduced DCs in the control vector group. Furthermore, human NEMO-K277A-, human NEMO-K277A-deltaV249-K255-, mouse NEMO-K270A- and IKK2-S177E-S181E-transduced DCs are shown to retain their upregulated CD86 for several weeks in culture. The secretion of IL-12p70 and TNF- α is found to be upregulated in the culture of DCs transduced with human NEMO-K277A-, human NEMO-K277A-deltaV249-K255-, mouse NEMO-K270A- and IKK2-S177E-S181E.

[00404] Following s.c. lentivector injection, transduced DCs are detected in the draining lymph nodes. A similar percentage of lymph node DCs (CD11c⁺/MHC class II⁺) are transduced after s.c. injection with either the control or the human NEMO-K277A-, human NEMO-K277A-deltaV249-K255-, mouse NEMO-K270A- and IKK2-S177E-S181E vector. However, there is an upregulation of CD86 on DCs in the human NEMO-K277A-, human NEMO-K277A-deltaV249-K255-, mouse NEMO-K270A- and IKK2-S177E-S181E-injected animals compared to the control vector-injected animals.

[00405] The ability of vectors encoding human NEMO-K277A-2A-Ii-OVA, human NEMO-K277A-deltaV249-K255-2A-Ii-OVA, mouse NEMO-K270A-2A-Ii-OVA, IKK2-S177E-S181E-2A-Ii-OVA and Ii-OVA to induce an Ova-specific CD8⁺T-cell response in mice after s.c. vaccination is examined. The vector dose of 5×10^5 i.u. is used. The human NEMO-K277A-2A-Ii-OVA, human NEMO-K277A-deltaV249-K255-2A-Ii-OVA, mouse NEMO-K270A-2A-Ii-OVA and IKK2-S177E-S181E-2A-Ii-OVA vaccinated mice show SIINFEKL/K^b pentamer-positive CD8⁺ T cells and IFN- γ -secreting CD8⁺ T cells as measured by intracellular fluorescence-activated cell sorting or ELISPOT assay.

[00406] Mice are inoculated with a lethal dose of EG7.OVA tumor cells before vaccinating them either with transduced DCs or with the human NEMO-K277A-, human NEMO-K277A-deltaV249-K255-, mouse NEMO-K270A- and IKK2-S177E-S181E-vectors directly. All mice are shown to develop tumors. After either transduced DC injection or direct lentivector injection, the number of tumor-free mice in the human NEMO-K277A-, human NEMO-K277A-deltaV249-K255-, mouse NEMO-K270A- and IKK2-S177E-S181E-group is higher than that in the control group. The efficacy of the NEMO-K277A-2A-Ii-OVA, human NEMO-K277A-deltaV249-K255-2A-Ii-OVA, mouse NEMO-K270A-2A-Ii-OVA, IKK2-

S177E-S181E-2A-Ii-OVA vectors is tested in a parasite protection model (Polley R et al, Infect. Immun. 74:773-776, 2016) using *L. donovani* expressing ovalbumin.

[00407] Use of human NEMO-K277A, human NEMO-K277A-deltaV249-K255, mouse NEMO-K270A and IKK2-S177E-S181E in vaccination

[00408] Antigen presenting cells collected in a single leukapheresis are transduced with adenoviral vector encoding human NEMO-K277A, human NEMO-K277A-deltaV249-K255, mouse NEMO-K270A and IKK2-S177E-S181E, followed by incubation with protein PA001, which contains the extracellular domain of human prostate-specific membrane antigen. Men with progressive mCRPC following ≤ 1 prior chemotherapy regimen are enrolled to evaluate three doses of the resulting vaccine (4×10^6 , 12.5×10^6 and 25×10^6 cells) administered intradermally every 2-4 weeks. There are no dose-limiting toxicities. Immune upregulation as well as anti-tumor activity are observed with PSA declines.

[00409] Construction and testing of humanized MPL CAR based on scFv fragment derived form 161 antibody

[00410] The murine monoclonal antibody 161 targets human MPL (Thrombopoietin receptor or TPO-R). To generate a CAR targeting MPL but with reduced immunogenicity, sequence of the scFV fragment comprising the antigen binding domain of the murine 161 antibody was humanized. The humanized 161 scFv fragment (SEQ ID NO: 891), designated hu-161-2, was cloned in the 2nd generation CAR backbone containing the 41BB costimulatory domain and CD3z activation domain (SEQ ID NO: 1582). Jurkat-NFAT-EGFP (J-N-G) cells were stably transduced with the lentivirus encoding the humanized MPL-hu-161-2 CAR construct. The parental and CAR-expressing Jurkats were subsequently cocultured with HEL cells and induction of EGFP expression monitored by FACS analysis after 4 h. Coculturing of Jurkat cells expressing MPL-hu-161-2 CAR construct with HEL cells led to increase in EGFP expression as compared to cells that had not been exposed to HEL cells, indicating the ability of humanized MPL-hu-161-2 CAR construct to recognize the target antigen and activate signaling. Essentially similar results are obtained when the experiment is repeated with a first generation CARs incorporating hu-161-2 scFV and coexpressing either vFLIP K13 (SEQ ID NO: 1286) or hNEMO-K277A mutant (SEQ ID NO: 1878).

[00411] Construction and testing of humanized MPL CAR based on scFv fragment derived form 175 and 111 antibody

[00412] The murine monoclonals 175 and 111 also bind human MPL. Therefore, the sequence of the scFV fragments comprising the antigen binding domain of these antibody is

humanized and used to make the corresponding 2nd generation CAR (CAR II) constructs (SEQ ID NOs: 1583 and 1584) as well as backbones 1 and 2 coexpressing vFLIP K13 (SEQ ID NO: 1287, 1288) and hNEMO-K277A (SEQ ID NOs: 1896 and 1897). The experiment is repeated as in the preceding example. Co-culturing of Jurkat cells expressing MPL-hu-175-2 and hu-111-2 CAR constructs with HEL cells led to increase in EGFP expression as compared to cells that had not been exposed to HEL cells, indicating the ability of humanized MPL-hu-175-2 and hu-111-2 CAR constructs to recognize the target antigen and activate signaling.

[00413] Construction and testing of CARs targeting CD70

[00414] A number of constructs targeting CD70 are constructed (SEQ ID NO: 9781-10086; and 7783-7789). The constructs are expressed in J-N-G and T cells and tested for T cell activation and cytotoxicity against CD70-expressing target cell lines RAJI and THP-1 using in vitro and in vivo assays.

[00415] Construction and testing of CARs targeting CD70, PTK7, kappa light chain, Claudin18A2, Ras/HLA-A2 complex, NY-ESO/HLA-A2 complex, Streptag and an epitope of CD43 expressed on leukemia cells.

[00416] CAR constructs are generated targeting PTK7, kappa light chain, Claudin18A2, Ras/HLA-A2 complex, NY-ESO/HLA-A2 complex, Streptag and an epitope of CD43 expressed on leukemia cells on either a 2nd generation backbone (*e.g.*, conventional CAR II) or backbones 1 and 2 co-expressing either vFLIP K13 or hNEMO-K277A. The experiment is repeated as in the preceding example. Coculturing of Jurkat cells expressing the different CAR constructs with their respective target cells led to increase in EGFP expression as compared to cells that had not been exposed to the target cells. Similarly, coculturing of T cells expressing the different CAR constructs with their respective target cells expressing GLuc led to increase in cell death as measured by increase in GLuc activity.

[00417] TFP targeting MPL

[00418] Several TFP based CARs are constructed targeting MPL based on hu-161-2 scFV as the antigen binding domain. The sequence of these TFP CAR constructs is shown in SEQ ID NO: 3526 to 3533. Jurkat-NFAT-EGFP (J-N-G) cells are transduced with lentiviruses encoding the TFP CARs targeting MPL and selected with puromycin. J-N-G cells expressing the TFP CARs targeting MPL are shown to induce EGFP expression upon co-culture with HEL.92.1.7 (HEL) cells for 4 hours. TFP CARs targeting MPL are also expressed in primary T cells and tested for their ability to induce lysis of HEL-GLuc cells upon co-culture for 4 hours. MPL TFP CAR constructs based on 175, 111, hu-175-2 and hu-

111-2 scFv (SEQ ID NO: 10476-10483) are similarly constructed and tested using J-N-G and primary T cells as described above for hu-161-2 based TFP CARs. J

[00419] TFP targeting other antigens

[00420] Next TFP CARs targeting a number of different antigens are constructed. In order to provide costimulation, the constructs also coexpress hNEMO-K277A. The constructs are expressed in J-N-G and primary T cells and tested for their ability to recognize cells expressing their target antigen using the assays described above. The TFP CARs expressing J-N-G cells are shown to induce EGFP expression upon co-culture with the target cell expressing their cognate antigen. T cells expressing these TFP CARs targeting different antigens are shown to induce cytotoxicity of the target cells expressing the corresponding antigen using the GLuc based cytotoxicity assay described above. **Table A** shows the target cell lines expressing the different target antigens that are used in the assay. Additional cell lines expressing the different target antigens are known in the art or can be genetically engineered to express a desired antigen by techniques known in the art. In the above example, the TFP constructs contain an accessory module that co-expresses hNEMO-K277A mutant to provide co-stimulation. In alternate embodiment, TFP constructs are also constructed that either lack an accessory module to provide costimulation or contain an accessory module which provides costimulation through the co-expression of other proteins, such as vFLIP K13. The experiment is repeated as above by expression of the TFP constructs in J-N-G and primary T cells with similar results.

[00421] Ab-TCR targeting MPL

[00422] Several Ab-TCR are constructed targeting MPL based on murine 161 scFV as the antigen binding domain. To improve the expression of TCR α and TCR β based Ab-TCR, specific mutations are introduced in their TCR receptor modules. The sequence of the TCR γ /TCR δ , wild-type TCR α /TCR β (labelled wt-op2) and mutant TCR α /TCR β (labelled SDVP-IAH) containing Ab-TCR constructs are shown in SEQ ID NO: 959 to 964. Jurkat-NFAT-EGFP (J-N-G) cells are transduced with lentiviruses encoding the Ab-TCRs targeting MPL (SEQ ID NO: 2091, 2397, 2703) and selected with puromycin. J-N-G cells expressing the Ab-TCRs targeting MPL are shown to induce EGFP expression upon co-culture with HEL cells for 4 hours, demonstrating the ability of Ab-TCRs targeting MPL to recognize MPL and activate signaling. Ab-TCRs targeting MPL are also expressed in primary T cells and tested for their ability to induce lysis of HEL-GLuc cells upon co-culture for 4 hours. T cells expressing MPL Ab-TCRs are shown to induce lysis of HEL-GLuc cells as measured by increase in GLuc activity. MPL Ab-TCR constructs based on murine 175 and 111 scFv (SEQ

ID NO: 10492-10493) are similarly constructed and tested using J-N-G and primary T cells as described above for 161 based Ab-TCRs.

[00423] Ab-TCRs targeting other antigens

[00424] Next Ab-TCRs targeting a number of different antigens are constructed. In order to provide costimulation, the constructs also coexpress hNEMO-K277A. The constructs are expressed in J-N-G and primary T cells and tested for their ability to recognize cells expressing their target antigen using the assays described above. The Ab-TCRs expressing J-N-G cells are shown to induce EGFP expression upon co-culture with the target cell expressing their cognate antigen. T cells expressing these Ab-TCRs targeting different antigens are shown to induce cytotoxicity of the target cells expressing the corresponding antigen using the GLuc based cytotoxicity assay described above. **Table A** shows the target cell lines expressing the different target antigens that are used in the assay. Additional cell lines expressing the different target antigens are known in the art or can be genetically engineered to express a desired antigen by techniques known in the art. In the above example, the Ab-TCR constructs contain an accessory module that co-expresses hNEMO-K277A mutant to provide co-stimulation. In alternate embodiments, Ab-TCR constructs are also constructed that either lack an accessory module to provide costimulation or contain an accessory module which provides costimulation through the co-expression of other proteins, such as vFLIP K13. The experiment is repeated as above by expression of the Ab-TCR constructs in J-N-G and primary T cells with similar results.

[00425] Flow Cytometry for CAR-Mediated Proliferation of Transduced CD8+ T Lymphocytes in Response to HIV-1 -Infected Target Cells

[00426] A number of CARs targeting HIV1 envelop glycoprotein are generated and are represented by SEQ ID NO: 8704-9349. The following assays are used to test their anti-HIV1 activity in vitro. The active constructs are used singly or in combination for the treatment of patients with HIV1 and AIDS.

[00427] HIV- 1 -infected T2 cells, which are MHC class I low due to a deletion in the transporter associated with processing (TAP) (Salter, et al. (1986) EMBO J 5:943-949) and previously shown to be suitable target cells for an HIV- 1 -specific CAR (Severino, et al. (2003) Virology 306:371-375), served as target cells. These are infected with an excess of HIV- 1 NL4-3 -based reporter virus containing a gene for murine CD24 (mCD24) in the vpr locus (Ali, et al. (2003) J Virol Methods 110: 137-142) to yield >90% infected cells by 3 or 4 days after infection, as previously described (Bennett, et al. (2007) J Virol 81 :4973-4980; Yang, et al. (1996) J Virol 70:5799-5806; and Yang, et al. (1997) J Virol 71 :3120-3128).

These are irradiated immediately before use with 10,000 rads in a cesium irradiator, as well as peripheral blood mononuclear cells from a healthy donor with 3,000 rads (feeder PBMCs). HIV1-CAR transduced primary CD8⁺ T lymphocytes are labeled with CellTrace Violet and washed according to manufacturer's directions (ThermoFisher Scientific, Grand Island, NY). In a 48 well plate well, 5×10^5 labeled transduced cells are added to 5×10^5 irradiated infected T2 cells and 2×10^6 irradiated feeder PBMCs, and cultured in 1 ml R10-50 for five days with a medium change after three days. Flow cytometry (LSR Fortessa II cytometer, BD Biosciences) was then performed with co-staining for human CD8 (PerCP-anti-human CD8, catalog #30130, Biolegend, San Diego, CA) and analysis of proliferation using FlowJo software (FlowJo, Ashland, OR). HIV1-CAR-transduced T cells are shown to proliferate when exposed to HIV-1-infected T2 cells.

