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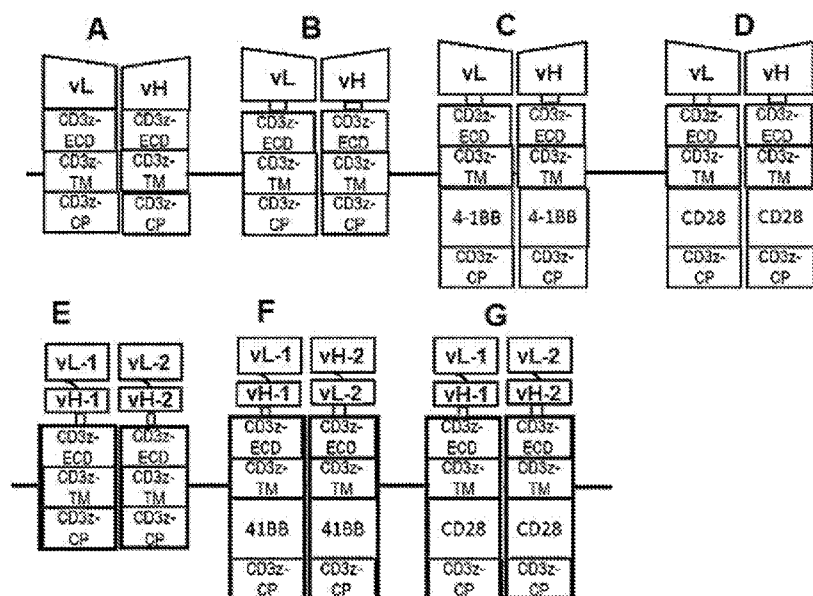


FIG. 1

(57) Abstract: The disclosure provides diverse antigen binding domains and platforms for construction of conventional and next generation chimeric antigen receptors for adoptive cellular therapies for cancer, infection, allergic, degenerative and immune disorders. Also provided are approaches for activation and expansion of immune T cells for adoptive cellular therapies for cancer, infection, allergic, degenerative and immune disorders.

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DIVERSE ANTIGEN BINDING DOMAINS, NOVEL PLATFORMS AND OTHER ENHANCEMENTS FOR CELLULAR THERAPY

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 62/679,741, filed June 1, 2018, the disclosures of which are incorporated herein for all purposes.

FIELD OF INVENTION

[0002] Provided herein are diverse antigen binding domains and novel platforms for construction of conventional and next generation chimeric antigen receptors for adoptive cellular therapies for cancer, infection, allergic, degenerative and immune disorders. Also provided are novel approaches for activation and expansion of immune T cells for adoptive cellular therapies for 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 June 1, 2019 and having 80,373,218 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] CARs are synthetic immune-receptors, which can redirect T cells to selectively kill tumor cells. 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. 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. 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.

[0005] Despite the success with CAR-T cells, there are several limitations to this approach, including toxicities such as "Cytokine release syndrome" (CRS) and neurotoxicities. The inclusion of costimulatory domain in the CAR construct results in non-physiological tonic signaling through the receptor, which in turn could contribute to their toxicity and lack of persistence.

[0006] 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.

SUMMARY

[0007] The following embodiments and aspects thereof are described and illustrated in conjunction with systems, compositions and methods which are meant to be exemplary and illustrative, not limiting in scope.

[0008] In certain embodiments, the disclosure provides compositions comprising genetically engineered effector cells (such as NK cells and T cells) that include polynucleotides that encode chimeric antigen receptors, synthetic immune receptors (SIRs) and the like that can be used on adoptive cell therapy for treatment of cancer, infectious, autoimmune and degenerative diseases.

[0009] In certain embodiments, the disclosure provides a platform of synthetic immune receptors, designated zSIRs, containing two CD3z chains. The polynucleotide sequences of the CD3z chains that can be used in the construction of zSIR are provided in, for example, SEQ ID NO: 67 and 71. The corresponding amino acid sequences are provided in SEQ ID NO: 4066 and 4070, respectively. The disclosure provides that the vL fragment of an antibody can be joined to one of the two CD3z chains and the vH fragment can be joined to the other CD3z chain. When the two such chains (*e.g.* vL-CD3z and vH-CD3z) are co-expressed in the same cell, the vL and vH fragments can bind their cognate antigen and transmit a T cell signal. In particular, T cells expressing such zSIR when exposed to a cell line expressing the cognate target antigen can activate NFAT signaling, induce IL2 production, promote T cell proliferation, promote T cell activation and exert cytotoxicity. The expression and activity of the zSIR can be further increased by incorporation of a linker between the vL/vH and the CD3z fragments. In particular, the IgCL (SEQ ID NO: 28 and 4027) and IgCH domains (SEQ ID NO: 29 and 4028) derived from antibodies serve as useful linkers between the vL/vH and CD3z fragments.

[0010] The disclosure further provides several new 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, Tri-TAC 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 **Table 3**. The SEQ ID Nos of the complementary determining regions (CDRs) of the light (vL) and heavy (vH) chains are shown in **Table 4**. The nucleic acid and amino acid SEQ IDs of exemplary conventional CARs (*i.e.*, 2nd generation CARs containing 41BB costimulatory domains), and next generation CARs (*e.g.*, SIRs, zSIRs, Ab-TCRs and TFP) based on these antigen binding domains are provided in **Table 6 and 7**. 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 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, PCT/US2016/058305, WO 2015/117229 A1 and PCT/US17/64379, which are incorporated herein by reference in their entirety. The immune cells expressing the CARs, both conventional and next generation 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 PCT/US2017/024843, WO 2014/160030 A2, WO 2016/187349 A1, PCT/US2016/058305, WO 2015/117229 A1 and PCT/US17/64379, which are incorporated herein by reference in their entirety.

[0011] The disclosure also provides a method of improving gene transfer using lentiviral vectors by coexpressing Vif protein and a CAR (*e.g.*, a conventional CAR, SIR, Ab-TCR, Tri-TAC or a recombinant TCR and the like) or Vif and any other therapeutic gene (*e.g.* β -globin gene for treatment of sickle cell anemia). An exemplary lentiviral vector (pLenti-EF1a-CD8SP-hu-CD19-USC1-LH4-vH-Gly-Ser-Linker-vL-Myc-CD8TM-BBz-2A-Vif) encoding a CAR and co-expressing Vif is provided in SEQ ID NO: 11268. In some embodiments, the Vif protein is provided in trans by co-expressing Vif in the packaging cells at the time of packaging of lentiviral vector. In such embodiment, the Vif protein is packaged along with the RNA encoding the lentiviral vector into the viral particles and is transferred to the target cells. The Vif protein can be expressed in the packaging cells by methods known in the art. In an exemplary embodiment, Vif protein is expressed in the packaging cells by co-transfecting a mammalian expression vector (*e.g.*, pCDNA3-Vif; SEQ ID NO: 11269)

encoding Vif with the lentiviral transfer vector encoding the gene(s) of interest (e.g. pLenti-EF1 α -CD8SP-MYC3-WT1-Ab13-vL-V5-[hTCRb-KACIAH]-F-P2A-SP-WT1-Ab13-vH-Myc4-[hTCRa-CSDVP]-F-F2A-PAC-DWPRE; SEQ ID NO: 151) and lentiviral packaging vector(s). Exemplary lentiviral packaging vector includes pMDLg/pRRE (Addgene plasmid 12251), which is a 3rd generation lentiviral packaging plasmid encoding Gag and Pol and also requires pRSV-Rev (Addgene#12253) and envelope expressing plasmid pMD2.G (Addgene#12259) for efficient packaging. Another lentiviral packaging vector is psPAX2 (Addgene plasmid#12260), which is a 2nd generation lentiviral packaging plasmid and can be used with envelope expressing plasmid pMD2.G (Addgene#12259) to package 2nd or 3rd generation lentiviral vectors. In an exemplary embodiment, a plasmid encoding Vif can be co-transfected with psPAX2 and pMD2.G plasmids to package a 2nd or a 3rd generation lentiviral vector. In an alternate exemplary embodiment, a plasmid encoding Vif can be co-transfected with pMDLg/pRRE, pRSV-Rev and pMD2.G plasmids to package a 3rd generation lentiviral vector. Vif can be also co-expressed from the same vector(s) encoding other lentiviral packaging proteins (e.g., gag, Pol and Rev). In an exemplary embodiment, the packaging plasmid psPAX2 is modified to also co-express Vif by methods known in the art. In an alternate exemplary embodiment, a 3rd generation lentiviral packaging plasmid encoding Gag and Pol is modified to also express Vif by fusing the nucleic acid sequence encoding Vif in frame with the nucleic acid sequence encoding Pol and separated from it by a P2A cleaveable linker sequence. In some embodiment, Vif is expressed in the packaging cells transiently while in other embodiments Vif is expressed in the packaging cells stably. In some embodiment, Vif is expressed in the target cells transiently while in other embodiments Vif is expressed in the target cells stably. In one embodiment, Vif is expressed in the target cells (e.g., T cells or stem cells) transiently by electroporation of a mammalian expression vector (e.g., pCDNA3-Vif; SEQ ID NO: 11269) encoding Vif or by electroporation of Vif polypeptide. Target cells (e.g., T cells or stem cells) transiently expressing Vif are subsequently infected with a lentiviral vector encoding a CAR or any therapeutic gene of interest (e.g., β globin).

[0012] The polyclonal nature of the immune response is key to its success in controlling various infections. In contrast, the current CAR therapies generally rely on targeting of a single antigen and/or single epitope of a single antigen. Loss of the targeted antigen or the targeted epitope is a frequent cause of failure of the current CAR therapies. To overcome this limitation, the disclosure provides CARs against multiple antigens and against multiple epitopes of a single antigen. These CARs can be used in suitable combinations to provide a

polyclonal and diverse adaptive immune response for the prevention or treatment of diseases, such as cancer, infectious diseases, autoimmune diseases, allergic diseases and degenerative diseases.

[0013] The disclosure also provides accessory modules that can be expressed in the adoptively transferred T cells (*e.g.*, CAR-T cells, TCR-T cell and TILs) to affect their survival, proliferation, activation, effector functions (*e.g.*, cytokines secretion, cytotoxicity *etc.*), exhaustion and *in vivo* persistence.

[0014] The disclosure provides at least one recombinant polynucleotide encoding at least one 1st generation or next generation chimeric antigen receptor (CAR), 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; and (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) wherein the binding domain is selected from a binding domain set forth in **Table 3**; (d) an optional third nucleic acid domain encoding a costimulatory domain; and an optional additional nucleic acid domain encoding an accessory module. In one embodiment, the first nucleic acid encodes partially or entirely at least one T-cell Receptor (TCR) chain as set forth in **Table 13**. In another or further embodiment, the first nucleic acid encodes at least one transmembrane domain in **Table 13** operably linked to the cytoplasmic domain of the TCR-type. In another or further embodiment, the polynucleotide encodes a CAR, wherein the CAR comprises: (i) a partial or entire T-cell receptor (TCR) constant chain having an amino acid sequence that has at least 75% sequence identity to a sequence selected from SEQ ID NO:4038 to 4063, 12602-12638, and which may comprise an optional costimulatory module; (ii) an optional linker; and (iii) one or more non-natural TCR antigen binding domain(s) linked to (a) selected from a binding domain set forth in **Table 3**; (iv) an optional accessor module; and (v) a dimer of a polypeptide comprising (i)-(iv). In another or further embodiment, the recombinant polynucleotide comprises a sequence encoding any one of the sequence in **Table 2**. In another or further embodiment, the accessory module comprises an amino acid sequence selected from SEQ ID NO: 4103-4117 and 4090-4096. In another or further embodiment, the encoded CAR comprises (1) any of CARs 1-16 of **Table 1** and/or (2) a backbone of **Table 2**; and (3) a binding domain of **Table 3**. In another or further embodiment, (i) is a CD3z TCR constant chain. In another or further

embodiment, the polynucleotide provides two first generation or next generation chimeric antigen receptors. In another or further embodiment, the polynucleotide encodes a dimer of CD3z constant chains.

[0015] The disclosure also provides at least one recombinant polynucleotide encoding at least one next generation chimeric antigen receptor (CAR), 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 CD3z protein having a sequence selected from the group consisting of SEQ ID NO:4064-4066, 4070-4072, and 4075-4078, 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; and (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) wherein the binding domain is selected from a binding domain set forth in **Table 3**; and (d) an optional third nucleic acid domain encoding a costimulatory module; and an optional additional nucleic acid encoding an accessory module. In another or further embodiment, the nucleic acid sequences encoding the endogenous CD3z protein are selected from the group consisting of SEQ ID NO: 67 and 71. In another or further embodiment, the at least one next generation CAR comprises two CARs each CAR comprising a CD3z chain. In another or further embodiment, a vL fragment of an antibody is operably linked to one of the two CD3z chains and a vH fragment of the antibody is operably linked to the other CD3z chain. In another or further embodiment, the vL and vH chains are selected from pairs in **Table 3 and 4** for a specific antigen target. In another or further embodiment, a linker is provided between the vL/vH and/or the CD3z chains. In another or further embodiment, an encoded linker is selected from the group consisting of IgCL (SEQ ID NO (DNA): 28 and SEQ ID NO (PRT): 4027) and IgCH domains (SEQ ID NO (DNA): 29 and SEQ ID NO (PRT): 4028). In another or further embodiment, further comprises the third nucleic acid domain encoding a costimulatory module. In another or further embodiment, the costimulatory module comprises a 41BB or CD28 protein. In another or further embodiment, the costimulatory module comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 4067 and 4068. In another or further embodiment, the costimulatory module comprises a signaling domain from any one or more of CD134 (OX40), Dap10, CD27, CD2, CD5, ICAM-1, LFA-1, Lck, TNFR-I, TNFR-II, Fas, CD30, CD40 and combinations thereof. In another or further embodiment, further comprises the

accessory module, wherein the accessory module comprises an amino acid sequence selected from SEQ ID NO: 4103-4117 and 4090-4096.

[0016] The disclosure also provides a recombinant cell expressing a homo- or hetero-dimer of a 1st generation or next generation chimeric antigen receptor (CAR), the homo- or hetero-dimer comprising: (a) a first 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 peptide linker; and (c) a second domain operably linked to the first domain, wherein the second domain comprises one or more non-natural TCR antigen binding domain(s) wherein the binding domain is selected from a binding domain set forth in **Table 3**; and (d) an optional third domain encoding a costimulatory module, and wherein the cell optionally comprises an accessory module, wherein the homo- or hetero-dimer associate on the surface of the recombinant cell. In another or further embodiment, the cell is transformed with the at least one recombinant polynucleotide as described herein. In another or further embodiment, the cell is a T-lymphocyte (T-cell). In another or further embodiment, the cell is a naïve T cells, a central memory T cells, an effector memory T cell, Treg or a combination thereof. In another or further embodiment, the cell is a natural killer (NK) cell, a hematopoietic stem cell (HSC), an embryonic stem cell, or a pluripotent stem cell. In another or further embodiment, the accessory module comprises an amino acid sequence selected from SEQ ID NO: 4103-4117 and 4090-4096. In another or further embodiment, the recombinant cell expresses or is engineered to express HIV1-vif.

[0017] The disclosure provides a chimeric antigen receptor (CAR) comprising (a) a first 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 peptide linker; and (c) a second domain operably linked to the first domain, wherein the second domain comprises one or more non-natural TCR antigen binding domain(s) wherein the binding domain is selected from a binding domain set forth in **Table 3**; and (d) an optional third domain encoding a costimulatory module. In another or further embodiment, the endogenous protein comprises a sequence selected from the group consisting of SEQ ID NO:4064-4066, 4070-4072, 4075-4078 and 12637. In another or further embodiment, the first nucleic acid encodes partially or entirely at least one T-cell Receptor (TCR) chain as set forth in **Table 13**. In another or further embodiment, the first

comprises a transmembrane domain in **Table 13** operably linked to the cytoplasmic domain of a corresponding TCR-type. In another or further embodiment, the CAR comprises: (i) a partial or entire T-cell receptor (TCR) constant chain having an amino acid sequence that has at least 75% sequence identity to a sequence selected from SEQ ID NO:4038 to 4063, 12602-12638, and which may comprise an optional costimulatory module.