[00428] Virus Suppression Assays

[00429] The ability of HIV1-CAR transduced CD8⁺ T lymphocytes and expanded and enriched clones thereof to suppress the replication of HIV-1 is tested as previously described (Yang, et al. (1997) PNAS USA 94: 11478-11483; and Yang, et al. (1997) J Virol 71 :3120-3128). HIV-1 strains tested is obtained from the NIH AIDS Reference and Reagent including 94US_3393 IN (catalog #11250), 90JJS873 (catalog #11251), 96TH_NP1538 (catalog #11252), 00TZ_A246 (catalog #11256). In brief, T1 cells transduced with human CCR5 are infected at a multiplicity of 0.1 tissue culture infectious doses per cell, and co-cultured in a 96-well plate with HIV1 CAR-transduced cells at a ratio of 5×10^4 to 1.25×10^4 cells respectively in 200 μ l of R1 0-50, or no effector cells as a control. The effector cells are confirmed to be >90% transduced. Each condition is run in triplicate, and viral replication is monitored using p24 quantitative ELISA (XpressBio, Frederick, MD). Exposure to HIV1 CAR cells is shown to lead to suppression of HIV1 as measured by p24 ELISA.

[00430] Effector cells expressing HIV1-CAR are also tested for antiviral activity against infected CD4⁺ cells. T2-CCR5 cells are infected with a panel of HIV-1 strains including primary R5-tropic isolates and cultured in the absence or presence of the HIV1-CAR transduced effector cells. Virus replication is assessed by measurement of p24 antigen between days 7 to 10 of culture. Suppression of replication is calculated as the difference of logio units of p24 between cultures without versus with effector cells, which is then normalized as the ratio to total replication without effector cells.

[00431] Chromium Release Killing Assays for CAR-Mediated Killing of HIV-1 - Infected Target Cells

[00432] T2-GLuc cells infected with HIV-1 strain NL4-3 as above are used as target cells for the HIV1-CAR transduced primary CD8⁺ T lymphocytes in Matador Assay or using standard ⁵¹Cr-release assays as previously described (Bennett, et al. (2007) J Virol 81 :4973-4980; Yang, et al. (1996) J Virol 70:5799-5806; and Bennett, et al. (2010) Aids 24:2619-2628). Briefly, infected and control uninfected T2 cells are ⁵¹Cr-labeled for 1 hour and incubated with or without effector CD8⁺ T lymphocytes for 4 hours at varying cell ratios in a 96-well U-bottom plate. Supernatants are then harvested for measurement of extracellular ⁵¹Cr by micro204-scintillation counting in 96 well plates. Spontaneous release is measured on target cells without effector cells, and maximal release is measured on target cells lysed with 2.5% Triton X-100. Specific lysis is calculated as: (experimental released chromium - spontaneous release) ÷ (maximal release - spontaneous release).

[00433] **Bispecific Antibodies targeting MPL**

[00434] Bispecific antibodies such as Bispecific T cell Engagers (BiTE) and Dual affinity retargeting (DART) can be used to retarget T cells to a target cell expressing a particular antigen.

[00435] A bispecific T cell engager based targeting MPL based on 161 scFv as the antigen binding domain is constructed. The sequence of this bispecific construct is shown in SEQ ID NO: 3736. The bispecific constructs contain a GGGSG-Streptagx2-Tag linker (SEQ ID NO: 287) but alternate linkers (*e.g.* SGGGS) can be used.

[00436] The bispecific construct was transfected in 293FT cells and supernatant containing the fusion protein collected after 48-96 hours. HEL-GLuc cells cultured with T cells in the presence of the MPL-161 bispecific fusion protein were shown to undergo cell lysis as determined by the GLuc assay as compared to the cells cultured with the bispecific fusion protein alone or T cells alone.

[00437] Bispecific antibodies encoding constructs based on 175, 111, hu-161-2, hu-175-2 and hu-111 scFv are next constructed and found to have activity in the HEL-GLuc cytotoxicity assay. Finally, bispecific antibodies targeting a number of other antigens, including PTK7, DLL3, TROP2, CD179a, CD179b, CD23, LAMP1, CDH1, CDH17, CD32, CDH19, HIV1-gp120 envelop glycoprotein *etc.*, are similarly constructed and are found to have activity when co-cultured with the target cell lines expressing their cognate antigen.

[00438] **Fig 4.** Activity of a Bispecific T cell engager targeting MPL and using a 161-scFv targeting domain. HEL-pLenti-hGluc and T cells were pre-incubated separately with the following supernatants at 4° C for 2h Medium alone and pLenti-161-StreptagII-CD3-Myc-His-P02 (042517-P02-SC). Post-incubation, cells were co-cultured in U-bottom 96-well plate

at an E:T ratio of 1:1 or 5:1 for 4h at 37 C. 50 μ l of cells+sup/well were transferred to 384 well plate in triplicate. hGLuc assay was performed using 15 μ l of CTZ assay buffer (1:100).

[00439] Expression and activity of TFPs in Jurkat cells lacking TCR α and TCR β expression.

[00440] Jurkat-NFAT-GFP (J-N-G) cells (T cell lymphoma) are infected with lentiviral vectors expressing gRNAs targeting TCR α and TCR β 1/ β 2 constant chains and coexpressing *Streptococcus Pyogenes* Cas9. The exemplary gRNA target sequences for TCR α chains are given in SEQ ID NO: 7754 and 7755. The exemplary gRNA target sequences for TCR β 1/ β 2 chains are given in SEQ ID NO: 7756-7758. In an alternative embodiment, the TCR α and TCR β 1/ β 2 constant chain loci are targeting using gRNA and TALONs as described in Knipping F *et al*, Molecular Therapy: Methods & Clinical Development, Vol 4, 2017. J-N-G cells lacking the expression of TCR α or TCR β 1/ β 2 chains are purified by cell sorting using antibodies directed against TCR/CD3 complex. Lentiviral vectors expressing TFPs directed against human MPL (SEQ ID NO: 2184, 2490, 2796) under EF1 α promoter are used to infect parental J-N-G cells (control) and those lacking the expression of TCR α or TCR β 1/ β 2 chains. Expression of TFPs in the cells is determined by immunostaining with Protein L staining and by staining with MPL-ECD-GGSG-NLuc-AcV5 fusion protein (SEQ ID NO: 4923). J-N-G parental cells show robust TFP expression on cell surface while J-N-G cells lacking TCR α or TCR β 1/ β 2 chains show poor to absent TFP expression. The different populations of J-N-G cells are exposed to HEL.92.1.7 target cells for 24 hours and examined for increase in NFAT-promoter driven GFP expression and IL2 production. J-N-G parental cells expressing MPL-specific TFPs show marked increase in GFP fluorescence and IL2 secretion upon co-culture with HEL.92.1.7 cells. In contrast, MPL-specific TFP-expressing J-N-G cells with absent TCR α or TCR β 1/ β 2 chains show weak to no GFP induction and IL2 secretion. Essentially similar results are obtained when the experiment is repeated with J-N-G parental and TCR α - or TCR β 1/ β 2-deficient cells upon expression of TFPs targeting CD19 (SEQ ID NO: 1913, 2219, 2525), CD20 (SEQ ID NO: 1945, 2251, 2557) and CD22 (SEQ ID NO: 1950, 2256, 2562) and upon coculture with RAJI and Nalm6 target cells.

[00441] Next lentiviral vectors expressing codon optimized TCR α constant chain (IgSP-[hTRAC-opt2]; SEQ ID NO: 1010) or TCR β constant chain (IgSP-[hTRBC-opt2]; SEQ ID NO: 1011) under EF1 α promoter are used to infect the different J-N-G cell populations. Expression of TCR α constant chain in MPL-specific TFP-expressing J-N-G cells in which the TCR α chain has been disrupted by gRNA mediated gene knock out results in

increased expression of TFP on the cell surface and induction of GFP expression and IL2 secretion upon co-culture with HEL.92.1.7 target cells. Similarly, expression of TCR β constant chain in MPL-specific TFP-expressing J-N-G cells in which the TCR β 1/ β 2 chain has been disrupted by gRNA-mediated gene knock out results in increased expression of TFP on the cell surface and induction of GFP expression and IL2 secretion upon co-culture with HEL.92.1.7 target cells

[00442] Expression and activity of Ab-TCR and cTCR/SIRs in Jurkat cells lacking TCR α and TCR β expression.

[00443] The above experiment is repeated with the exception that expression cassettes encoding Ab-TCRs and SIRs targeting human CD19 are used in place of TFPs targeting CD19. The Ab-TCRs targeting CD19 is represented by SEQ ID NO: 3124. The cTCR/SIRs targeting CD19 are represented by SEQ ID NO: 3878-3880. Expression of Ab-TCR, cTCR and SIR in J-N-G cells lacking TCR α or TCR β 1/ β 2 expression is shown to results in increased expression and activity as compared to their expression in parental J-N-G cells.

[00444] Allogeneic and off-the-shelf T cells expressing CAR, TFP and Ab-TCR of the disclosure

[00445] Allogeneic or off-the-shelf CAR-T cells are generated by decreasing or eliminating the expression of endogenous TCR α and/or TCR β chain using TALON, CRISP/Cas9 or other nucleases.

[00446] The MPL-specific TFP cassettes (SEQ ID Nos: 3527, 3529, 3531) are cloned in targeting constructs designed for targeting into the TRAC genomic locus and containing the right and left homology arms derived from TRAC genomic sequences. A polyadenylation sequence is inserted downstream of the stop codon of the TFPs. The schematic of the targeting construct and the targeting strategy is shown in **Fig. 5A**. The sequences of the targeting constructs are provided in SEQ ID NO: 3858, 7773 and 7776. The targeting constructs are cloned in an integration defective lentiviral vector (IDLV) and an Adeno-Associated Viral (AAV) vector. The constructs are directed to the TRAC locus in purified human T cells using CRISP/Cas9 (**Fig. 5A**) as described in techniques known in the art and using the TRAC gRNA sequence (SEQ ID NO: 7751):

5'C*A*G*GGUUCUGGAUAUCUGUGUUUAGAGCUAGAAAUAGCAAGUAAAAU
AAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU* U* U*
U-3'. Asterisk (*) represents 2' -O-methyl 3' phosphorothioate. Exemplary techniques to deliver targeting constructs to the TRAC locus using IDLV and AAV are described in Knipping *F et al*, Molecular Therapy: Methods & Clinical Development, Vol 4, 2017 and

Eyquem J *et al* Nature, 543(7643):113-117, 2017, respectively. In parallel, purified human T cells are also transduced using the conventional approach with lentiviral vectors encoding the corresponding TFP constructs (SEQ ID NO: 2184, 2490, 2796) under EF1 α promoter to generate cells expressing the different TFPs. Expression of TCR $\alpha\beta$ and TFPs in the T cells is determined by immunostaining with TCR/CD3 antibodies and Protein L staining, respectively. Expression of TFPs on T cells is also examined by staining with MPL-ECD-GGSG-NLuc-AcV5 fusion protein (SEQ ID NO: 4923). The different populations of T cells are exposed to HEL.92.1.7-GLuc target cells and are compared using *in vitro* and *in vivo* assays of cell activation, proliferation, cytokine production (*e.g.*, IL2), target cell lysis, senescence, exhaustion, *in vivo* expansion, *in vivo* persistence and *in vivo* anti-tumor activity. TFPs show impaired expression on T cells surface and reduced or absent activity (*e.g.*, T cell activation, proliferation, cytokine production and cytotoxicity *etc.*) in T cell when their expression is directed to the TRAC locus as compared to when they are expressed using lentiviral vectors. It is next tested if expression of TCR α constant chain (TRAC chain) can be used to restore TFP expression and signaling and activity in T cells in which the endogenous TRAC genomic locus has been disrupted by the TFP expression cassette. For this purpose, targeting constructs are constructed that coexpress TFPs with an accessory module encoding a TCR α constant chain with an amino terminal signal peptide (IgSP) (**Fig. 5B**). The accessory module is separated from the TFP encoding sequence by a Furine-SGSG-P2A linker. The nucleotide sequence of exemplary targeting constructs coexpressing a TCR α constant chain with TFP constructs targeting MPL are shown in SEQ ID NOs: 3859, 7774, and 7777). The nucleotide sequence of the TCR α constant chain in these constructs is codon optimized and differs from the endogenous TCR α constant chain in its nucleotide sequence. In an alternate embodiment, the TRAC chain is codon optimized and carries amino acid substitutions that are known to enhance the expression of human TCR α constant chain. The nucleotide sequence of exemplary exogenous TRAC chains that can be used to allow re-expression of TCR/CD3 complex in T cells in which the expression of endogenous TCR α gene has been down-regulated or eliminated by targeting are shown in SEQ ID NO: 3886 to 3894. The exogenous TRAC can be expressed in T cells either by itself (SEQ ID NO: 1010) or it can be co-expressed with the TFP expression cassettes using a single vector.

[00447] The MPL-specific TFP constructs (SEQ ID Nos: 3858, 7773 and 7776) and TFP-TRAC constructs (SEQ ID NOs: 3859, 7774, and 7777) are cloned in the IDLV and AAV vector and are directed to the TRAC locus essentially as described previously.

[00448] The cells expressing the constructs are exposed to HEL.92.1.7-GLuc target cells and tested in functional assays as described above. T cells in which the TFP-TRAC constructs are directed to the TRAC locus show better expression of TFP on cell surface as compared to T cells in which the TFP constructs alone (*i.e.* without coexpression of the exogenous TRAC chain) are directed to the TRAC locus. In addition, T cells in which the TFP-TRAC constructs are directed to the TRAC locus show greater proliferation, activation, cytokine (*e.g.*, IL2 and TNF α) production, cytotoxicity, *in vivo* expansion, *in vivo* anti-tumor activity against the target cells as compared to T cells in which the TFP constructs alone (*i.e.* without coexpression of the exogenous TRAC chain) are directed to the TRAC locus.

[00449] The expression and activity of TFPs is also restored in T cells in which the endogenous TRAC locus has been disrupted by designing the targeting cassette such that TFP cassette is followed in frame by a 2A cleavable linker, a signal peptide (*e.g.*, a CD8 signal peptide or an IgH signal peptide) and the first exon of the TCR α chain (TRAC) (**Fig 5C**). The nucleotide sequence of exemplary targeting constructs is shown in SEQ ID NO: 3860, 7775 and 7778. In this embodiment, the TRAC protein is produced by the endogenous TRAC chain whose cell surface expression is facilitated by the signal peptide provided in the targeting cassette.