[0018] The disclosure provides a polynucleotide encoding the chimeric antigen receptor as described above and herein.

[0019] The disclosure also provides a vector comprising the polynucleotide(s) described herein.

[0020] The disclosure also provides a virus comprising the polynucleotide(s) as described herein. In another or further embodiment, the virus is a retrovirus, an adenovirus, an adeno-associated virus, a lentivirus, a pox virus or a herpes virus.

[0021] The disclosure also provides a pharmaceutical composition comprising: any one or more of the inventions described herein and a pharmaceutically acceptable carrier.

[0022] The disclosure also provides a method for treating cancer comprising: providing the composition, a recombinant cell of the disclosure and administering a therapeutically effective amount of the composition or cell to the subject so as to treat cancer. In another or further embodiment, the cancer is blood cancer. In another or further embodiment, the blood cancer is any one or more of acute myeloid leukemia, chronic myeloid leukemia, myelodysplastic syndrome, lymphoma, multiple myeloma and acute lymphocytic leukemia. In another embodiment, the cancer is a solid tumor.

[0023] In one embodiment, provided herein is an isolated nucleic acid encoding a SIR (*i.e.*, a next generation CAR), wherein the antigen specific domain of the SIR targets CD19 and the SIR optionally expresses a codon optimized variant of K13-vFLIP (K13-opt). In exemplary embodiments, the sequences of isolated nucleic acid fragments targeting CD19 are set forth in SEQ ID NOs: 14056-14059 and 14109-14112. In exemplary embodiments, the sequences of isolated polypeptide targeting CD19 and optionally coexpressing K13-vFLIP are set forth in SEQ ID NOs: 15800-15803 and 15853-15856. In some embodiments, the vL and vH fragments targeting CD19 are described in **Table 3** and set forth in SEQ ID Nos (DNA): 12662, 12693 and 12656 and 12687 and SEQ ID Nos (PRT): 14406, 14437 and 14400 and 14431. Also provided herein are polypeptides encoded by nucleic acids encoding SIR and optionally encoding K13-vFLIP, wherein the antigen specific domain of the SIR targets CD19. Further provided herein are vectors encoding nucleic acids encoding SIR and K13-vFLIP, wherein the antigen specific domain of the SIR targets CD19. In exemplary embodiments, a vector

encoding a SIR targeting CD19 is provided in SEQ ID NO: 12641. Also provided herein are genetically engineered cells (such as T cells, NKT cells) comprising vectors encoding nucleic acids encoding SIR and K13-vFLIP, wherein the antigen specific domain of the SIR targets CD19. Also provided are methods for treatment and prevention of a disease where the disease causing or disease associated cells express CD19.

[0024] In one embodiment, provided herein is an isolated nucleic acid encoding a SIR, wherein the antigen specific domain of the SIR targets MPL and the SIR optionally expresses a codon optimized variant of K13-vFLIP (K13-opt). In exemplary embodiments, the sequences of isolated nucleic acid fragments targeting MPL are set forth in SEQ ID NOs: 13791-13792 and 13844-13845. In exemplary embodiments, the sequences of isolated polypeptide targeting MPL and optionally coexpressing K13-vFLIP are as forth in SEQ ID NOs: 15535-15536 and 15588-15589. In some embodiments, the vL and vH fragments targeting MPL are described in **Table 3** and set forth in SEQ ID Nos (DNA): 12665, 12696 and 12658 and 12689 and SEQ ID Nos (PRT): 14409, 14440 and 14402 and 14433. Also provided herein are polypeptides encoded by nucleic acids encoding SIR and optionally encoding K13-vFLIP, wherein the antigen specific domain of the SIR targets MPL. Further provided herein are vectors encoding nucleic acids encoding SIR and K13-vFLIP, wherein the antigen specific domain of the SIR targets MPL. In exemplary embodiments, a vector encoding a SIR targeting MPL is provided in SEQ ID NO: 14384. Also provided herein are genetically engineered cells (such as T cells, NKT cells) comprising vectors encoding nucleic acids encoding SIR and optionally encoding K13-vFLIP, wherein the antigen specific domain of the SIR targets MPL. Also provided are methods for treatment and prevention of a disease where the disease causing or disease associated cells express MPL.

[0025] In one embodiment, provided herein is an isolated nucleic acid encoding a SIR, wherein the antigen specific domain of the SIR targets BCMA and the SIR optionally expresses a codon optimized variant of K13-vFLIP (K13-opt). In exemplary embodiments, the sequences of isolated nucleic acid fragments targeting BCMA are set forth in SEQ ID NOs: 12890-12893, 12943-12946, 12996-12999, 13049-13052 and 12837-12840. In exemplary embodiments, the sequences of isolated polypeptide targeting BCMA and optionally coexpressing K13-vFLIP are as forth in SEQ ID NOs: 14634-14637, 14687-14690, 14740-14743, 14793-14796, and 14581-14584. In some embodiments, the vL and vH fragments targeting BCMA are described in **Table 3** and set forth in SEQ ID Nos (DNA): 12670 and 12701, 12669 and 12700, 12671-12702, 12657 and 12688, 12654 and 12685 and SEQ ID Nos (PRT): 14414 and 14445, 14413 and 14444, 14415 and 14446, 14398 and 14429, and 14401 and 14432. Also provided herein

are polypeptides encoded by nucleic acids encoding SIR and optionally encoding K13-vFLIP, wherein the antigen specific domain of the SIR targets BCMA. Further provided herein are vectors encoding nucleic acids encoding SIR and K13-vFLIP, wherein the antigen specific domain of the SIR targets BCMA. In exemplary embodiments, vectors encoding a SIR targeting BCMA are provided in SEQ ID NO: 14378 and 14385. Also provided herein are genetically engineered cells (such as T cells, NKT cells) comprising vectors encoding nucleic acids encoding SIR and optionally encoding K13-vFLIP, wherein the antigen specific domain of the SIR targets BCMA. Also provided are methods for treatment and prevention of a disease where the disease causing or disease associated cells express BCMA.

[0026] In one embodiment, provided herein is an isolated nucleic acid encoding a SIR, wherein the antigen specific domain of the SIR targets MSLN and the SIR optionally expresses a codon optimized variant of K13-vFLIP (K13-opt). In exemplary embodiments, the sequences of isolated nucleic acid fragments targeting MSLN are set forth in SEQ ID NOs: 14268-14269, 14321-14322, and 14374-14375. In exemplary embodiments, the sequences of isolated polypeptide targeting MSLN and optionally coexpressing K13-vFLIP are as forth in SEQ ID NOs: 16012-16013, 16065-16066 and 16118-16119. In some embodiments, the vL and vH fragments targeting MSLN are described in **Table 3** and set forth in SEQ ID Nos (DNA): 12668 and 12699, 12667 and 12698, and 12666-12697 and SEQ ID Nos (PRT): 14412 and 14443, 14411 and 14442, and 14410 and 14441. Also provided herein are polypeptides encoded by nucleic acids encoding SIR and optionally encoding K13-vFLIP, wherein the antigen specific domain of the SIR targets MSLN. Further provided herein are vectors encoding nucleic acids encoding SIR and K13-vFLIP, wherein the antigen specific domain of the SIR targets MSLN. In exemplary embodiments, vectors encoding a SIR targeting MSLN are provided in SEQ ID NO: 14381 and 14383. Also provided herein are genetically engineered cells (such as T cells, NKT cells) comprising vectors encoding nucleic acids encoding SIR and optionally encoding K13-vFLIP, wherein the antigen specific domain of the SIR targets MSLN. Also provided are methods for treatment and prevention of a disease where the disease causing or disease associated cells express MSLN.