[00450] The alloreactivity of the TFP-TRAC-expressing T cells which lack the expression of native TCR α chain but in which the TFP cell surface expression and activity is rescued by the expression of TCR α constant chain is tested using mixed lymphocyte culture reaction using irradiated T cells derived from an allogeneic donor. TFP-TRAC-expressing T cells which lack the expression of native TCR α show markedly reduced to absence of alloreactivity as measured by proliferative response as compared to T cells in which TFPs are expressed using lentiviral vectors. The ability of TFP-TRAC-expressing human T cells which lack the expression of native TCR α to induce Graft *vs* Host Disease (GVHD) is examined by administration of 5 million TFP-TRAC-expressing TCR α -deficient T cells per animal into immunodeficient NSG mice (Jackson Lab). Animals are observed for over 90 days. Human T cells in which the TFP-TRAC-cassettes are directed to the TRAC locus show markedly reduced to absence of Graft *vs* Host Disease (GVHD) when infused into immunodeficient NSG mice (Jackson Lab) while GVHD is observed in animals given T cells in which TFP are expressed using lentiviral vectors. The ability of TFP-TRAC-expressing TCR α -deficient T human T cells to induce Graft *vs* Host Disease (GVHD) is also examined by administration of 1 million cells per kilogram into allogeneic human recipients who have received lymphodepleting chemotherapy. Allogeneic T cells in which the TFP-TRAC cassettes are

directed to the TRAC locus show markedly reduced incidence and severity of Graft vs Host Disease (GVHD) when given to allogeneic recipients.

[00451] Essentially similar results are obtained when the experiment is repeated with T cells in which the TFPs are directed to the TRBC locus. The target sequence of gRNA targeting TRBC are shown in SEQ ID NO: 7756-7758. These gRNAs are used in combination with *Streptococcus Pyogenes* Cas9 using methods known in the art. Directing the TFP expression cassettes to the TRBC genomic locus is shown to result in impaired activity of the MPL-specific TFPs. However, the activity of TFPs is restored by coexpression of exogenous TCR β constant chain (TRBC). The nucleotide sequence of exemplary exogenous TRBC chains that can be used to restore TCR/CD3 complex signaling function are shown in SEQ ID NO: 3899-3910. The exogenous TRBC can be expressed in T cells either by itself (SEQ ID NO: 1011) or it can be co-expressed with the TFP expression cassettes from a single vector. The nucleotide sequence of exemplary constructs coexpressing a TRBC chain with TFP constructs targeting MPL are shown in SEQ ID NO: 3537, 3539 and 3541. These TFP expression constructs can be cloned in suitable TRBC targeting vectors using techniques known in the art. In an alternate embodiment, the expression of TRBC can be restored in T cells in which the endogenous TRBC locus has been disrupted by designing the targeting cassette such that TFP cassette is followed in frame by a 2A cleavable linker, a signal peptide (*e.g.*, a CD8 signal peptide or an IgH signal peptide) and the first exon of the TCR β chain (TRBC).

[00452] **Directing the Ab-TCR constructs to the TRAC locus**

[00453] Two Ab-TCR constructs targeting CD19 based on FMC63 scFv are generated in lentiviral vector (SEQ ID NO: 3837) driven by EF1 α promoter. The nucleotide sequences of these constructs, CD8SP-FMC63-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-FMC63-vH-[IgG1-CH1-TCRa-SDVP-6MD] and CD8SP-FMC63-vL-[IgCL-TCRg-6MD]-F-P2A-SP-FMC63-vH-[IgG1-CH1-TCRd-6MD] are represented by the nucleotide sequences encoding the Ab-TCR component of SEQ ID NO: 3124 and 3324. Primary human T cells are infected with the corresponding lentiviral supernatants and assayed for the cell surface expression of the Ab-TCRs using FLAG-CD19-ECD-GGSG-NLuc-AcV5 supernatant (SEQ ID NO: 1014) and for cytotoxicity against RAJI-GLuc cells. T cells expressing the Ab-TCRs show modest expression and activity. The expression of the Ab-TCRs is directed to the TRAC locus essentially as described by Eyquem *J et al* (Nature, 543(7643):113-117, 2017) using gene targeting constructs (see, **Fig. 6**) and represented by SEQ ID NO: 3861-3864. The targeting construct contains a splice acceptor (SA), followed by a F2A coding sequence, the Ab-TCR

cassette, flanked by sequences homologous to the TRAC locus (LHA and RHA, left and right homology arm). In cassettes A and B (SEQ ID NO: 3861-3862), the nucleotide sequence coding the Ab-TCR expression cassettes are followed by a stop codon, polyA sequences, Exon 1 of TRAC and the sequence homologous to the *TRAC* locus (RHA: right homology arm). In cassette C, nucleotide sequence coding the Ab-TCR expression cassette is followed by a stop codon, Exon 1 of TRAC and the sequence homologous to the *TRAC* locus (RHA: right homology arm) but without a poly A sequence so that the transcript carries at its 3' end the TRAC gene and its polyadenylation sequence. In cassette D, the Ab-TCR cassette lacks its own TCR α module and extends only upto the IgG1-CH1 region, which is fused in frame to 3' half of the first exon of TRAC. Thus, in this construct, the TCR α module is encoded by the genomic TRAC locus containing part of exon 1, and whole of exon 2 and exon 3. Cassette E resembles cassette D except that the RHA in the targeting construct carries mutations (SDVP) that can enhance the expression of TRAC. T cells in which the Ab-TCR cassettes are directed to the TRAC locus uniform and physiological expression and long-term persistence and activity of the transgene as determined using *in vivo* and *in vitro* assays as compared to Ab-TCR cassettes expressed using lentiviral vectors. The Ab-TCR expressing T cells are purified by staining with PE-Protein L followed by flow sorting. The alloreactivity of the Ab-TCR-expressing T cells is tested using mixed lymphocyte culture reaction using irradiated T cells derived from an allogeneic donor. T cells in which the Ab-TCRs are directed to the TRAC locus show markedly reduced to absence of alloreactivity as measured by proliferative response as compared to T cells in which Ab-TCRs are expressed using lentiviral vectors. The ability of Ab-TCR expressing human T cells to induce Graft vs Host Disease (GVHD) is examined by administration of 5 million Ab-TCR expressing T cells per animal into immunodeficient NSG mice (Jackson Lab). Animals are observed for over 90 days. Human T cells in which the Ab-TCR cassettes are directed to the TRAC locus show markedly reduced to absence of Graft vs Host Disease (GVHD) when infused into immunodeficient NSG mice (Jackson Lab) while GVHD is observed in animals given T cells in which Ab-TCRs are expressed using lentiviral vectors. The ability of Ab-TCR expressing human T cells to induce Graft vs Host Disease (GVHD) is also examined by administration of Ab-TCR expressing T cells (1 million cells per kilogram) into allogeneic human recipients. Allogeneic T cells in which the Ab-TCR cassettes are directed to the TRAC locus show markedly reduced incidence and severity of Graft vs Host Disease (GVHD) when given to allogeneic recipients. Essentially similar results are obtained using T cells in which Ab-TCR are expressed by directing the expression cassettes to the TRBC locus.

[00454] **Directing the chimeric TCR or Synthetic Immune Receptors (SIR) constructs to the TRAC locus**

[00455] Three cTCRs (or SIR) constructs targeting CD19 based on FMC63 scFv are generated in lentiviral vector (SEQ ID NO: 3837) driven by EF1 α promoter. The nucleotide sequences of these constructs are represented by SEQ ID NO: 3878, 3879 and 3880, respectively. They all have the same vL and vH regions. While the SEQ ID NO: 3880 has the wild-type nucleotide sequence of TCR α and TCR β constant chains, the SEQ ID NO: 3878 and 3879 have codon optimized sequences. The SEQ ID NO: 3878 further carries several amino acid substitutions to enhance the expression and base-pairing of the TCR α and TCR β constant chains. Primary human T cells are infected with the corresponding lentiviral supernatants and assayed for the cell surface expression of the SIR using FLAG-CD19-ECD-GSG-NLuc-AcV5 supernatant (SEQ ID NO: 1014) and for cytotoxicity against RAJI-GLuc cells. The cTCR/SIR construct with SEQ ID NO: 3880 is not found to express well or to induce target cell lysis. The cTCR/SIR are also directed to the TRAC locus essentially as described by Eyquem J *et al* (Nature, 543(7643):113-117, 2017) using exemplary gene targeting constructs (see, **Fig. 7**) represented by SEQ ID NO: 3865 to 3868, and 3873. The targeting construct contains a splice acceptor (SA), followed by a F2A coding sequence, the Ab-TCR cassette, flanked by sequences homologous to the TRAC locus (LHA and RHA, left and right homology arm). In cassettes A and B (SEQ ID NO: 3865, 3866 and 3873), the nucleotide sequence coding the SIR expression cassettes are followed by a stop codon, poly A sequences, Exon 1 of TRAC and the sequence homologous to the *TRAC* locus (RHA: right homology arm). In cassette E, nucleotide sequence coding the SIR expression cassette is followed by a stop codon, Exon 1 of TRAC and the sequence homologous to the *TRAC* locus (RHA: right homology arm) but without an intervening poly A sequence so that the transcript carries at its 3' end the TRAC gene and its polyadenylation sequence. In cassette D, the SIR cassette lacks its own TCR α module and extends only upto the FMC63-vH region, which is fused in frame to the first exon of TRAC present in the targeting construct. Thus, in this construct, the TCR α module is encoded by the genomic TRAC locus containing part of exon 1, and whole of exons 2 and 3. Cassette F resembles cassette E except that the RHA in the targeting construct carries mutations (CSDVP) in the exons 1 and 2 of TRAC that can enhance the expression of TRAC. T cells in which the cTCR/SIR cassettes are directed to the TRAC locus (SEQ ID NO: 3873) show uniform and physiological expression and long-term persistence and activity of the transgene as determined using *in vivo* and *in vitro* assays as compared to cTCR/SIR cassettes expressed using lentiviral vectors. Other cTCRs (SEQ ID

NO: 3865-3868) also show uniform expression and activity when directed to the TRAC locus. The cTCR and SIR expressing T cells are purified by staining with FITC conjugated CD3 antibody and PE-Protein L followed by flow sorting. The alloreactivity of the cTCR- and SIR-expressing T cells is tested using mixed lymphocyte culture reaction using irradiated T cells derived from an allogeneic donor. T cells in which the cTCR/SIR are directed to the TRAC locus show markedly reduced to absence of alloreactivity as measured by proliferative response as compared to T cells in which cTCR/SIR are expressed using lentiviral vectors. The ability of cTCR/SIR expressing human T cells to induce Graft vs Host Disease (GVHD) is examined by administration of 5 million cTCR/SIR expressing T cells per animal into immunodeficient NSG mice (Jackson Lab). Animals are observed for over 90 days. Human T cells in which the cTCR/SIR cassettes are directed to the TRAC locus show markedly reduced to absence of Graft vs Host Disease (GVHD) when infused into immunodeficient NSG mice (Jackson Lab) while GVHD is observed in animals given T cells in which cTCR/SIR are expressed using lentiviral vectors. The ability of cTCR and SIR expressing human T cells to induce Graft vs Host Disease (GVHD) is also examined by administration of cTCR/SIR expressing T cells (1 million cells per kilogram) into allogeneic human recipients who have received lymphodepleting chemotherapy. Allogeneic T cells in which the cTCR/SIR cassettes are directed to the TRAC locus show markedly reduced incidence and severity of Graft vs Host Disease (GVHD) when given to allogeneic recipients. Essentially similar results are obtained using T cells in which cTCR and SIR are expressed by directing the expression cassettes to the TRBC locus.

[00456] Directing a TCR or a cTCR/SIR constructs to the TRAC locus

[00457] A TCR construct and a cTCRs (or SIR) construct targeting NY-ESO-1/HLA-A2 complex are generated in lentiviral vector (SEQ ID NO: 3837) and are based on TCR NYESO-1G4 and TCR mimic antibody NYESO-35-15. The nucleotide sequences of these constructs are represented by SEQ ID NO: 3883 and 3882, respectively. The two constructs are also targeted to the TRAC locus using the targeting constructs represented by SEQ ID NO: 3874-3877. The design of the targeting construct is shown in **Fig. 8**. T cells in which the NY-ESO-1 TCR or cTCR is directed to the TRAC locus show uniform expression of the transgene, good recognition of target cells expressing NY-ESO-1/HLA-A2 complex and perform equally well or better than T cells in which the above constructs are expressed using lentiviral mediated gene transfer using *in vivo* assays. Human T cells in which the NY-ESO-1 TCR or cTCR is directed to the TRAC locus also show reduced alloreactivity in mixed lymphocyte reaction and reduced GVHD in NSG mice xenograft model as compared to the T

cells in which the the NY-ESO-1 or cTCR are expressed using lentiviral mediated gene transfer.

[00458] Directing a single chain cTCR/SIR construct to the TRAC locus

[00459] Single chan cTCR/SIRs in which FMC63-scFv is attached to codon optimized TCRA constant chain or codon optimized plus murinized TCRA constant chain (SEQ ID NO: 3881) are expressed in T cells using lentiviral vector and show poor expression and activity. The same constructs are directed to the TRAC locus using the targeting constructs shown in **Fig. 9** and represented by SEQ ID NO: 3869-3872. T cells in which the single chain cTCRs/SIRs are directed to the the TRAC locus show uniform expression and activity of the cTCR when assayed using the assays described previously. In addition, T cells in which the single chain cTCR/SIR are directed to the TRAC locus show reduced incidence of alloreactivity using MLR and reduced incidence of GVHD using NSG mice xenograft model as compared to the T cells in which the the NY-ESO-1 or cTCR are expressed using lentiviral mediated gene transfer.

[00460] In the above examples, the CAR/TFP/Ab-TCR/TCR/cTCRs are directed to the TRAC locus. Essentially a similar procedure can be used to direct the CAR/TFP/Ab-TCR/TCR/cTCR or an accessory module to the TCBC, CD3 ϵ , CD3 δ , CD3 γ , and CD3 ζ loci using techniques known in the art.