[0027] In one embodiment, provided herein is an isolated nucleic acid encoding a SIR, wherein the antigen specific domain of the SIR targets CD22 and the SIR optionally expresses a codon optimized variant of K13-vFLIP (K13-opt). In exemplary embodiments, the sequences of isolated nucleic acid fragments targeting CD22 are set forth in SEQ ID NOs: 13314-13317, 13420-13423, 13473-13476 and 14215-14218. In exemplary embodiments, the sequences of isolated polypeptide targeting CD22 and optionally coexpressing K13-vFLIP are as forth in

SEQ ID NOs: 15058-15061, 15164-15167, 15217-15220, and 15959-15962. In some embodiments, the vL and vH fragments targeting CD22 are described in **Table 3** and set forth in SEQ ID Nos (DNA): 12663 and 12694, 12655 and 12686, 12643 and 12674, 12652 and 12683 and SEQ ID Nos (PRT): 14407 and 14438, 14399 and 14430, 14387 and 14418, 14396 and 14427. Also provided herein are polypeptides encoded by nucleic acids encoding SIR and optionally encoding K13-vFLIP, wherein the antigen specific domain of the SIR targets CD22. Further provided herein are vectors encoding nucleic acids encoding SIR and K13-vFLIP, wherein the antigen specific domain of the SIR targets CD22. In exemplary embodiments, a vector encoding a SIR targeting CD22 is provided in SEQ ID NO: 12640. Also provided herein are genetically engineered cells (such as T cells, NK cells) comprising vectors encoding nucleic acids encoding SIR and optionally encoding K13-vFLIP, wherein the antigen specific domain of the SIR targets CD22. Also provided are methods for treatment and prevention of a disease where the disease causing or disease associated cells express CD22.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] **Figure 1** depicts a schematic representation of different zSIRs. CD3z-ECD, CD3z-TM, CD3z-CP refers to the extracellular, transmembrane and cytoplasmic domains of CD3z. 4-1BB and CD28 refers to the cytoplasmic costimulatory domains of 4-1BB and CD28.

[0029] **Figure 2A-B** depicts induction of IFN γ upon co-culture of CAR-T cells of the disclosure with RAJI cells (Figure 2A) and Nalm6 cells (Figure 2B).

[0030] **Figure 3** depicts the *in vivo* efficacy of CAR-T cells of the disclosure in a xenograft model of RAJI cells as measured using bioluminescence imaging.

[0031] **Figure 4** depicts the *in vivo* efficacy of CAR-T cells of the disclosure in a xenograft model of Nalm6 cells as measured using bioluminescence imaging.

DETAILED DESCRIPTION

[0032] 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.

[0033] 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.

[0034] 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.”

[0035] 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.

[0036] 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.

[0037] 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**) of WO2019067805 and in WO 2017/070608 A1 which are incorporated herein by reference. Exemplary Ab-TCRs targeting BCMA and co-expressing an accessory module encoding NEMO-K277A are provided in SEQ ID NO: 4382-4383 (**Table 6**). 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.

[0038] The term “accessory module” refers to an element that is co-expressed with a CAR (including next generation CAR such as SIR, zSIR, Ab-TCR, Tri-TAC, TFP *etc.*) and/or rTCR to increase, decrease, regulate or modify the expression or activity of a CAR/rTCR or CAR/rTCR-expressing cells. Exemplary accessory modules include any one or more of 41BBL, CD40L, HIV1-Vif, 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, hNEMO-K277A, shRNA targeting Brd4 and combination thereof. The accessory module can be co-expressed with the CAR/rTCR and the like using a single vector or using two or more different vectors. In some embodiments, the accessory modules reduce or prevent toxicity associated with CARs and/of TCRs and the like. In some embodiments, the accessory module improves the efficiency of lentiviral mediated gene transfer.

[0039] 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.

[0040] 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 such as sdAb (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 mini bodies).

[0041] 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.

[0042] 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.

[0043] The term "anticancer effect" or "anti-tumor 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 a CAR, SIR, TFP, Ab-TCR, Tri-Tac, zSIR and the like in prevention of the occurrence of cancer in the first place.

[0044] "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.

[0045] The term "antigen" or "Ag" refers to a molecule that provokes an immune response.

[0046] The term "antigen presenting cell" or "APC" refers to any cell that expresses on its surface an antigen that can be recognized by an immune cell or antibody that binds to an immune cell. For example, a CD19 expressing B lymphocyte can serve as an antigen presenting cell for a T cell expressing a CAR directed against CD19. An APC may present an antigen independent of an MHC molecule or in context of an MHC molecule. The APC may present the antigen in complex with major histocompatibility complexes (MHC's). The T-cells may recognize these MHC-antigen complexes using their T-cell receptors (TCRs). In alternate embodiment, an APCs may present an antigen on its surface that is recognized by a natural (*e.g.*, CD28 or 41BB) or a synthetic (*e.g.*, CAR, SIR, zSIR, Ab-TCR, Tri-Tac, or TFP *etc.*) receptor expressed on T cells independent of MHC.

[0047] The term "antigen presenting substrate" or "APS" refers to any substrate such as a bead, a microbead, a plate, or any matrix that displays a foreign antigen on its surface. In one embodiment, an APS may present an antigen on its surface that is recognized by a natural (*e.g.*, CD28 or 41BB) or synthetic (*e.g.*, a conventional CAR, a SIR, a zSIR, an Ab-TCR, a TFP) receptor expressed on T cells. In an exemplary embodiment, beads coated with the extracellular domain of CD19 on their surface can serve as APS for T cells expressing a CD19-directed conventional CAR, SIR, zSIR, Ab-TCR or TFP.

[0048] 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.

[0049] 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.).

[0050] An "antigen binding domain" or "antigen binding module" or "antigen binding segment" refers to a polypeptide or peptide that due to its primary, secondary or tertiary sequence and or 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, a non-immunoglobulin binding protein, a ligand or a receptor.

[0051] "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). Antibodies and affinities can be phenotypically characterized and compared using functional assays (*e.g.*, flow cytometry assay and Topanga assay).

[0052] The term "Association constant (K_a)" is defined as the equilibrium constant of the association of a receptor and ligand or antibody and antigen.

[0053] The term "autoantigen" refers to an endogenous antigen that stimulates production of an autoimmune response, such as production of autoantibodies. Examples of autoantigens include, but are not limited to, desmoglein 1, desmoglein 3, and fragments thereof.

[0054] 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.

[0055] 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.

[0056] As used herein, the term “binding domain” or “antibody molecule” refers to a protein, *e.g.*, an immunoglobulin chain or fragment thereof, 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.

[0057] “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 the 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. The epitope bound by the antigen binding domain of a conventional CAR or a next generation CAR (*e.g.*, SIR, zSIR, TFP, Tri-Tac or Ab-TCR) 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 hydrophathy 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 hydrophathy 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.

[0058] 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. Biol. 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. Biol. 196:901-917 (1987); and MacCallum *et al.*, J. Mol. Biol. 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).

[0059] The term "framework region" refers to the art-recognized portions of an antibody variable region that exist between the more divergent (*i.e.*, hypervariable) CDRs.

[0060] Amino acid sequence modifications of the binding molecules described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the vL and/or vH fragments of a conventional CAR or a next generation CAR (*e.g.*, SIR, zSIR and the like). Such modifications include, for example, deletions from, and/or insertions into, and/or substitutions of, residues within the amino acid sequences of the binding molecules. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the binding molecules, such as changing the number or position of glycosylation sites. Preferably, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids may be substituted in a CDR, while 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 25 amino acids may be substituted in the framework regions (FRs). The substitutions are preferably conservative substitutions as described herein. Additionally, or alternatively, 1, 2, 3, 4, 5, or 6 amino acids may be inserted or deleted in each of the CDRs (of course, dependent on their length), while 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 25 amino acids may be inserted or deleted in each of the FRs.

[0061] Preferably, amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 residues to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. An insertional variant of the binding molecule includes the fusion to the N- or C-terminus of the antibody to an enzyme or a fusion to a polypeptide which increases the serum half-life of the antibody.

[0062] Another type of variant is an amino acid substitution variant. These variants have preferably at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid residues in the binding molecule replaced by a different residue. The sites of greatest interest for substitutional mutagenesis include the CDRs of the heavy and/or light chain, in particular the hypervariable regions, but FR alterations in the heavy and/or light chain are also contemplated.

[0063] For example, if a CDR sequence encompasses 6 amino acids, it is envisaged that one, two or three of these amino acids are substituted. Similarly, if a CDR sequence encompasses 15 amino acids it is envisaged that one, two, three, four, five or six of these amino acids are substituted.

[0064] Generally, if amino acids are substituted in one or more or all of the CDRs of the heavy and/or light chain, it is preferred that the then-obtained "substituted" sequence is at least 60%, more preferably 65%, even more preferably 70%, particularly preferably 75%,

more particularly preferably 80% identical to the “original” CDR sequence. This means that it is dependent of the length of the CDR to which degree it is identical to the “substituted” sequence. For example, a CDR having 5 amino acids is preferably 80% identical to its substituted sequence in order to have at least one amino acid substituted. Accordingly, the CDRs of the binding molecule may have different degrees of identity to their substituted sequences, *e.g.*, CDRL1 may have 80%, while CDRL3 may have 90%.

[0065] Preferred substitutions (or replacements) are conservative substitutions. However, any substitution (including non-conservative substitution or one or more from the “exemplary substitutions” listed herein below) is envisaged as long as the binding molecule retains its capability to bind to the target antigen and/or its CDRs have an identity to the then substituted sequence (at least 60%, more than 65%, more than 70%, typically more than 75%, or more than 80% identical to the “original” CDR sequence).

[0066] Non-conservative substitutions will entail exchanging a member of one for another class. Any cysteine residue not involved in maintaining the proper conformation of the binding molecule may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability (particularly where the antibody is an antibody fragment such as an Fv fragment).

[0067] The SEQ IDs of the CDRs of the exemplary vL and vH segments that can be used to constitute the antigen binding domains of a CAR (*e.g.*, a 2nd generation CAR, a SIR, a zSIR, an Ab-TCR, Tri-Tac or a TFP) of the disclosure targeting different antigens are provided in **Table 4**.

[0068] 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 radioimmunoprecipitation assay (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.

[0069] "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), testicular cancer, lung 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.

[0070] "Chemotherapeutic agents" are compounds that are known to be of use in chemotherapy for cancer.

[0071] "Chimeric antigen receptors" (CAR) are artificial T cell receptors contemplated for use as a therapy for cancer, using a technique called adoptive cell transfer. CARs are constructed specifically to stimulate T cell activation and proliferation in response to a specific antigen to which the CAR binds. The term "Chimeric Antigen Receptor" or alternatively 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 aspect, the costimulatory molecule is chosen from the costimulatory molecules described herein, *e.g.*, 4-1BB (*i.e.*, CD137), CD27 and/or CD28. In one aspect the CAR comprises an optional leader sequence at the amino-terminus (N-ter) of the CAR fusion protein. In one aspect, 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. Typically, "CAR-T cells" are used, which refer to T-cells that have been engineered to contain a chimeric antigen receptor. Thus, T lymphocytes bearing such CARs are generally referred to as CAR-T lymphocytes. A second generation CAR targeting CD19 and comprising a CD8 signal

peptide, an antigen binding domain based on CD19-AM1 scFv, a CD8 hinge and transmembrane domain, a 4-1BB costimulatory domain and a CD3z stimulatory domain is represented by SEQ ID NO: 799. A CAR in which the 4-1BB costimulatory domain is replaced by a different costimulatory domain (*e.g.*, CD28 or CD27) is also referred to as a conventional CAR. To overcome the limitation of conventional CARs, several alternative designs or next generation CARs have been described, including TCR receptor fusion proteins or TFP (WO 2016/187349 A1), antibody TCR or AbTCRs (PCT/US2016/058305). Tri-TAC (WO 2015/117229 A1) and synthetic immune receptors or SIR (US 62/429,597 and PCT/US17/64379). As used herein, the term “CAR” or “CARs” also encompasses newer approaches (*i.e.*, TFP, AbTCR, Tri-Tac, SIR and zSIR *etc.*) to conferring antigen specificity onto cells. The present disclosure provides several novel antigen binding domains that can be used for the generation of CARs. Although not explicitly described, it is envisioned that these antigen binding domain(s) (*e.g.*, scFv, vL, vH, or vHH *etc.*) can be used to generate the conventional first and second generation CARs as well as newer approaches (*i.e.*, TFP, AbTCR, Tri-Tac, SIR and zSIR *etc.*) to conferring antigen specificity onto cells. Thus, the vL and vH fragments of a given antigen binding domain can be used to generate a double chain SIR, a double chain Ab-TCR or a double chain zSIR when these fragments are fused to the two constant chains (*e.g.*, TCRa/b or TCRg/d) comprising a SIR, Ab-TCR or zSIR. The vL and vH fragment of the same antigen binding domain can be joined via a flexible linker to generate a scFv which in turn can be used to generate a conventional first or second generation CAR, a TFP or a Tri-TAC using methods known in the art.

[0072] “Codon optimization” or “controlling for species codon bias” refers to the preferred codon usage of a particular host cell

[0073] As used herein, “co-express” refers to expression of two or more genes. Genes may be nucleic acids encoding, for example, a single protein or a chimeric protein as a single polypeptide chain. For example, the zSIR described herein may be encoded by a single polynucleotide chain and synthesized as single polypeptide chain, which is subsequently cleaved into different polypeptides, each representing a distinct functional unit. In some embodiments, where the zSIR consists of two or more functional polypeptide units, the different functional units are coexpressed using one or more polynucleotide chains. 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.*). In another embodiment, a Ser-Gly-Ser-Gly (SGSG) motif (SEQ ID NO: 86-87 and 4085-86) 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, in some embodiments, a furine cleavage site (RAKR) (SEQ ID NO: 88-90 and 4087-4089) is added upstream of the SGSG motifs to facilitate cleavage of the residual 2A peptide following translation. The polynucleotides encoding the different units of a zSIR may be linked by IRES (Internal Ribosomal Entry Site) sequences. Alternately, the different functional units of a zSIR 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 sequences of cleavable linkers are provided in SEQ ID NO: 80 to SEQ ID NO: 85.

[0074] It will be recognized that proteins can have identity or homology to one another and retain similar or identical functions. For example, the disclosure includes CD3z chains 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.

[0075] The term a "costimulatory molecule" 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 molecules include, but are not limited to an MHC class I molecule, BTLA and a Toll ligand receptor, as well as OX40, CD27, CD28, CD8, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), 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 costimulatory intracellular signaling domain can be the intracellular portion of a costimulatory molecule. 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.

[0076] The term “disease-specific antigen” or “disease-associated antigen” or “disease causing antigen” refers to an antigen expressed on cells that contribute to the development of a disease.

[0077] The term “disease-causing cell” or “disease-associated cell” refers to a cell that contribute to the development of a disease. Exemplary disease causing cells include cancer cells and virally infected cells. Non-cancerous cells, such as B lymphocytes and T lymphocytes, have been associated with the pathogenesis of immune, allergy, degenerative and infectious diseases and are also considered disease causing cells.

[0078] The term “disease-supporting antigen” refers to an antigen expressed on cells that support the survival, proliferation, persistence or activity of disease causing cells. In some embodiments, the disease-supporting antigen is an antigen present on stromal cells. 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. Exemplary stromal cell antigens include bone marrow stromal cell antigen 2 (BST2), fibroblast activation protein (FAP) and tenascin.

[0079] 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.

[0080] “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.

[0081] The phrase "disease associated with expression of a target antigen" or "disease associated antigen" 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.

[0082] "Disease targeted by genetically modified cells" as used herein encompasses the targeting of any cell involved in any manner in any disease by a genetically modified cells that hones to the disease or a target tissue or cell type, irrespective of whether the genetically modified cells target diseased cells or healthy cells to effectuate a therapeutically beneficial result.

[0083] The term "Dissociation constant (K_d)" is defined as the equilibrium constant of the dissociation of a receptor–ligand interaction.

[0084] The term "encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (*e.g.*, rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene, cDNA, or RNA, encodes a protein if transcription and

translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

[0085] Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. The phrase nucleotide sequence that encodes a protein or a RNA may also include in trans to the extent that the nucleotide sequence encoding the protein may in some version contain an intron(s).

[0086] The term "effective amount" or "therapeutically effective amount" are used interchangeably herein, and refer to an amount of a compound, formulation, material, or composition, as described herein effective to achieve a particular biological result.

[0087] The term "endogenous", "native" or "naturally occurring" refers to any material from or produced inside an organism, cell, tissue or system. It also 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.

[0088] The term "exogenous" refers to any material introduced from or produced outside an organism, cell, tissue or system.

[0089] The term "expression" refers to the transcription and/or translation of a particular nucleotide sequence driven by a promoter and/or other regulatory elements.