[00461] A shorter EF1 α promoter retains strong promoter activity in T cells and is suitable for adoptive cellular therapy

[00462] Use of strong viral promoters in adoptive cellular therapy applications carries the risk of activation of downstream oncogenes and development of cancer. As such, human Elongation Factor 1 α (EF1 α) promoter is frequently used in adoptive cellular therapy applications as it provides strong expression and is human in origin. A limitation of EF1 α promoter, however, is its relatively large size. Although a mini-EF1 α promoter has been described, it is much weaker as compared to the EF1 α promoter. To determine whether an internal deletion in the EF1 α promoter would allow shortening of its length while preserving its promoter strength, a SacII fragment was deleted from the EF1 α promoter. The nucleotide sequence of the resulting EF1 α -D-SacII promoter is presented in SEQ ID NO: 3842.

Lentiviral vectors encoding a CD19-directed FMC63-BBz CAR were constructed in the vectors with the wild-type EF1 α promoter (SEQ ID NO: 3840) or EF1 α -D-SacII promoter (SEQ ID NO: 3839). The vectors also co-expressed EGFP and blasticidin resistance gene via 2A linkers. Lentiviruses were generated in 293FT cells and used to infect J-N-G cells. Infected cells were selected with blasticidin and then tested for their ability to induce EGFP

expression upon co-culture with CD19+ve RAJI cells. Near equivalent induction of EGFP expression was observed in J-N-G cells transduced with either lentiviral construct. In addition, near equivalent expression of the FMC63-BBz CAR was observed on the surface of J-N-G cells transduced with either construct as determined by binding with CD19-ECD-GGS-NLuc fusion protein. These results demonstrate that the EF1 α -D-SacII promoter can be used for adoptive cellular therapy applications. The results further demonstrate that the EF1 α -D-SacII promoter is not more prone to silencing than the wild-type EF1 α promoter and can be used for long-term transgene expression.

[00463] Use of water soluble Dasatinib Salt for control of Cytokine release

syndrome and neurological complications observed during adoptive cellular therapy

[00464] Dasatinib is a poorly water soluble drug and commercial Dasatinib is a monohydrate and reported to have solubility of 8 $\mu\text{g/mL}$ at 24 °C. As patients with CRS and neurological complications have difficulty taking the oral form of Dasatinib, water soluble form of Dasatinib is desirable. Water soluble salts of Dasatinib have been described in WO2015107545 A1. Injectable compositions comprising soluble salts of Dasatinib methane sulphonate monohydrate can be prepared according to the method of WO2015107545 A1 and used to treat patients with CRS and neurological complications associated with administration of CAR-T cells and Blinatumomab. The dose of Dasatinib methane sulphonate monohydrate can be titrated up to achieve an effective plasma concentration. In an exemplary embodiment, the plasma concentration of Dasatinib is kept higher than 10 nM, 20 nM, 50 nM, 100 nM, 200 nM or 300 nM. In another exemplary embodiment, the plasma concentration of Dasatinib is kept higher than 5 ng/ml, 15 ng/ml, 25 ng/ml, 50 ng/ml or 75 ng/ml. Finally, Dasatinib methane sulphonate monohydrate dissolved in normal saline can be also used for intra-thecal administration in patients with neurological complications from CAR-T cells and Blinatumomab. In an exemplary embodiment, the intra-thecal dose of Dasatinib methane sulphonate is adjusted to achieve CSF concentration higher than 10 nM, 20 nM, 50 nM, 100 nM, 200 nM or 300 nM. In an exemplary embodiment, the intra-thecal dose of Dasatinib methane sulphonate is adjusted to achieve CSF concentration higher than higher than 5 ng/ml, 15 ng/ml, 25 ng/ml, 50 ng/ml or 75 ng/ml.

[00465] Use of autologous T cells expressing conventional CARs and backbones 1-72 targeting multiple antigens for adoptive cell therapy

[00466] Patients with many different diseases, including infectious diseases (*e.g.*, HIV1, EBB, CMV, HTLV1, etc), degenerative diseases (*e.g.*, Alzheimer's disease), allergic diseases (*e.g.*, chronic idiopathic urticarial) and multiple cancers will be enrolled in an IRB

approved phase I clinical trial of immunotherapy with adoptively transferred autologous CAR-T cells coexpressing NEMO-K277A (backbone 2) targeting different disease-causing or disease-associated antigens. The CAR for different diseases will be selected based on the known expression of their target antigen in the disease-causing or disease-associated cells. Where possible, the expression of the CAR target on the disease causing or disease associated cells will be confirmed by binding with Antigen binding domain-GGS-NLuc fusion protein in which the antigen binding domain of the CAR is fused to non-secretory form of NLuc protein via a flexible linker. Alternatively, immunohistochemistry or flow cytometry using commercially available antibodies will be used to confirm the expression of the target antigen of the CAR on disease-causing or disease-associated cells. T cells will be collected from the subjects using leukopheresis, transduced with the appropriate lentivirus vectors and expanded *ex vivo* using CD3/CD28 beads in a closed system. After the resulting cell products have undergone quality control testing (including sterility and tumor specific cytotoxicity tests), they will be cryopreserved. CAR-T cell products will be administered to the subjects as described in the preceding example. Clinical and laboratory correlative follow-up studies can then be performed at the physician's discretion. Essentially a similar approach is used to test CARs in other backbones described in this disclosure.

[00467] Use of allogeneic T cells expressing conventional CARs and backbones 1-72 targeting multiple antigens for adoptive cell therapy

[00468] Patients with many different diseases, including infectious diseases (*e.g.*, HIV1, EBB, CMV, HTLV1, etc), degenerative diseases (*e.g.*, Alzheimer's disease), allergic diseases (*e.g.*, chronic idiopathic urticarial) and multiple cancers will be enrolled in an IRB approved phase I clinical trial of immunotherapy with adoptively transferred allogeneic CAR-T cells targeting different disease-causing or disease-associated antigens. The CAR for different diseases will be selected based on the known expression of their target antigen in the disease-causing or disease-associated cells. Where possible, the expression of the CAR target on the disease causing or disease associated cells will be confirmed by binding with Antigen binding domain-GGS-NLuc fusion protein in which the antigen binding domain of the CAR is fused to non-secretory form of NLuc protein via a flexible linker. Alternatively, immunohistochemistry or flow cytometry using commercially available antibodies will be used to confirm the expression of the target antigen of the CAR on disease-causing or disease-associated cells. T cells will be collected from a healthy donor using leukopheresis. The CAR expression cassette (SEQ ID NO: 1900 to SEQ ID NO: 2205) are cloned in the targeting vector and the CAR module is directed to the TRAC locus in the T cells essentially

as described by (Eyquem J et al, Nature, 543(7643):113-117). T cells lacking CD3 expression on the surface are selected by immunomagnetic purification and then expanded ex vivo using CD3/CD28 beads in a closed system. After the resulting cell products have undergone quality control testing (including sterility and tumor specific cytotoxicity tests), they will be cryopreserved. CAR-T cell products will be administered to the subjects as described in the preceding example. Clinical and laboratory correlative follow-up studies can then be performed at the physician's discretion. Essentially a similar approach is used to test CARs in other backbones, including CARs that co-express TCR α constant chain (TRAC) lacking the V α domain, described in this disclosure.

[00469] CAR-T Cell Hepatic Arterial Infusion

[00470] In addition to intravenous infusion, T cells expressing the conventional CARs and backbones 1-72 described in this invention can be infused intra-arterially to provide high concentration of CAR-T cells in a local area or organ involved with a disease. In the following example, this approach is used in case of a patient with hepatic metastases from a gastrointestinal cancer which expresses Folate Receptor alpha (FR1). Essentially a similar approach can be used for intra-arterial infusion of T cells expressing conventional CARs and backbones 1-72 targeting other tumor antigens.

[00471] A mapping angiogram will be performed via a right common femoral artery approach at baseline. The gastroduodenal and right gastric arteries, in addition to other potential sources of extrahepatic perfusion, will be embolized with microcoils. The same arterial access procedure will be carried out for administration of T cells expressing the construct CD8SP-FR1-huMov19-(vL-vH)-Myc-z-P2A- hNEMO-K277A-T2A-PAC (SEQ ID NO: 1727). The T cells will be collected from the patient on day 0 and will be infected with lentivirus encoding the construct CD8SP-FR1-huMov19-(vL-vH)-Myc-z-P2A-hNEMO-K277A-T2A-PAC and expanded as described in the previous examples. The CAR-T cells will be given in a dose escalating fashion on day 14 (10^7 CAR-T cells), day 28 (10^8 CAR-T cells) and day 44 (10^9 CAR-T cells). The CAR- T cells will be injected manually via a 60cc syringe at a rate of <2 cc/second. The total volume of infusion will be approximately 100 cc. Angiography with calibrated contrast rate will be performed after the first infusion of 50 cc and at completion of the CAR-T infusion to confirm preserved arterial flow. Infusions will be delivered into the proper hepatic artery when possible. Certain patients may have aberrant hepatic arterial anatomy, where either the right or left hepatic artery does not arise from the proper hepatic artery. In such cases the dose of CAR-T cells will be split based upon lobar

volume calculations. In such cases, split doses will be delivered separately into the right and left hepatic arteries to ensure proportionate CAR-T delivery to both hepatic lobes.

[00472] Intraperitoneal administration of CAR-T cells

[00473] CAR-T cells can also be administered intraperitoneally, essentially as described in Koneru M et al (Journal of Translational Medicine; 2015; 13:102). In the following example, this approach is used in patients with peritoneal involvement with ovarian cancer which expresses Folate Receptor alpha (FR1). Essentially a similar approach can be used for intra-peritoneal infusion of CAR-T cells targeting other tumor antigens described in this disclosure.

[00474] A screening informed consent will be offered to patients with recurrent high-grade serous ovarian cancer to test their cancer for the expression of FR1. After expression of FR1 is confirmed by immunohistochemistry, then patients will have a leukapheresis product obtained from peripheral blood. Excess platelet and red blood cell contamination will be removed from the leukapheresis product and the product will be frozen. In the treatment phase of the study, the leukapheresis product will be thawed and washed. Subsequently, CD3⁺ T cells will be isolated from the thawed leukapheresis product by magnetic separation using CD3/CD28 beads. Activated T cells will be lentivirally transduced with the CD8SP-FR1-huMov19-(vL-vH)-Myc-z-P2A-hNEMO-K277A-T2A-PAC construct and further expanded using CD3/CD28 bead expansion protocol.

[00475] Patients with recurrent high-grade serous ovarian, primary peritoneal or fallopian tube carcinoma shown to express FR1 antigen confirmed by immunohistochemistry (IHC) analysis of banked (paraffin embedded) or freshly biopsied tumor will potentially be eligible for the study.

[00476] The phase I dose-escalation dosing will be used in the trial. Cohorts of 3–6 patients will be infused with escalating doses of modified T cells to establish the maximum tolerated dose (MTD). There will be four planned dose levels: 3×10^5 , 1×10^6 , 3×10^6 , and 1×10^7 CAR-T cells/kg. Cohorts I and II will be treated with 3×10^5 CAR-T cells/kg but patients in cohort II will also receive lymphodepleting cyclophosphamide. Cohorts II-V will receive escalating doses of the modified T cells following pretreatment with cyclophosphamide. Lymphodepleting cyclophosphamide dosed at 750 mg/m² will be administered 2–4 days prior to the initial T cell infusion. A standard 3 + 3 dose escalation schema will be followed.

[00477] An IP catheter will be placed prior to T cell infusion. Patients will be admitted to the inpatient unit of the hospital prior to their first infusion of CAR T cells and will remain

hospitalized until at least 3 days after the second infusion of CAR T cells. The first cohort of patients to be treated, and the first patient treated in each subsequent cohort, will be admitted to the intensive care unit (ICU); subsequent patients may be admitted to the medical oncology inpatient service (subject to the clinical judgment of the treating physician).

[00478] Patients will receive a single dose of lymphodepleting cyclophosphamide (750 mg/m² IV) chemotherapy 2 to 4 days prior to initiating treatment with CAR-modified T cells. The transduced T cells will be quality tested for number, purity, viability, and sterility prior to infusion. All patients will receive 50% of the genetically modified T cell dose intravenously. Patients will be closely monitored for toxicities. One to 3 days later, the remaining dose of T cells will be administered as an IP infusion. At least 3 patients will be treated at dose level 1, with an accrual of no more than 2 patients per month within each dose level. All patients treated in the preceding cohort will be observed for a minimum of 4 weeks from the day of the initial T cell infusion before escalation to the next cohort occurs. Blood samples will be obtained from all patients prior to and following treatment to assess toxicity, therapeutic effects, and survival of the genetically modified T cells.

[00479] **Use of CAR-T cells for intratumoral injection**

[00480] CAR-T cells can also be administered intra-tumorally, essentially as described in Brown CE, et al, Clin Cancer Res. 2015 September 15; 21(18): 4062–4072. In the following example, this approach will be used in case of patients with recurrent glioblastoma (GBM) which expresses IL13Ra2. Essentially a similar approach can be used for intra-tumoral injection of T cells expressing conventional CARs or conventional CARs expressing accessory modules (backbones 1-72) targeting other tumor antigens.

[00481] A pilot safety and feasibility study will be conducted to test CD8SP-IL13Ra2-Hu108-(vL-vH)-Myc-z-P2A-hNEMO-K277A-T2A-PAC (SEQ ID NO: 1769) expressing T cells in recurrent GBM. All participating patients will be required to give written informed consent. The clinical protocol will be approved by the University of Southern California Institutional Review Board and conducted under an Investigational New Drug Application, and registered at ClinicalTrials.gov. Eligible patients will include adults (18-70 yrs) with recurrent or refractory unifocal supratentorial grade III or IV glioma whose tumors do not show communication with ventricles/CSF pathways and are amenable to resection. Participation in this trial will be independent of IL13Ra2 (or IL13Ra2) tumor antigen status. Patients will be enrolled following initial diagnosis of high-grade glioma (WHO grade III or IV), at which time they will undergo leukapheresis for collection of peripheral blood mononuclear cells (PBMC). These cells will be used to engineer T cells to express the

construct CD8SP-IL13Ra2-Hu108-(vL-vH)-Myc-z-P2A-hNEMO-K277A-T2A-PAC containing the puromycin resistance gene (PAC) following infection with the corresponding lentiviral vector as described in the previous examples. Alternatively, the CAR-T cells could be generated following infection with a retroviral vector or using sleeping beauty transposon or by transfection of IVT mRNA. Subsequently, the release tested therapeutic CAR-T cells will be cryopreserved and stored for later use. At the time of first recurrence of the tumor, the research participant will undergo resection of tumor along with placement of a Rickham reservoir/catheter. Concurrently, the therapeutic CAR-T cells will be thawed, re-expanded *in vitro* using CD3/CD28 beads based rapid expansion protocol. Following recovery from surgery and post baseline MR imaging, the CAR-T cells will be administered directly into the resection cavity via the indwelling catheter, essentially as described (Brown CE, *et al*, Clin Cancer Res. 2015 21(18): 4062–4072). Cells will be manually injected into the Rickham reservoir using a 21 gauge butterfly needle to deliver a 2 mL volume over 5-10 minutes, followed by 2 mL flush with preservative free normal saline over 5 minutes. The protocol treatment plan will specify an intra-patient dose escalation schedule with a target of 12 CAR T cell doses administered intracranially over a 5 week period comprised of weekly treatment cycles. During cycles 1, 2, 4 and 5, T cell infusions will be performed on days 1, 3 and 5 of the cycle week, and week 3 will be a rest cycle. For safety, in cycle 1 an intrapatient dose escalation strategy, with CAR T cell doses of 10^7 , 5×10^7 and 10^8 cells per infusion administered on days 1, 3 and 5 respectively, will be used and this will be followed by 9 additional CAR T cell infusions of 10^8 cells over 4 weeks. Imaging to assess response will be performed during the week 3 rest cycle and after week 5. The guidelines provided in the NCI Common Toxicity Criteria version 2.0 (<https://ctep.info.nih.gov/>) will be followed for the monitoring of toxicity and adverse event reporting.