[0090] 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. 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 polylysine compound, liposome, and the like. Examples of viral transfer vectors include, but are not limited to, adenoviral vectors, adeno-associated virus vectors, retroviral vectors, lentiviral vectors, and the like.

[0091] 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 adeno-associated viruses) that incorporate the recombinant polynucleotide.

[0092] 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.

[0093] 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. 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 adeno-associated viruses) that incorporate the recombinant polynucleotide.

[0094] The term "functional polypeptide unit (FPU)" of, *e.g.*, a zSIR, refers to a polypeptide comprising an amino terminal signal sequence functionally linked to an antigen binding domain and, *e.g.*, a CD3z chain. For example, the antigen binding domain is located between the signal sequence and the CD3z chain.

[0095] The term "functional portion" when used in reference to, *e.g.*, a zSIR refers to any part or fragment of a polypeptide, *e.g.*, the zSIR, which part or fragment retains the biological activity of the desired molecule, *e.g.*, of the zSIR, of which it is a part (*e.e.*, the parent zSIR). For example, functional portions encompass those parts of a zSIR 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 zSIR. In reference to the parent zSIR, the functional portion can comprise, for instance, about 10%, 25%, 30%, 50%, 68%, 80%, 90%, 95%, or more, of the parent zSIR.

[0096] "Genetically modified cells", "redirected cells", "genetically engineered cells" or "modified cells" as used herein refer to cells that have been modified to express a CAR (*e.g.*, a conventional 2nd generation CAR, TFP, AbTCR, SIR, Tri-Tac and zSIR), or a recombinant TCR. For example, a genetically modified T-lymphocyte that expresses a CAR or a zSIR is a genetically modified cell.

[0097] 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.

[0098] "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, and natural killer T (NKT) cells.

[0099] "Immune receptor expressing 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 and expresses one or more immune receptors, such as, for example, an endogenous TCR, a recombinant TCR or a CAR. Examples of immune receptor expressing cells include T cells, *e.g.*, alpha/beta T cells and gamma/delta T cells and NKT cells.

[00100] "Immune effector function or immune effector response," as that term is used herein, refers to function or response, *e.g.*, of an immune effector cell, that enhances or promotes an immune attack of a target cell, *e.g.*, 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.

[00101] An "intracellular signaling 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, for example, an immune effector function of the CAR (*e.g.*, 2nd generation CAR, TFP, AbTCR, SIR, Tri-TAC and/or zSIR) containing cell. Examples of immune effector function include cytolytic activity and helper activity, including the secretion of cytokines. The TCR $\alpha/\beta/\gamma/\delta$ chains do not have an intracellular signaling domain of their own but transmit a signal by associating with other chains of the TCR signaling complex (*e.g.*, CD3z, CD3e, CD3d and CD3g) that possess a signaling domain. 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.

[00102] 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.

[00103] As used herein, the term “linker” (also “linker domain” or “linker region”) refers to an oligo or polypeptide that joins together two or more domains or regions of a CAR (*e.g.*, 2nd generation CAR, TFP, AbTCR, SIR and zSIR) disclosed herein. The linker can be anywhere from 1 to 500 amino acids 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. 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. Exemplary embodiments of linkers with non-flexible linkers are EAAAK (SEQ ID NO: 4011), E-coil (SEQ ID NO: 4009), K-coil (SEQ ID NO: 4010), or PG4SP (SEQ ID NO: 4007). In other embodiments, the linker joining the antigen binding domain and the CD3z chains of a zSIR share similar length. In other embodiments, the linker joining the antigen binding domain and the CD3z chains of a zSIR differ in length by no more than 20 amino acids, typically by no more than 10 amino acids, preferably by no more than 5 amino acids, more preferably by no more than 2 amino acids. In some embodiments, the linker joining the antigen binding domain and the CD3z chains of a zSIR have the identical or similar amino acid composition. Exemplary linkers with identical composition are PG4SP (SEQ ID NO: 4007) and PG4SP-v2 (SEQ ID NO: 4008). In some embodiments, the linkers joining the antigen binding domain and the CD3z chains of a zSIR are PG4SP (DNA SEQ ID NO: 8; PRT SEQ ID NO: 4007) and PG4SP-v2 (DNA SEQ ID NO: 9; PRT SEQ ID NO: 4008).

[00104] In some embodiments, the linkers joining the antigen binding domains and the CD3z chains of a zSIR are derived from antibodies. In one embodiment, the linker joining a vL region and a CD3z chain of a zSIR is IgCL (DNA SEQ ID NO: 28; PRT SEQ ID NO: 4027) and the linker joining a vH region and a CD3z chain of a zSIR is IgG1-CH1 (DNA SEQ ID NO: 29 and PRT SEQ ID NO: 4028). In some embodiments, the linker joining the respective antigen binding domain and the CD3z chain of a zSIR are IgCL (DNA SEQ ID NO: 28; PRT SEQ ID NO: 4027) and IgG2-0C-CH1 (DNA SEQ ID NO: 30; PRT SEQ ID NO: 4029). In some embodiments, the linker may comprise an epitope tag. In some embodiments, the epitope tag is selected from the group consisting of a MYC tag, a V5 tag, a AcV5 tag, a StreptagII, a FLAG tag, or HA. In some embodiments, the non-cleavable linker is of a length sufficient to ensure that two adjacent domains do not sterically interfere with

one another. In one embodiment of the disclosure, three amino acid residues (Gly-Ser-Gly) are added to the carboxy-terminal of the linkers (*e.g.*, Myc tag or V5 tag) that are located between the antigen binding domain and the CD3 ζ chains of the zSIR. In certain embodiments, the linkers may carry additional sequences, such as restriction enzyme sites.

[00105] The term "flexible polypeptide linker" as used in refers to a peptide linker that consists of amino acids such as, for example, 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, 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:5). In another embodiment, the linkers include multiple repeats of (Gly₂Ser), (GlySer) or (Gly₃Ser). Also included within the scope of the disclosure are linkers described in W02012/138475 (incorporated herein by reference).

[00106] The term "lentivirus" refers to a genus of the Retroviridae family. HIV, SIV, and FIV are all examples of lenti viruses.

[00107] 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. Other examples of lentivirus vectors are pLENTI-EF1 α (SEQ ID NO: 129), pLENTI-EF1 α -DWPRE (SEQ ID NO: 130) and pCCLc-MNDU3 (SEQ ID NO: 12639).

[00108] As used herein a "non-naturally occurring TCR antigen binding domain" refers to a binding domain operably linked to a TCR constant region or a CD3 ζ chain 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 constant chain or a CD3 ζ chain 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.

[00109] The term "operably 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.

[00110] "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.

[00111] 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).

[00112] 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 to form a polymer of amino acids.

[00113] "Refractory" as used herein refers to a disease, *e.g.*, cancer, that does not respond to a treatment. In embodiments, a refractory cancer can be resistant to a treatment before or at the beginning of the treatment. In other embodiments, the refractory cancer can become resistant during a treatment. A refractory cancer is also called a resistant cancer.

[00114] "Relapsed" as used herein refers to the return of a disease (*e.g.*, cancer) or the signs and symptoms of a disease such as cancer after a period of improvement, *e.g.*, after prior treatment of a therapy, *e.g.*, cancer therapy

[00115] Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention.

[00116] The term "retrovirus vector" or "retroviral 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. Other example of a retrovirus vector is MSCV-Bgl2-AvrII-Bam-EcoR1-Xho-BstB1-Mlu-Sal-ClaI.I03 (SEQ ID NO: 131).

[00117] 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. An example of a Sleeping Beauty Transposon Vector is pSBbi-Pur (SEQ ID NO: 133). Other examples of Sleeping Beauty Transposon Vectors encoding a SIR are provided in SEQ ID NO: 134 and SEQ ID NO: 135.

[00118] The term "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 disclosure, a scFv is also described as vL-Gly-Ser-Linker-vH. For example, FMC63-vL-Gly-Ser-Linker-FMC63-vH refers to a scFv containing the vL and vH fragments of FMC63 monoclonal antibody linked via a linker consisting of Gly and Ser residues. Alternatively, a scFv is also described as (vL+vH). For example, FMC6-(vL+vH) refers to an scFv containing the vL and vH fragments of FMC63 antibody linked via a linker in which the vL fragment is located at the N-terminal.

[00119] 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.

[00120] The term "Synthetic Immune Receptor" or alternatively a "SIR" refers to a polypeptide, typically two polypeptides (*e.g.*, a hetero- or homo-dimer) 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. SIR have been described in PCT/US17/64379. 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 and antigens or ligand cognate 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 TCRA or hTCR-alpha or hTCR α or hTCRA or C α), human T cell receptor-beta1 (TCR-beta1 or TCR β 1 or TCRb1 or hTCR-beta1 or hTCR β 1 or hTCRb1 or C β 1), human T cell receptor-beta 2 (TCR-beta2 or TCR β 2 or TCRb2 or hTCR-beta2 or hTCR β 2 or hTCRb2 or C β 2 also designated TCR-beta, TCR β or TCRb or C β), human Pre-T cell receptor alpha ((preTCR-alpha or preTCR α or preTCRA or preC α), human T cell receptor-gamma (TCR-gamma or TCR γ or TCRg or or hTCR-gamma or hTCR γ or hTCRg or hTCR γ 1 or hTCRgammal, or C γ), or human T cell receptor-delta (TCR-delta or TCR δ or TCR δ or hTCR-delta 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 nucleic acid sequences of exemplary TCR constant chains are provided in SEQ ID NO: 39-64 (**Table 5**). The amino acid sequences of exemplary TCR constant chains are provided in SEQ ID NO: 4038-4063 (**Table 5**). 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.

[00121] The term “stimulation,” refers to a primary response induced by binding of a stimulatory molecule (*e.g.*, a TCR/CD3 complex or SIR) with its cognate ligand (or target antigen in the case of a SIR) 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.

[00122] 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 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 the antigen binding domain BCMA-Am06-HL targeting BCMA described in this disclosure and co-expressing an accessory module encoding NEMO-K277A are provided in SEQ ID

NO: 4384-4387 (**Table 6**). Exemplary TFPs incorporating different antigen binding domains described in this disclosure and co-expressing an accessory module encoding NEMO-K277A are provided in **Table 7**. The SEQ ID Nos, antigen binding domains and target antigens of these TFPs can be determined by referring to **Table 6** as the order of the different constructs (*i.e.*, CAR class) listed in **Table 7** is the same as the order of constructs (*i.e.*, CAR class) listed in **Table 6**. 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.*

[00123] 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.

[00124] 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).

[00125] 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}), tissue resident T cells, α/β T cells, γ/δ T cells, iPSC-derived T cells, synthetic T cells or combinations thereof.

[00126] 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 colony counts of the infectious agent, amelioration of various physiological symptoms associated with a disease condition, prevention of the occurrence of disease in the first place or in the prevention of relapse of the disease.

[00127] "Treatment" and "treating," as used herein refer to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the condition as well as those prone to have the condition or those in whom the condition is to be prevented.

[00128] The term "zeta" (or defined by the greek symbol "ζ") or alternatively "zeta chain", "CD3-zeta" or "TCR-zeta" is defined as the protein provided as GenBank Accession 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 Accession 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 DNA SEQ ID NO: 101 and PRT SEQ ID NO: 4100.

[00129] Provided herein are compositions comprising a CAR and optional one or more accessory modules and method of using same to treat diseases, including cancer. As described herein, specific combinations of CARs (**Table 1**) and accessory modules as described in **Table 2** define a 'backbone' (**Table 2**).

[00130] **Table 1:** CAR architectures. First generation CARs (conventional CAR I or CAR I) have an antigen specific domain (ASD), an intracellular signaling domain (ISD) (*e.g.* CD3z) and no costimulatory domain. The TCR fusion proteins (TFP) are next generation CARs that are described in WO 2016/187349 A1 but resemble conventional CAR I in having an antigen specific domain (ASD) and an intracellular signaling domain. Second generation CARs (Conventional CAR 2 or CAR II) have an antigen specific domain (ASD), one costimulatory domain (*e.g.* 41BB or CD28) and an intracellular signaling (ISD) domain (*e.g.* CD3z). Third generation CARs (Conventional CAR 3 or CAR III) have an antigen specific domain (ASD), two costimulatory domains (*e.g.* 41BB and CD28) and an intracellular signaling (ISD) domain (*e.g.* CD3z). AbTCRs 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 a TCR constant chain and result in activation of T cell signaling. Synthetic immune receptors are next generation cTCR and are described in US 62/429,597 and PCT/US017/064379. SIRs can be single chain, one and half, or double chain receptors consisting of one or more antigen binding domains that are fused to one or more TCR constant chains and result in activation of T cell signaling upon ligand-binding. zSIRs are described in this application.

[00131] The zSIRs are a novel platform of synthetic immune receptors (SIRs) containing two CD3-zeta (CD3z) chains. The nucleic acid and amino acid sequences of the CD3z chains that can be used in the construction of zSIR are provided in DNA SEQ ID Nos: 67 and 71 and PRT SEQ ID Nos: 4066 and 4072. The disclosure provides that the vL fragment of an antibody can be joined to one of the two CD3z chains and the vH fragment can be joined to the other CD3z chain. When the two such chains (*e.g.* vL-CD3z and vH-CD3z) are co-expressed in the same cell, the vL and vH fragments can associate together, recognize their cognate antigen or binding partner and transmit a T cell signal. In particular, T cells expressing such zSIR when exposed to a cell line expressing the target antigen can activate NFAT signaling, induce IL2 production and exert cytotoxicity. The expression and activity of the zSIR can be further increased by incorporation of a linker between the vL/vH and the CD3z fragments. In particular, the IgCL and IgCH domains derived from antibodies serve as useful linkers between the vL/vH and CD3z fragments. Exemplary linkers that can be used in construction of zSIRs are provided in SEQ ID NOs: 4004 to 4037 (**Table 5**). Provided in **Figure 1** are schematic examples of zSIRs contemplated by the disclosure.

[00132] For example, zSIR1, the vL fragment of an scFV is joined to one CD3z-ECD-TM-CP (extracellular, transmembrane and cytoplasmic domain) and the vH fragment joined to a second CD3zECDTMCP. An exemplary zSIR1 is provided in SEQ ID NO: 425. In zSIR2, one ASD (*e.g.*, scFV fragment) is joined to one CD3zECDTMCP (extracellular, transmembrane and cytoplasmic domain) and the second ASD is joined to a second CD3zECDTMCP. An exemplary zSIR2 is provided in SEQ ID NO: 3961. The two ASD may target the same or different antigens or different epitopes of the same antigen. An exemplary zSIR2 in which the two ASD target two different antigens is provided in SEQ ID NO: 3962. An exemplary zSIR2 in which the two ASD target two epitopes of the same antigens is provided in SEQ ID NO: 3961. In zSIR3, the vL fragment of an scFV is joined to one CD3zECDTMCP (extracellular, transmembrane and cytoplasmic domain) via the cL linker (SEQ ID NOs: 28 and 4027) derived from an immunoglobulin and the vH fragment joined to a second CD3zECDTMCP via a CH1 linker (SEQ ID NOs: 29 and 4028). An exemplary zSIR3 is CD8-hCD19-EUK5-13-vL-IgCL-Bam-CD3zECDTMCP-opt-F-P2A-Spe-SP-Bst-hCD19-EUK5-13-vH-IgG1-CH1-KPN-CD3zECDTMCP-opt2-F-F2A-Xba-PAC (SEQ ID NO: 3955). Other linkers that can be used in the construction of a zSIR are listed in **Table 5**.