[00482] Use of CAR-T cells for ex-vivo purging of bone marrow or peripheral blood stem cell preparation prior to transplant

[00483] CAR T cells can be used to purge the bone marrow or peripheral blood stem cell preparation of cancer cells prior to stem cell transplant. In the following example, CD8SP-CS1-HuLuc64-(vL-vH)-Myc-z-P2A-hNEMO-K277A-T2A-PAC (SEQ ID NO: 1699) expressing T cells will be used to purge bone marrow or peripheral blood stem cells obtained from a patient with multiple myeloma prior to autologous stem cell (or bone marrow) transplant.

[00484] Patient will undergo leukopheresis to collect peripheral blood mononuclear cells (PBMC). T cells will be purified using CD3 beads. These cells will be used to engineer

T cells to express the CD8SP-CS1-HuLuc64-(vL-vH)-Myc-z-P2A- hNEMO-K277A-T2A-PAC CAR following infection with the corresponding lentiviral vector as described in the previous examples. Subsequently, the release-tested therapeutic CAR-T cells will be cryopreserved and stored for later use or used fresh. Bone marrow cells and peripheral blood progenitor cell products will be collected from a patient with multiple myeloma following standard procedures. For mobilization of peripheral blood stem cells, patients will receive cyclophosphamide, 3 gm/m² followed by G-CSF, 10 µg/kg subcutaneously each day beginning 24 h after cyclophosphamide until pheresis is complete. Peripheral blood stem cells will be collected once the peripheral blood CD34+-cell count is 15 cells/µl. The collection goal will be to process three blood volumes per day until a minimum of 2.0 times 10⁶ CD34+ cells/kg are reached after processing. The bone marrow and peripheral blood stem cell products will be optionally depleted of Red Blood Cells and/or enriched for CD34 expressing cells using CliniMACS Prodigy® System from Miltenyi Biotec and following the manufacturer's recommendations. The products will be used for *ex-vivo* purging fresh or cryopreserved. For purging, the bone marrow or peripheral blood stem cell products will be cocultured with thawed CAR-T cells at an effector to target ratio ranging from 5: 1 to 30:1 for 4 to 24 hours in XVIVO medium (Lonza) supplanted with 100 IU recombinant human-IL2. Cells will be cultured at 37°C, in a 5% CO₂ humidified incubator. At the end of the coculture period, an aliquot of the cells will be taken for sterility and quality testing (including measurement of CFU-GM and flow cytometry for CD34 and CD138 positive cells). The remaining sample will be administered intravenously to the patient who has previously received myeloablative chemotherapy (*e.g.*, high dose Melphalan in two divided doses of 70 mg/m² for a total dose of 140 mg/m²).

[00485] Use of Bispecific T cell engagers

[00486] Proteins encoded by the Bispecific T cell engagers are expressed in Hela cells using the constructs having the SEQ ID Nos listed in **Table 13**. The proteins are purified using Metal affinity tag or StrepTag II columns using standard protein purification techniques. The purified proteins are tested in phase I clinical trials. Patients are selected based on the expression of the target antigens of the bispecific antibodies using different assays known in the art. The bispecific antibodies are administered by 24 hour infusion. The guidelines provided in the NCI Common Toxicity Criteria version 2.0 ([http\[s://\]ctep.ifo.nih.gov/](http[s://]ctep.ifo.nih.gov/)) are followed for the monitoring of toxicity and adverse event reporting.

[00487] Use of CAR combinations

[00488] Patients with mesothelioma and glioblastomas are administered T cells infected with lentiviruses encoding the following combination of CARs targeting Mesothelin (expressed on mesothelioma), IL13Ra2 (expressed on Glioblastomas) and hematopoietic markers (CD19, CD20, CD22, BCMA). The T cells are either of the wild-type TCR chains or have the TCR α chain knocked out by CRISP/Cas9 approach. It is observed that coexpression in the same T cells with the wild-type TCR chains of a CAR targeting mesothelin with a CAR targeting CD19, CD20, CD22 or BCMA results in increased T cell expansion in vivo as compared to expression of Mesothelin alone. Essentially similar results are obtained with CAR targeting glioblastoma. However, in T cells that are defective in TCR chains, coexpression of TFP based CARs targeting CD20 (SEQ ID NO: 9660) fail to induce in vivo expansion while co-expression of SIR (SEQ ID NO: 9668) or Ab-TCR (SEQ ID NO: 9676) based CARs successfully induces T cells expansion.

[00489] **Table 15. Effect of CAR combination on in vivo T cell expansion**

Disease	T cells TCR status	Target antigen of 1st CAR	SEQ ID of 1st CAR	Target antigen of 2nd CAR	SEQ ID of 2nd CAR	T cell Expansion
Mesothelioma	Wild Type	Mesothelin	1505	None		Poor
Mesothelioma	Wild Type	Mesothelin	1505	CD19	1016	Good
Mesothelioma	Wild Type	Mesothelin	1505	CD19	1607	Good
Mesothelioma	Wild Type	Mesothelin	1505	CD20	1631	Good
Mesothelioma	Wild Type	Mesothelin	1505	CD22	1644	Good
Mesothelioma	Wild Type	Mesothelin	1505	BCMA	1624	Good
Glioblastoma	Wild Type	IL13Ra2	1493	None		Poor
Glioblastoma	Wild Type	IL13Ra2	1493	CD19	1016	Good
Glioblastoma	Wild Type	IL13Ra2	2075	CD19	1607	Good
Glioblastoma	Wild Type	IL13Ra2	2381	CD20	1631	Good
Glioblastoma	Wild Type	IL13Ra2	2687	CD22	1644	Good
Glioblastoma	Wild Type	IL13Ra2	2687	BCMA	1624	Good
Glioblastoma	TCR-alpha-ve	IL13Ra2	2075	CD20	9660	Poor
Glioblastoma	TCR-alpha-ve	IL13Ra2	2381	CD20	9660	Poor
Glioblastoma	TCR-alpha-ve	IL13Ra2	2687	CD20	9660	Poor
Glioblastoma	TCR-alpha-ve	IL13Ra2	1493	None		Poor
Glioblastoma	TCR-alpha-ve	IL13Ra2	1493	CD20	9668	Good
Glioblastoma	TCR-alpha-ve	IL13Ra2	2075	CD20	9676	Good
Glioblastoma	TCR-alpha-ve	IL13Ra2	2381	CD20	9668	Good
Glioblastoma	TCR-alpha-ve	IL13Ra2	2687	BCMA	9362	Good
Glioblastoma	TCR-alpha-ve	IL13Ra2	2687	BCMA	9362	Good

[00490] The various methods and techniques described above provide a number of ways to carry out the application. Of course, it is to be understood that not necessarily all objectives or advantages described can be achieved in accordance with any particular embodiment described herein. Thus, for example, those skilled in the art will recognize that the methods can be performed in a manner that achieves or optimizes one advantage or group of advantages as taught herein without necessarily achieving other objectives or advantages as taught or suggested herein. A variety of alternatives are mentioned herein. It is to be understood that some preferred embodiments specifically include one, another, or several features, while others specifically exclude one, another, or several features, while still others mitigate a particular feature by inclusion of one, another, or several advantageous features.

[00491] Furthermore, the skilled artisan will recognize the applicability of various features from different embodiments. Similarly, the various elements, features and steps discussed above, as well as other known equivalents for each such element, feature or step, can be employed in various combinations by one of ordinary skill in this art to perform methods in accordance with the principles described herein. Among the various elements, features, and steps some will be specifically included and others specifically excluded in diverse embodiments.

[00492] A number of embodiments have been set forth above to illustrate the disclosure. The following claims further set forth what the Applicants regard as their invention.

WHAT IS CLAIMED IS:

1. An immune cell or immune cell population thereof expressing (i) at least one non-naturally occurring immune receptor and (ii) at least one non-naturally occurring agent that selectively activates the NF- κ B signaling pathway.
2. The immune cell or immune cell population thereof of claim 1, where the at least one non-naturally occurring immune receptor comprises at least one antigen-binding domain and at least one transmembrane domain.
3. The immune cell or immune cell population thereof of claim 1, where the at least one non-naturally occurring immune receptor is capable of recruiting at least one TCR associated signaling module.
4. The immune cell or immune cell population thereof of claim 1, where the at least one non-naturally occurring immune receptor is a chimeric antigen receptor (CAR) or a recombinant TCR.
5. The immune cell or immune cell population thereof of claim 2, wherein the at least one antigen-binding domain of the at least one non-naturally occurring immune receptor binds to an antigen selected from a group consisting of CD5; CD19; CD123; CD22; CD30; CD171; CS1 (also referred to as CD2 subset 1, CRACC, MPL, SLAMF7, CD319, and 19A24); C-type lectin-like molecule-1 (CLL-1 or CLECL1); CD33; epidermal growth factor receptor variant III (EGFRviii); ganglioside G2 (GD2); ganglioside GD3 (aNeu5Ac(2-8)aNeu5Ac(2-3)bDGalp(1-4)bDGlc(1-1)Cer); TNF receptor family member B cell maturation (BCMA); Tn antigen ((Tn Ag) or (GalNAc α -Ser/Thr)); prostate-specific membrane antigen (PSMA); Receptor tyrosine kinase-like orphan receptor 1 (ROR1); Fms Like Tyrosine Kinase 3 (FLT3); Tumor-associated glycoprotein 72 (TAG72); CD38; CD44v6; a glycosylated CD43 epitope expressed on acute leukemia or lymphoma but not on hematopoietic progenitors, a glycosylated CD43 epitope expressed on non-hematopoietic cancers, Carcinoembryonic antigen (CEA); Epithelial cell adhesion molecule (EPCAM); B7H3 (CD276); KIT (CD117); Interleukin-13 receptor subunit alpha-2 (IL-13Ra2 or CD213A2); Mesothelin; Interleukin 11 receptor alpha (IL-11Ra); prostate stem cell antigen (PSCA); Protease Serine 21 (Testisin or PRSS21); vascular endothelial growth factor receptor 2 (VEGFR2); Lewis(Y) antigen; CD24; Platelet-derived growth factor receptor beta

(PDGFR-beta); Stage-specific embryonic antigen-4 (SSEA-4); CD20; Folate receptor alpha (FRa or FR1); Folate receptor beta (FRb); Receptor tyrosine-protein kinase ERBB2 (Her2/neu); Mucin 1, cell surface associated (MUC1); epidermal growth factor receptor (EGFR); neural cell adhesion molecule (NCAM); Prostase; prostatic acid phosphatase (PAP); elongation factor 2 mutated (ELF2M); Ephrin B2; fibroblast activation protein alpha (FAP); insulin-like growth factor 1 receptor (IGF-I receptor), carbonic anhydrase IX (CAIX); Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2); glycoprotein 100 (gp100); oncogene fusion protein consisting of breakpoint cluster region (BCR) and Abelson murine leukemia viral oncogene homolog 1 (Abl) (bcr-abl); tyrosinase; ephrin type-A receptor 2 (EphA2); sialyl Lewis adhesion molecule (sLe); ganglioside GM3 (aNeu5Ac(2-3)bDCIalp(l-4)bDGlcpl(l-1)Cer); transglutaminase 5 (TGS5); high molecular weight-melanoma associated antigen (HMWMAA); o-acetyl-GD2 ganglioside (OAcGD2); tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related (TEM7R); claudin 6 (CLDN6); thyroid stimulating hormone receptor (TSHR); G protein coupled receptor class C group 5, member D (GPRC5D); chromosome X open reading frame 61 (CXORF61); CD97; CD179a; anaplastic lymphoma kinase (ALK); Polysialic acid; placenta-specific 1 (PLAC1); hexasaccharide portion of globoH glycosphingolipid (GloboH); mammary gland differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3 (ADRB3); pannexin 3 (PANX3); G protein-coupled receptor 20 (GPR20); lymphocyte antigen 6 complex, locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame Protein (TARP); Wilms tumor protein (WT1); Cancer/testis antigen 1 (NY-ESO-1); Cancer/testis antigen 2 (LAGE-1a); Melanoma-associated antigen 1 (MAGE-A1); ETS translocation-variant gene 6, located on chromosome 12p (ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, Member 1A (XAGE1); angiopoietin-binding cell surface receptor 2 (Tie 2); melanoma cancer testis antigen-1 (MAD-CT-1); melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; tumor protein p53 (p53); p53 mutant; prostatein; survivin; telomerase; prostate carcinoma tumor antigen-1 (PCT A-1 or Galectin 8), melanoma antigen recognized by T cells 1 (MelanA or MART1); Rat sarcoma (Ras) mutant; human Telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoints; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease, serine 2 (TMPRSS2) ETS fusion gene); N-Acetyl glucosaminyl-transferase V (NA17); paired box protein Pax-3 (PAX3); Androgen receptor; Cyclin B1; v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Tyrosinase-related protein 2 (TRP-2); Cytochrome

P450 1B 1 (CYP1B 1); CCCTC-Binding Factor (Zinc Finger Protein)-Like (BORIS or Brother of the Regulator of Imprinted Sites), Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3); Paired box protein Pax-5 (PAX5); proacrosin binding protein sp32 (OY-TESI); lymphocyte-specific protein tyrosine kinase (LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint 2 (SSX2); Receptor for Advanced Glycation Endproducts (RAGE-1); renal ubiquitous 1 (RUI); renal ubiquitous 2 (RU2); legumain; human papilloma virus E6 (HPV E6); human papilloma virus E7 (HPV E7); intestinal carboxyl esterase; heat shock protein 70-2 mutated (mut hsp70-2); CD79a; CD79b; CD72; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR or CD89); Leukocyte immunoglobulin-like receptor subfamily A member 2 (LILRA2); CD300 molecule-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2); lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); and immunoglobulin lambda-like polypeptide 1 (IGLL1), MPL, Biotin, c-MYC epitope Tag, CD34, LAMP1 TROP2, GFRalpha4, CDH17, CDH6, NYBR1, CDH19, CD200R, SleA (CA19.9; Sialyl Lewis X Antigen); Fucosyl-GM1, PTK7, gpNMB, CDH1-CD324, DLL3, CD276/B7H3, IL11Ra, IL13Ra2, CD179b-IGL11, TCRgamma-delta, NKG2D, CD32 (FCGR2A), Tn ag, Tim1-/HVCRI, CSF2RA (GM-CSFR-alpha), TGFbetaR2, Lewis Ag, TCR-beta1 chain, TCR-beta2 chain, TCR-gamma chain, TCR-delta chain, FITC, Leutenizing hormone receptor (LHR), Follicle stimulating hormone receptor (FSHR), Gonadotropin Hormone receptor (CGHR or GR), CCR4, GD3, SLAMF6, SLAMF4, HIV1 envelope glycoprotein, HTLV1-Tax, CMV pp65, EBV-EBNA3c, KSHV K8.1, KSHV-gH, influenza A hemagglutinin (HA), GAD, PDL1, Guanylyl cyclase C (GCC), auto antibody to desmoglein 3 (Dsg3), auto antibody to desmoglein 1 (Dsg1), HLA, HLA-A, HLA-A2, HLA-B, HLA-C, HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, HLA-DR, HLA-G, IgE, CD99, Ras G12V, Tissue Factor 1 (TF1), AFP, GPRC5D, Claudin18.2 (CLD18A2 or CLDN18A.2), P-glycoprotein, STEAP1, Liv1, Nectin-4, Cripto, gpA33, BST1/CD157, low conductance chloride channel, and an antigen recognized by TNT antibody.

6. The immune cell or immune cell population thereof of claim 1, wherein the at least one non-naturally occurring agent capable of selectively activating NF- κ B pathway is selected from the group consisting of vFLIP K13, K13-opt, a NEMO mutant, a NEMO-fusion protein, IKK1-S176E-S180E, IKK2-S177E-S181E, RIP, IKK α , IKK β , Tcl-1, MyD88-L265,

any NF- κ B activating protein or protein fragment, any inhibitor of an inhibitor of NF- κ B pathway, any gene editing system capable of selectively activating NF- κ B, any homolog or variant thereof and any combination thereof.

7. The immune cell or immune cell population thereof of claim 1, wherein the at least one non-naturally occurring agent capable of selectively activating NF- κ B pathway is of non-viral origin.

8. The immune cell or immune cell population thereof of claim 1, wherein the at least one non-naturally occurring agent capable of selectively activating NF- κ B pathway is a gene editing system.

9. The immune cell or immune cell population thereof of claim 1, wherein the at least one non-naturally occurring agent capable of selectively activating NF- κ B pathway induces oligomerization of NEMO/IKK γ .

10. The immune cell or immune cell population thereof of claim 1, wherein the at least one non-naturally occurring agent capable of selectively activating NF- κ B pathway induces activation of the IKK complex.

11. The immune cell or immune cell population thereof of claim 1, wherein at least one the non-naturally occurring agent capable of selectively activating NF- κ B pathway does not activate the AKT pathway.

12. The immune cell or immune cell population thereof of claim 1, wherein the at least one non-naturally occurring agent capable of selectively activating NF- κ B pathway is expressed in a constitutive or inducible manner.

13. The immune cell or immune cell population thereof of claim 1, wherein the at least one non-naturally occurring agent capable of selectively activating NF- κ B pathway is expressed transiently.

14. The immune cell or immune cell population thereof of claim 1, wherein the at least one non-naturally occurring agent capable of selectively activating NF- κ B pathway is expressed stably.
15. The immune cell or immune cell population thereof of claim 1, wherein the activity of the at least one non-naturally occurring agent capable of selectively activating NF- κ B pathway is controlled post-translationally through contacting the cell with a compound.
16. The immune cell or immune cell population thereof of claim 1, where the at least one non-naturally occurring agent capable of selectively activating NF- κ B pathway is expressed as a fusion construct with one or more copies of a switch domain.
17. The immune cell or immune cell population thereof of claim 9, wherein the activity of the at least one non-naturally occurring agent capable of selectively activating NF- κ B pathway is controlled at the post-translational level by administration of therapeutically effective amount of a compound that induces dimerization of the switch domain.
18. The immune cell or immune cell population thereof of claim 16 or 17, where the switch domain comprises one or more copies of a FKBP12 domain.
19. The immune cell or immune cell population thereof of claim 15, wherein the compound is AP20187 or Rimiducid or a homolog thereof.
20. The immune cell or immune cell population thereof of claim 1, wherein the immune cell is a T-lymphocyte (T-cell), a CAR-T cell, a TCR-expressing T cell, a tumor infiltrating lymphocyte (TIL), a tissue resident lymphocyte, a stem cell, an induced pluripotent stem cell or a Natural Killer (NK) cell.
21. The immune cell or immune cell population thereof of claim 1, wherein the immune cell has been engineered to lack a functional native T-Cell Receptor (TCR) signaling complex and/or β 2 microglobulin.
22. The immune cell or immune cell population thereof of claim 1 and 21, wherein the at least one non-naturally occurring immune receptor and/or the at least one agent capable of

selectively activating NF- κ B signaling pathway are cloned into an endogenous TCR gene such that the expression of the at least one non-naturally occurring immune receptor and/or the at least one agent capable of selectively activating NF- κ B signaling pathway are under control of the endogenous regulatory elements/promoter for the TCR gene.

23. The immune cell or immune cell population thereof of claim 1 that is used for the prevention and treatment of a disease selected from the group of a cancer, infectious disease, immune disease, and allergic disease.

24. The immune cell or immune cell population thereof of claim 1, where at least one polynucleotide encodes the at least one non-naturally occurring immune receptor and the at least one non-naturally occurring agent capable of selectively activating NF- κ B signaling pathway are expressed from a single promoter.

25. The immune cell or immune cell population thereof of claim 1, where at least one polynucleotide encoding the at least one non-naturally occurring immune receptor and the at least one non-naturally occurring agent capable of selectively activating NF- κ B signaling pathway are expressed using two or more separate promoters.

26. The immune cell or immune cell population thereof of claim 24 or 25, wherein the at least one polynucleotide comprises a first nucleic acid coding sequence encoding the at least one non-naturally occurring immune receptor separated from a second nucleic acid sequence encoding the non-naturally occurring agent capable of selectively activating NF- κ B such that upon expression of the first and second nucleic acid coding sequences that non-naturally occurring immune receptor and non-naturally occurring agent capable of selectively activating NF- κ B are not physically or chemically linked.

27. The immune cell or immune cell population thereof of claim 24 or 25, wherein the at least one non-naturally occurring immune receptor and/or the at least one non-naturally occurring agent capable of selectively activating NF- κ B coding polynucleotide(s) are cloned into an endogenous TCR gene such that the at least one non-naturally occurring immune receptor and/or at least one non-naturally occurring agent capable of selectively activating NF- κ B are under control of the endogenous regulatory elements/promoter for the TCR gene.

28. The immune cell or immune cell population thereof of claims 21 or 27, wherein one or more constant chains of the TCR genes are functionally re-expressed.
29. At least one recombinant polynucleotide encoding at least one non-naturally occurring immune receptor, the at least one recombinant polynucleotide comprising:
- (a) a first nucleic acid domain encoding a partial or entire transmembrane and/or cytoplasmic domain and optionally the extracellular domain of an endogenous protein, wherein the endogenous protein is expressed on the surface of lymphocytes and triggers the activation and/or proliferation of the lymphocyte;
 - (b) optionally a polynucleotide a linker;
 - (c) a second nucleic acid domain operably linked to the first nucleic acid domain, wherein the second nucleic acid domain encodes one or more non-natural TCR antigen binding domain(s);
 - (d) an optional third nucleic acid domain encoding a costimulatory domain; and
 - (e) an optional additional nucleic acid domain encoding an accessory module.
30. At least one recombinant polynucleotide comprising:
- a) a first nucleic acid encoding a non-naturally occurring immune receptor; and
 - b) a second nucleic acid encoding an accessory module comprising a selective NF- κ B activator.
31. The at least one recombinant polynucleotide of claim 30, wherein the first nucleic acid and the second nucleic acid are separated by an oligonucleotide linker encoding a cleavable peptide linker.
32. The at least one recombinant polynucleotide of claim 30, comprising two recombinant polynucleotide such that the first nucleic acid and second nucleic acid are expressed from separate vectors.
33. The at least one recombinant polynucleotide of claim 30, wherein the selective NF- κ B activator is a non-naturally occurring selective NF- κ B activator.

34. The at least one polynucleotide of claim 30, where the non-naturally occurring immune receptor is selected from the group consisting of a CAR, an Ab-TCR, a TFP, a cTCR, a SIR and a recombinant TCR.
35. The at least one polynucleotide of claim 30, wherein the non-naturally occurring immune receptor comprises an (i) an extracellular antigen specific domain, (ii) a transmembrane domain, and (iii) an optional intracellular signaling domain comprising an immunoreceptor tyrosine-based activation motif (ITAM), wherein (iii) is located at the C-terminus of the non-naturally occurring immune receptor.
36. The at least one polynucleotide of claim 30, wherein upon expression of the first and second nucleic acids sequences the non-naturally occurring immune receptor and selective NF- κ B activator polypeptide are not physically or chemically linked.
37. The at least one polynucleotide of claim 35, wherein the extracellular antigen-specific domain binds to any one or more of CD5; CD19; CD123; CD22; CD30; CD171; CS1 (also referred to as CD2 subset 1, CRACC, MPL, SLAMF7, CD319, and 19A24); C-type lectin-like molecule-1 (CLL-1 or CLECL1); CD33; epidermal growth factor receptor variant III (EGFRviii); ganglioside G2 (GD2); ganglioside GD3 (aNeu5Ac(2-8)aNeu5Ac(2-3)bDGalp(1-4)bDGlc(1-1)Cer); TNF receptor family member B cell maturation (BCMA); Tn antigen ((Tn Ag) or (GalNAc α -Ser/Thr)); prostate-specific membrane antigen (PSMA); Receptor tyrosine kinase-like orphan receptor 1 (ROR1); Fms Like Tyrosine Kinase 3 (FLT3); Tumor-associated glycoprotein 72 (TAG72); CD38; CD44v6; a glycosylated CD43 epitope expressed on acute leukemia or lymphoma but not on hematopoietic progenitors, a glycosylated CD43 epitope expressed on non-hematopoietic cancers, Carcinoembryonic antigen (CEA); Epithelial cell adhesion molecule (EPCAM); B7H3 (CD276); KIT (CD117); Interleukin-13 receptor subunit alpha-2 (IL-13Ra2 or CD213A2); Mesothelin; Interleukin 11 receptor alpha (IL-11Ra); prostate stem cell antigen (PSCA); Protease Serine 21 (Testisin or PRSS21); vascular endothelial growth factor receptor 2 (VEGFR2); Lewis(Y) antigen; CD24; Platelet-derived growth factor receptor beta (PDGFR-beta); Stage-specific embryonic antigen-4 (SSEA-4); CD20; Folate receptor alpha (FRa or FR1); Folate receptor beta (FRb); Receptor tyrosine-protein kinase ERBB2 (Her2/neu); Mucin 1, cell surface associated (MUC1); AFP/MHC complex; epidermal growth factor receptor (EGFR); neural cell adhesion molecule (NCAM); Prostase; prostatic acid phosphatase (PAP); elongation factor 2

mutated (ELF2M); Ephrin B2; fibroblast activation protein alpha (FAP); insulin-like growth factor 1 receptor (IGF-I receptor); carbonic anhydrase IX (CAIX); Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2); glycoprotein 100 (gp100); oncogene fusion protein consisting of breakpoint cluster region (BCR) and Abelson murine leukemia viral oncogene homolog 1 (Abl) (bcr-abl); tyrosinase; ephrin type-A receptor 2 (EphA2); sialyl Lewis adhesion molecule (sLe); ganglioside GM3 (aNeu5Ac(2-3)bDCIalp(l- 4)bDGlcpl(l- 1)Cer); transglutaminase 5 (TGS5); high molecular weight-melanoma associated antigen (HMWMAA); o-acetyl-GD2 ganglioside (OAcGD2); tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related (TEM7R); claudin 6 (CLDN6); thyroid stimulating hormone receptor (TSHR); G protein coupled receptor class C group 5, member D (GPRC5D); chromosome X open reading frame 61 (CXORF61); CD97; CD179a; anaplastic lymphoma kinase (ALK); Polysialic acid; placenta-specific 1 (PLAC1); hexasaccharide portion of globoH glycosphingolipid (GloboH); mammary gland differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3 (ADRB3); pannexin 3 (PANX3); G protein-coupled receptor 20 (GPR20); lymphocyte antigen 6 complex, locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame Protein (TARP); Wilms tumor protein (WT1); WT1/MHC I complex; Cancer/testis antigen 1 (NY-ESO-1); NY-ESO-1/MHC I complex; Cancer/testis antigen 2 (LAGE-1a); Melanoma-associated antigen 1 (MAGE-A1); ETS translocation-variant gene 6, located on chromosome 12p (ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, Member 1A (XAGE1); angiopoietin-binding cell surface receptor 2 (Tie 2); melanoma cancer testis antigen-1 (MAD-CT-1); melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; tumor protein p53 (p53); p53 mutant; prostein; survivin; telomerase; prostate carcinoma tumor antigen-1 (PCT A-1 or Galectin 8), melanoma antigen recognized by T cells 1 (MelanA or MARTI); Rat sarcoma (Ras) mutant; human Telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoints; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease, serine 2 (TMPRSS2) ETS fusion gene); N-Acetyl glucosaminyl-transferase V (NA17); paired box protein Pax-3 (PAX3); Androgen receptor; Cyclin B1; v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Tyrosinase-related protein 2 (TRP-2); Cytochrome P450 1B 1 (CYP1B 1); CCCTC-Binding Factor (Zinc Finger Protein)-Like (BORIS or Brother of the Regulator of Imprinted Sites), Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3); Paired box protein Pax-5 (PAX5); proacrosin binding protein sp32 (OY-TESI); lymphocyte-specific

protein tyrosine kinase (LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint 2 (SSX2); Receptor for Advanced Glycation Endproducts (RAGE-1); renal ubiquitous 1 (RUI); renal ubiquitous 2 (RU2); legumain; human papilloma virus E6 (HPV E6); HPV E6/MHC I complex; human papilloma virus E7 (HPV E7); HPV E7/MHC I complex; AFP/MHC I complex; Ras/MHC I complex; intestinal carboxyl esterase; heat shock protein 70-2 mutated (mut hsp70-2); CD79a; CD79b; CD72; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR or CD89); Leukocyte immunoglobulin-like receptor subfamily A member 2 (LILRA2); CD300 molecule-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2); lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); and immunoglobulin lambda-like polypeptide 1 (IGLL1), MPL, Biotin, c-MYC epitope Tag, CD34, LAMP1 TROP2, GFRalpha4, CDH17, CDH6, NYBR1, CDH19, CD200R, Slea (CA19.9; Sialyl Lewis Antigen); Fucosyl-GM1, PTK7, gpNMB, CDH1-CD324, DLL3, CD276/B7H3, IL11Ra, IL13Ra2, CD179b-IGL11, TCRgamma-delta, NKG2D, CD32 (FCGR2A), Tn ag, Tim1-/HVCR1, CSF2RA (GM-CSFR-alpha), TGFbetaR2, Lews Ag, TCR-beta1 chain, TCR-beta2 chain, TCR-gamma chain, TCR-delta chain, FITC, Leutenizing hormone receptor (LHR), Follicle stimulating hormone receptor (FSHR), Gonadotropin Hormone receptor (CGHR or GR), CCR4, GD3, SLAMF6, SLAMF4, HIV1 envelope glycoprotein, HTLV1-Tax, CMV pp65, EBV-EBNA3c, KSHV K8.1, KSHV-gH, influenza A hemagglutinin (HA), GAD, PDL1, Guanylyl cyclase C (GCC), auto antibody to desmoglein 3 (Dsg3), auto antibody to desmoglein 1 (Dsg1), HLA, HLA-A, HLA-A2, HLA-B, HLA-C, HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, HLA-DR, HLA-G, IgE, CD99, Ras G12V, Tissue Factor 1 (TF1), AFP, GPRC5D, Claudin18.2 (CLD18A2 or CLDN18A.2), P-glycoprotein, STEAP1, Liv1, Nectin-4, Cripto, gpA33, BST1/CD157, low conductance chloride channel, and an antigen recognized by TNT antibody.

38. The at least one polynucleotide of claim 30, wherein the selective NF- κ B activator is selected from the group consisting of vFLIP K13, a NEMO mutant, a NEMO-fusion protein, IKK1-S176E-S180E, IKK2-S177E-S181E, RIP, FKBPx2-RIP-ID, IKK1, FKBPx2-IKKa, IKK2, FKBPx2-IKK2, Tcl-1, MyD88-L265, any NF- κ B activating protein or protein fragment, any inhibitor of an inhibitor of NF- κ B pathway, a gene editing system capable of

selectively activating NF- κ B, an RNA interference system that selectively activating NF- κ B and any combination thereof.

39. The at least one polynucleotide of claim 37, where the selective NF- κ B activator is expressed as a fusion construct with one or more copies of FKBP domain.

40. The polynucleotide of claim 35, wherein the extracellular antigen specific domain is selected from the group consisting of:

- the variable region of the heavy chain (vH) of an antibody or a fragment thereof specific for a predefined target antigen;
- the variable region of the light chain (vL) of an antibody or a fragment thereof specific for a predefined target antigen;
- a single chain variable fragment (scFv) or a fragment thereof specific for a predefined target antigens;
- an antibody fragment (*e.g.*, Fv, a Fab, a (Fab')₂) specific for a predefined target antigen;
- a single domain antibody (SDAB) fragments specific for a predefined target antigen;
- a camelid vHH domain specific for a predefined target antigen;
- a non-immunoglobulin antigen binding scaffolds specific for a predefined target antigen;
- a receptors specific or a fragment thereof for a predefined target antigen;
- a ligands or a fragment thereof specific for a predefined target antigen;
- a bispecific-antibody, -antibody fragment, -scFV, -vHH, -SDAB, -non-immunoglobulin antigen binding scaffold, -receptor or -ligand specific for one or more predefined target antigens; and
- an autoantigen or a fragment thereof.

41. At least one vector comprising the at least one polynucleotide of any one of claims 30-40.

42. The at least one vector of claim 41, wherein the vector is selected from the group consisting of a DNA vector, an RNA vector, a plasmid, a lentivirus vector, adenoviral vector, AAV vector, a retrovirus vector, a baculovirus vector, a sleeping beauty transposon vector, and a piggybac transposon vector.

43. An immune effector cell or stem cell comprising at least one recombinant polynucleotide of any of claims 30-40.
44. An immune effector cell or stem cell comprising that at least one vector of claim 41.
45. An immune effector cell or stem cell comprising the at least one vector of claim 42.
46. An antigen presenting cell comprising at least one vector of claim 41.
47. The immune effector cell or stem cell of any one of claims 43-45, wherein the immune effector cell is a human T cell, a human NKT cell or a synthetic T cell, NK cell, or a stem cell that can give rise to an immune effector cell, optionally, wherein the T cell is diacylglycerol kinase (DGK) and/or Ikaros deficient and/or Brd4 deficient.
48. A method to (i) extend the life span of an immune cell expressing, (ii) stimulate proliferation of an immune cell, (iii) stimulate cytokine production by an immune cell, (iv) enhance antigen presentation by an immune cell, (v) protect an immune cell from apoptosis, the method comprising transfecting or transforming the immune cells with a polynucleotide encoding a selective NF- κ B activator or a NF- κ B specific stimulatory polypeptide.
49. The method of claim 48, wherein the selective NF- κ B activator or a NF- κ B specific stimulatory polypeptide is selected from the group consisting of vFLIP K13, K13-opt, a NEMO mutant, a NEMO-fusion protein, IKK1-S176E-S180E, IKK2-S177E-S181E, RIP, IKK α , IKK β , Tcl-1, MyD88-L265, any NF- κ B activating protein or protein fragment, any inhibitor of an inhibitor of NF- κ B pathway, any homolog or variant thereof and any combination thereof.
50. The method of claim 49, wherein the selective NF- κ B activator or a NF- κ B specific stimulatory polypeptide is expressed in a constitutive or inducible manner.
51. The method of claim 50, wherein the selective NF- κ B activator or a NF- κ B specific stimulatory polypeptides controlled post-translationally through contacting the T cell with a compound.

52. The method of claim 50, where the selective NF- κ B activator or a NF- κ B specific stimulatory polypeptide is expressed as a fusion construct with one or more copies of FKBP domain.
53. The method of claim 52, wherein the activity of the selective NF- κ B activator or a NF- κ B specific stimulatory polypeptide is controlled at the post-translational level by administration of therapeutically effective amount of a compound that induces dimerization of the FKBP domain.
54. The method of claim 51, wherein the compound is AP20187 or rimiducid.
55. A method of making a non-naturally occurring immune receptor-expressing immune effector cell, comprising introducing at least one vector of claim 41 or at least one recombinant polynucleotide of claim 29 into an immune effector cell or a hematopoietic stem cell or progenitor cell that can give rise to an immune effector cell, under conditions such that a non-naturally occurring immune receptor is expressed and the immune effector cell comprises (i) extended life span, (ii) improved T cell proliferation, and/or (iii) reduced apoptosis compared to a CAR-T cell lacking an NF κ B specific stimulatory polypeptide.
56. The method of claim 55, further comprising:
- a) providing a population of immune effector cells; and
 - b) removing T regulatory cells from the population, thereby providing a population of T regulatory-depleted cells;
- wherein steps a) and b) are performed prior to introducing the vector or recombinant polynucleotide encoding the CAR and/or NF κ B specific stimulatory polypeptide to the population.
57. The method of claim 56, wherein the T regulatory cells are removed from the cell population using an anti-CD25 antibody, or an anti-GITR antibody.
58. The method of claim 55, further comprising:
- a) providing a population of immune effector cells; and

b) enriching P-glycoprotein (P-gp or Pgp; MDR1, ABCB1, CD243)-positive cells from the population, thereby providing a population of P-glycoprotein (P-gp or Pgp; MDR1, ABCB1, CD243)-enriched cells;

wherein steps a) and b) are performed prior to or after introducing the vector or recombinant polynucleotide encoding the CAR and/or NFkB specific stimulatory polypeptide.

59. The method of claim 58, wherein the P-glycoprotein positive cells are enriched using any one or more of the methods selected from the group consisting of:

- i) immunoselection using one or a cocktail of P-glycoprotein specific antibodies,
- ii) staining with one or more of fluorescent dyes that are substrates of P-glycoprotein, tetramethylrhodamine methyl ester (TMRM), Adriamycin and actinomycin-D) under conditions at which P-glycoprotein is active as a pump and enriching for cells that stain less with the dye,
- iii) selection of cells that are resistant to phototoxic compounds that are substrates of P-glycoprotein, such as any one or more of TH9402, 2-(4,5-dibromo-6-amino-3-imino-3H-xanthen-9-yl)-benzoic acid methyl ester hydrochloride, 2-(4,5-dibromo-6-amino-3-imino-3H-xanthen-9-yl)-benzoic acid ethyl ester hydrochloride, 2-(4,5-dibromo-6-amino-3-imino-3H-xanthen-9-yl)-benzoic acid octyl ester hydrochloride, 2-(4,5-dibromo-6-amino-3-imino-3H-xanthen-9-yl)-benzoic acid n-butyl ester hydrochloride, 2-(6-ethyl amino-3-ethyl imino-3H-xanthen-9-yl)-benzoic acid n-butyl ester hydrochloride, or derivatives thereof or combinations thereof, and
- iv) selection of cells that are resistant to cytotoxic compounds that are substrates of P-glycoprotein, such as vincristine, vinblastine, taxol, paclitaxel, mitoxantrone, etoposide, adriamycin, daunorubicin and actinomycin-D.

60. A method of generating a population of RNA-engineered cells comprising introducing *in vitro* transcribed RNA or RNAs or synthetic RNA or RNAs into a cell or population of cells, where the RNA or RNAs comprises a recombinant polynucleotide or polynucleotides of claim 30.

61. A method of providing anti-disease immunity in a subject comprising administering to the subject an effective amount of the immune effector cell or a stem cell that can give rise to an immune effector cell of any one of claims 43-47, wherein the cell is an autologous T

cell or an allogeneic T cell, or an autologous NKT cell or an allogeneic NKT cell or an autologous or an allogeneic hematopoietic stem cell or an autologous or an allogeneic iPSC that can give rise to an immune effector cell.

62. The method of claim 61, wherein the allogeneic T cell or allogeneic NKT cell or hematopoietic stem cell or iPSC lacks expression or has low expression of a functional TCR or a functional HLA.

63. A composition comprising an immune effector cell or a stem cell that can generate immune effector cells comprising a non-naturally occurring immune receptor and a selective NFkB activator, wherein the non-naturally occurring immune receptor comprises an antigen binding domains that bind to a disease-associated antigen associated said disease-associated antigen is selected from a group consisting of: CD5, CD19, CD123, CD22, CD30, CD171; CS-1 (also referred to as CD2 subset 1, CRACC, SLAMF7, CD319, and 19A24); C-type lectin-like molecule-1 (CLL-1 or CLECL1); CD33; epidermal growth factor receptor variant III (EGFRviii); ganglioside G2 (GD2); ganglioside GD3 (aNeu5Ac(2-8)aNeu5Ac(2-3)bdGalp(l-4)bdGlcP(l-l)Cer); TNF receptor family member B cell maturation (BCMA); Tn antigen ((Tn Ag) or (GalNAc α -Ser/Thr)); prostate-specific membrane antigen (PSMA); Receptor tyrosine kinase-like orphan receptor 1 (ROR1); FmsLike Tyrosine Kinase 3 (FLT3); Tumor-associated glycoprotein 72 (TAG72); CD38; CD44v6; a glycosylated CD43 epitope expressed on acute leukemia or lymphoma but not on hematopoietic progenitors, a glycosylated CD43 epitope expressed on non-hematopoietic cancers, Carcinoembryonic antigen (CEA); Epithelial cell adhesion molecule (EPCAM); B7H3 (CD276); KIT (CD117); Interleukin-13 receptor subunit alpha-2 (IL-13Ra2 or CD213A2); Mesothelin; Interleukin 11 receptor alpha (IL-11Ra); prostate stem cell antigen (PSCA); Protease Serine 21 (Testisin or PRSS21); vascular endothelial growth factor receptor 2 (VEGFR2); Lewis(Y) antigen; CD24; Platelet-derived growth factor receptor beta (PDGFR-beta); Stage-specific embryonic antigen-4 (SSEA-4); CD20; Folate receptor alpha; Receptor tyrosine-protein kinase ERBB2 (Her2/neu); Mucin 1, cell surface associated (MUC1); epidermal growth factor receptor (EGFR); neural cell adhesion molecule (NCAM); Prostase; prostatic acid phosphatase (PAP); elongation factor 2 mutated (ELF2M); Ephrin B2; fibroblast activation protein alpha (FAP); insulin-like growth factor 1 receptor (IGF-I receptor), carbonic anhydrase IX (CAIX); Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2); glycoprotein 100 (gp100); oncogene fusion protein consisting of breakpoint cluster region (BCR) and Abelson murine

leukemia viral oncogene homolog 1 (Abl) (bcr-abl); tyrosinase; ephrin type-A receptor 2 (EphA2); Fucosyl GM1; sialyl Lewis adhesion molecule (sLe); ganglioside GM3 (aNeu5Ac(2-3)bDCIalp(1-4)bDGlcp(1-1)Cer); transglutaminase 5 (TGS5); high molecular weight-melanoma-associated antigen (HMWMAA); o-acetyl-GD2 ganglioside (OAcGD2); tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related (TEM7R); claudin 6 (CLDN6); thyroid stimulating hormone receptor (TSHR); G protein coupled receptor class C group 5, member D (GPRC5D); chromosome X open reading frame 61 (CXORF61); CD97; CD179a; anaplastic lymphoma kinase (ALK); Polysialic acid; placenta-specific 1 (PLAC1); hexasaccharide portion of globoH glycosphingolipid (GloboH); mammary gland differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3 (ADRB3); pannexin 3 (PANX3); G protein-coupled receptor 20 (GPR20); lymphocyte antigen 6 complex, locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame Protein (TARP); Wilms tumor protein (WT1); Cancer/testis antigen 1 (NY-ESO-1); Cancer/testis antigen 2 (LAGE-1a); Melanoma-associated antigen 1 (MAGE-A1); ETS translocation-variant gene 6, located on chromosome 12p (ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, Member 1A (XAGE1); angiopoietin-binding cell surface receptor 2 (Tie 2); melanoma cancer testis antigen-1 (MAD-CT-1); melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; tumor protein p53 (p53); p53 mutant; prostein; surviving; telomerase; prostate carcinoma tumor antigen-1 (PCT A-1 or Galectin 8), melanoma antigen recognized by T cells 1 (MelanA or MART1); Rat sarcoma (Ras) mutant; human Telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoints; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease, serine 2 (TMPRSS2) ETS fusion gene); N-Acetyl glucosaminyl-transferase V (NA17); paired box protein Pax-3 (PAX3); Androgen receptor; Cyclin B1; v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Tyrosinase-related protein 2 (TRP-2); Cytochrome P450 1B 1 (CYP1B 1); CCCTC-Binding Factor (Zinc Finger Protein)-Like (BORIS or Brother of the Regulator of Imprinted Sites), Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3); Paired box protein Pax-5 (PAX5); proacrosin binding protein sp32 (OY-TES1); lymphocyte-specific protein tyrosine kinase (LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint 2 (SSX2); Receptor for Advanced Glycation End products (RAGE-1); renal ubiquitous 1 (RUI); renal ubiquitous 2 (RU2); legumain; human papilloma virus E6 (HPV E6); human papilloma virus E7 (HPV E7); intestinal carboxyl esterase; heat shock protein 70-2 mutated (mut hsp70-2); CD79a;

CD79b; CD72; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR or CD89); Leukocyte immunoglobulin-like receptor subfamily A member 2 (LILRA2); CD300 molecule-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2); lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); and immunoglobulin lambda-like polypeptide 1 (IGLL1), MPL, Biotin, c-MYC epitope Tag, CD34, LAMP1 TROP2, GFRalpha4, CDH17, CDH6, NYBR1, CDH19, CD200R, SleA (CA19.9; Sialyl Lewis Antigen) Fucosyl-GM1, PTK7, gpNMB, CDH1-CD324, DLL3, CD276/B7H3, IL11Ra, IL13Ra2, CD179b-IGL1, ALK TCRgamma-delta, NKG2D, CD32 (FCGR2A), CSPG4-HMW-MAA, Tim1-/HVCR1, CSF2RA (GM-CSFR-alpha), TGFbetaR2, VEGFR2/KDR, Lews Ag, TCR-beta1 chain, TCR-beta2 chain, TCR-gamma chain, TCR-delta chain, FITC, Leutenizing hormone receptor (LHR), Follicle stimulating hormone receptor (FSHR), Chorionic Gonadotropin Hormone receptor (CGHR), CCR4, SLAMF6, SLAMF4, HIV1 envelope glycoprotein, HTLV1-Tax, CMV pp65, EBV-EBNA3c, influenza A hemagglutinin (HA), GAD, PDL1, Guanylyl cyclase C (GCC), KSHV-K8.1 protein, KSHV-gH protein, auto-antibody to desmoglein 3 (Dsg3), autoantibody to desmoglein 1 (Dsg1), HLA, HLA-A, HLA-A2, HLA-B, HLA-C, HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, HLA-DR, HLA-G, IGE, CD99, RAS G12V, Tissue Factor 1 (TF1), AFP, GPRC5D, claudin18.2 (CLD18A2 OR CLDN18A.2)), P-glycoprotein, STEAP1, LIV1, NECTIN-4, CRIPTO, GPA33, BST1/CD157, low conductance chloride channel, and antigen recognized by TNT antibody.

64. A method of treating or preventing a disease associated with expression of a disease-associated antigen in a subject, comprising administering to the subject an effective amount of an immune effector cell comprising a non-naturally occurring immune receptor and a selective NFkB activator, wherein the non-naturally occurring immune receptor comprises an antigen binding domains that bind to a disease-associated antigen associated said disease-associated antigen is selected from a group consisting of: CD5, CD19; CD123; CD22; CD30; CD171; CS-1 (also referred to as CD2 subset 1, CRACC, SLAMF7, CD319, and 19A24); C-type lectin-like molecule-1 (CLL-1 or CLECL1); CD33; epidermal growth factor receptor variant III (EGFRviii); ganglioside G2 (GD2); ganglioside GD3 (aNeu5Ac(2-8)aNeu5Ac(2-3)bDGalp(1-4)bDGlc(1-1)Cer); TNF receptor family member B cell maturation (BCMA); Tn antigen ((Tn Ag) or (GalNAc α -Ser/Thr)); prostate-specific membrane antigen (PSMA);

Receptor tyrosine kinase-like orphan receptor 1 (ROR1); FmsLike Tyrosine Kinase 3 (FLT3); Tumor-associated glycoprotein 72 (TAG72); CD38; CD44v6; a glycosylated CD43 epitope expressed on acute leukemia or lymphoma but not on hematopoietic progenitors, a glycosylated CD43 epitope expressed on non-hematopoietic cancers, Carcinoembryonic antigen (CEA); Epithelial cell adhesion molecule (EPCAM); B7H3 (CD276); KIT (CD117); Interleukin-13 receptor subunit alpha-2 (IL-13Ra2 or CD213A2); Mesothelin; Interleukin 11 receptor alpha (IL-11Ra); prostate stem cell antigen (PSCA); Protease Serine 21 (Testisin or PRSS21); vascular endothelial growth factor receptor 2 (VEGFR2); Lewis(Y) antigen; CD24; Platelet-derived growth factor receptor beta (PDGFR-beta); Stage-specific embryonic antigen-4 (SSEA-4); CD20; Folate receptor alpha; Receptor tyrosine-protein kinase ERBB2 (Her2/neu); Mucin 1, cell surface associated (MUC1); epidermal growth factor receptor (EGFR); neural cell adhesion molecule (NCAM); Prostase; prostatic acid phosphatase (PAP); elongation factor 2 mutated (ELF2M); Ephrin B2; fibroblast activation protein alpha (FAP); insulin-like growth factor 1 receptor (IGF-I receptor), carbonic anhydrase IX (CAIX); Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2); glycoprotein 100 (gp100); oncogene fusion protein consisting of breakpoint cluster region (BCR) and Abelson murine leukemia viral oncogene homolog 1 (Abl) (bcr-abl); tyrosinase; ephrin type-A receptor 2 (EphA2); Fucosyl GM1; sialyl Lewis adhesion molecule (sLe); ganglioside GM3 (aNeu5Ac(2-3)bDCIalp(l-4)bDGlcpl(l-1)Cer); transglutaminase 5 (TGS5); high molecular weight-melanomaassociated antigen (HMWMAA); o-acetyl-GD2 ganglioside (OAcGD2); tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related (TEM7R); claudin 6 (CLDN6); thyroid stimulating hormone receptor (TSHR); G protein coupled receptor class C group 5, member D (GPRC5D); chromosome X open reading frame 61 (CXORF61); CD97; CD179a; anaplastic lymphoma kinase (ALK); Polysialic acid; placenta-specific 1 (PLAC1); hexasaccharide portion of globoH glycosphingolipid (GloboH); mammary gland differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3 (ADRB3); pannexin 3 (PANX3); G protein-coupled receptor 20 (GPR20); lymphocyte antigen 6 complex, locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame Protein (TARP); Wilms tumor protein (WT1); Cancer/testis antigen 1 (NY-ESO-1); Cancer/testis antigen 2 (LAGE-1a); Melanoma-associated antigen 1 (MAGE-A1); ETS translocation-variant gene 6, located on chromosome 12p (ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, Member 1A (XAGE1); angiopoietin-binding cell surface receptor 2 (Tie 2); melanoma cancer testis antigen-1 (MAD-CT-1); melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen

1; tumor protein p53 (p53); p53 mutant; prostein; surviving; telomerase; prostate carcinoma tumor antigen-1 (PCT A-1 or Galectin 8), melanoma antigen recognized by T cells 1 (MelanA or MART1); Rat sarcoma (Ras) mutant; human Telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoints; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease, serine 2 (TMPRSS2) ETS fusion gene); N-Acetyl glucosaminyl-transferase V (NA17); paired box protein Pax-3 (PAX3); Androgen receptor; Cyclin B1; v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Tyrosinase-related protein 2 (TRP-2); Cytochrome P450 1B 1 (CYPIB 1); CCCTC-Binding Factor (Zinc Finger Protein)-Like (BORIS or Brother of the Regulator of Imprinted Sites), Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3); Paired box protein Pax-5 (PAX5); proacrosin binding protein sp32 (OY-TES1); lymphocyte-specific protein tyrosine kinase (LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint 2 (SSX2); Receptor for Advanced Glycation End products (RAGE-1); renal ubiquitous 1 (RUI); renal ubiquitous 2 (RU2); legumain; human papilloma virus E6 (HPV E6); human papilloma virus E7 (HPV E7); intestinal carboxyl esterase; heat shock protein 70-2 mutated (mut hsp70-2); CD79a; CD79b; CD72; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR or CD89); Leukocyte immunoglobulin-like receptor subfamily A member 2 (LILRA2); CD300 molecule-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2); lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); and immunoglobulin lambda-like polypeptide 1 (IGLL1), MPL, Biotin, c-MYC epitope Tag, CD34, LAMP1 TROP2, GFRalpha4, CDH17, CDH6, NYBR1, CDH19, CD200R, Slea (CA19.9; Sialyl Lewis Antigen) Fucosyl-GM1, PTK7, gpNMB, CDH1-CD324, DLL3, CD276/B7H3, IL11Ra, IL13Ra2, CD179b-IGL11, ALK TCRgamma-delta, NKG2D, CD32 (FCGR2A), CSPG4-HMW-MAA, Tim1-/HVCR1, CSF2RA (GM-CSFR-alpha), TGFbetaR2, VEGFR2/KDR, Lews Ag, TCR-beta1 chain, TCR-beta2 chain, TCR-gamma chain, TCR-delta chain, FITC, Leutenizing hormone receptor (LHR), Follicle stimulating hormone receptor (FSHR), Chorionic Gonadotropin Hormone receptor (CGHR), CCR4, SLAMF6, SLAMF4, HIV1 envelope glycoprotein, HTLV1-Tax, CMV pp65, EBV-EBNA3c, influenza A hemagglutinin (HA), GAD, PDL1, Guanylyl cyclase C (GCC), KSHV-K8.1 protein, KSHV-gH protein, auto-antibody to desmoglein 3 (Dsg3), autoantibody to desmoglein 1 (Dsg1), HLA, HLA-A, HLA-A2, HLA-B, HLA-C, HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, HLA-

DR, HLA-G, IGE, CD99, RAS G12V, Tissue Factor 1 (TF1), AFP, GPRC5D, claudin18.2 (CLD18A2 OR CLDN18A.2)), P-glycoprotein, STEAP1, LIV1, NECTIN-4, CRIPTO, GPA33, BST1/CD157, low conductance chloride channel, and antigen recognized by TNT antibody,

thereby treating the subject or preventing a disease in the subject.

65. The use or method of claim 63 or 64, wherein the disease associated with expression of the disease associated antigen is selected from the group consisting of a proliferative disease, a precancerous condition, a cancer, and a non-cancer related indication associated with expression of the disease-associated antigen.

66. The use or method of claim 65, wherein the cancer is a hematologic cancer chosen from one or more of chronic lymphocytic leukemia (CLL), acute leukemias, acute lymphoid leukemia (ALL), B-cell acute lymphoid leukemia (B-ALL), T-cell acute lymphoid leukemia (T-ALL), chronic myelogenous leukemia (CML), B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma, primary effusion lymphoma, follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin's lymphoma, Hodgkin's lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, or pre-leukemia.

67. The use or method of claim 65, wherein the cancer is selected from the group consisting of colon cancer, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, cancer of the small intestine, cancer of the esophagus, melanoma, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, solid tumors of childhood, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal

pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, Merkel cell cancer, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers, combinations of said cancers, and metastatic lesions of said cancers.

68. The use or method of claim 65, wherein the disease is associated with infection by a virus including but not limited to HIV1, HIV2, HTLV1, Epstein Barr virus (EBV), cytomegalovirus (CMV), adenovirus, adeno-associated virus, BK virus, Human Herpesvirus 6, Human Herpesvirus 8 influenza virus, parainfluenza virus, avian flu virus, MERS and SARS coronaviruses, Crimean Congo Hemorrhagic fever virus, rhino virus, enterovirus, Dengue virus, West Nile virus, Ebola virus, Marburg virus, Lassa fever virus, zika virus, RSV, measles virus, mumps virus, rhino virus, varicella virus, herpes simplex virus 1 and 2, varicella zoster virus, HIV-1, HTLV1, Hepatitis virus, enterovirus, hepatitis B virus, Hepatitis C virus, Nipah and Rift valley fever viruses, Japanese encephalitis virus, Merkel cell polyomavirus, or is associated with infection with mycobacterium tuberculosis, atypical mycobacteria species, Pneumocystis jirovecii, toxoplasmosis, rickettsia, nocardia, aspergillus, mucor, or candida.

69. The use or method of claim 65, wherein the disease is an immune or degenerative disease including but not limited to diabetes mellitus, multiple sclerosis, rheumatoid arthritis, pemphigus vulgaris, ankylosing spondylitis, Hoshimoto's thyroiditis, SLE, sarcoidosis, scleroderma, mixed connective tissue disease, graft versus host disease or Alzheimer's disease.