[00133] In another embodiment, a costimulatory domain is also incorporated in the the CD3z chain(s) of a zSIR. Exemplary costimulatory domains include costimulatory domains of 41BB (SEQ ID NO: 69 and SEQ ID NO: 4068) and CD28 (SEQ ID NO:69 and SEQ ID

NO; 4067). CD3z chains containing 41BB (BB) (see, schematic “C”, above) and CD28 (see, schematic “D”, above) costimulatory domains are presented in SEQ ID NO (DNA): 76-79 and SEQ ID NO: (PRT): 4075-4078. An exemplary zSIR with CD3z containing CD28 constimulatory domains is presented by CD8SP-BCMA-Am06-HL-vL-[CD3zECDTM-28z-opt]-F-P2A-SP-BCMA-Am06-HL-vH-[CD3zECDTM-28z-opt2] (SEQ ID NO (DNA): 3971 and (SEQ ID NO (PRT): 7971). An exemplary zSIR with CD3z containing 41BB constimulatory domains is presented by CD8SP-BCMA-Am06-HL-vL-[CD3zECDTM-BBz-opt]-F-P2A-SP-BCMA-Am06-HL-vH-[CD3zECDTM-BBz-opt2] (SEQ ID NO (DNA): 3972 and (SEQ ID NO (PRT): 7972). zSIRs 4-9 resemble zSIRs 1-3 except substitution of CD3zECDTMCP with CD3zECDTM-BBz or with CD3zECDTM-28z domains.

[00134] **Table 1**

Table 1 Exemplary CARs							
CAR 1	CAR 1 or CAR I (including TFP)	ASD	HR	TMD	ISD		
CAR 2	CAR 2 (CAR II)	ASD	HR	TMD	CSD	ISD	
CAR 3	CAR 3 (CAR III)	ASD	HR	TMD	CSD-I	CSD-II	ISD
CAR 4	AbTCR	vL-cL	TCRD(1)	2A	vH-CH1	TCRD (II)	
CAR 5	Double Chain cTCR/SIR	vL	TCR-C(1)	2A	vH	TCR-C (II)	
CAR 6	One & Half Chain cTCR/SIR		TCR-C(1)	2A	ASD	TCR-C (II)	
CAR 7	zSIR1	vL	CD3zECD TMCP	2A	vH	CD3zECD TMCP	
CAR 8	zSIR2	ASD	CD3zECD TMCP	2A	ASD	CD3zECD TMCP	
CAR 9	zSIR3	vL-cL	CD3zECD TMCP	2A	vH-CH1	CD3zECD TMCP	
CAR 10	zSIR4	vL	CD3zECD TM-BBz	2A	vH	CD3zECD TM-BBz	
CAR 11	zSIR5	vL	CD3zECD TM-28z	2A	vH	CD3zECD TM-28z	
CAR 12	zSIR6	ASD	CD3zECD TM-BBz	2A	ASD	CD3zECD TM-BBz	
CAR 13	zSIR7	ASD	CD3zECD TM-28z	2A	ASD	CD3zECD TM-28z	
CAR 14	zSIR8	vL-cL	CD3zECD TM-BBz	2A	vH-CH1	CD3zECD TM-BBz	
CAR 15	zSIR9	vL-cL	CD3zECD TM-28z	2A	vH-CH1	CD3zECD TM-28z	

[00135] **Table 2: Exemplary Backbones**

Backbone	CAR Component	Accessory Module		
		NAME	SEQ ID (DNA)	SEQ ID (PRT)
Backbone 1	CAR I	K13-vFLIP	108	4107

Backbone 2	CAR I	FKBPX2-K13	113	4112
Backbone 3	CAR I	tBCMA	97	4096
Backbone 4	CAR I	HIV-1 Vif	118	4117
Backbone 5	CAR II	K13-vFLIP	108	4107
Backbone 6	CAR II	FKBPX2-K13	113	4112
Backbone 7	CAR II	tBCMA	97	4096
Backbone 8	CAR II	HIV-1 Vif	118	4117
Backbone 9	CAR III	K13-vFLIP	108	4107
Backbone 10	CAR III	FKBPX2-K13	113	4112
Backbone 11	CAR III	tBCMA	97	4096
Backbone 12	CAR III	HIV-1 Vif	118	4117
Backbone 13	AbTCR	K13-vFLIP	108	4107
Backbone 14	AbTCR	FKBPX2-K13	113	4112
Backbone 15	AbTCR	tBCMA	97	4096
Backbone 16	AbTCR	HIV-1 Vif	118	4117
Backbone 17	DC-cTCR/SIR	K13-vFLIP	108	4107
Backbone 18	DC-cTCR/SIR	FKBPX2-K13	113	4112
Backbone 19	DC-cTCR/SIR	tBCMA	97	4096
Backbone 20	DC-cTCR/SIR	HIV-1 Vif	118	4117
Backbone 21	OHC-cTCR/SIR	K13-vFLIP	108	4107
Backbone 22	OHC-cTCR/SIR	FKBPX2-K13	113	4112
Backbone 23	OHC-cTCR/SIR	tBCMA	97	4096
Backbone 24	OHC-cTCR/SIR	HIV-1 Vif	118	4117
Backbone 25	zSIR1	K13-vFLIP	108	4107
Backbone 26	zSIR1	FKBPX2-K13	113	4112
Backbone 27	zSIR1	tBCMA	97	4096
Backbone 28	zSIR1	HIV-1 Vif	118	4117
Backbone 29	zSIR2	K13-vFLIP	108	4107
Backbone 30	zSIR2	FKBPX2-K13	113	4112
Backbone 31	zSIR2	tBCMA	97	4096
Backbone 32	zSIR2	HIV-1 Vif	118	4117
Backbone 33	zSIR3	K13-vFLIP	108	4107
Backbone 34	zSIR3	FKBPX2-K13	113	4112
Backbone 35	zSIR3	tBCMA	97	4096
Backbone 36	zSIR3	HIV-1 Vif	118	4117
Backbone 37	zSIR4	K13-vFLIP	108	4107
Backbone 38	zSIR4	FKBPX2-K13	113	4112
Backbone 39	zSIR4	tBCMA	97	4096
Backbone 40	zSIR4	HIV-1 Vif	118	4117
Backbone 41	zSIR5	K13-vFLIP	108	4107
Backbone 42	zSIR5	FKBPX2-K13	113	4112
Backbone 43	zSIR5	tBCMA	97	4096
Backbone 44	zSIR5	HIV-1 Vif	118	4117
Backbone 45	zSIR6	K13-vFLIP	108	4107
Backbone 46	zSIR6	FKBPX2-K13	113	4112

Backbone 47	zSIR6	tBCMA	97	4096
Backbone 48	zSIR6	HIV-1 Vif	118	4117
Backbone 49	zSIR7	K13-vFLIP	108	4107
Backbone 50	zSIR7	FKBPX2-K13	113	4112
Backbone 51	zSIR7	tBCMA	97	4096
Backbone 52	zSIR7	HIV-1 Vif	118	4117
Backbone 53	zSIR8	K13-vFLIP	108	4107
Backbone 54	zSIR8	FKBPX2-K13	113	4112
Backbone 55	zSIR8	tBCMA	97	4096
Backbone 56	zSIR8	HIV-1 Vif	118	4117
Backbone 57	zSIR9	K13-vFLIP	108	4107
Backbone 58	zSIR9	FKBPX2-K13	113	4112
Backbone 59	zSIR9	tBCMA	97	4096
Backbone 60	zSIR9	HIV-1 Vif	118	4117

[00136] **TABLE 3: Sequence listing of vL, vH and scFv Fragments targeting different antigens that are used in the construction of CARs**

Antigen Target	Antigen binding domain	vL		vH		scFv	
		DNA SEQ ID	PRT SEQ ID	DNA SEQ ID	PRT SEQ ID	DNA SEQ ID	PRT SEQ ID
BCMA	BCMA-Am14-HL	155	4118	229	4192	303	4266
BCMA	BCMA-Am08-HL	156	4119	230	4193	304	4267
BCMA	BCMA-Am06-HL	157	4120	231	4194	305	4268
CD19	hu-CAT18-1-HL	158	4121	232	4195	306	4269
CD19	CAT17-HL	159	4122	233	4196	307	4270
CD22	hu-HA22-1	160	4123	234	4197	308	4271
CD19	CD19-DART1	161	4124	235	4198	309	4272
CD20	hu-Ubli-1-v4	162	4125	236	4199	310	4273
Integrin B7	Hu-IntB7-MMG49	163	4126	237	4200	311	4274
BCMA	BCMA-BB-CAR02	164	4127	238	4201	312	4275
Her2	Her2-169	165	4128	239	4202	313	4276
Her2	Her2-XMT-1520	166	4129	240	4203	314	4277
Her2	Her2-XMT-1518	167	4130	241	4204	315	4278
Her2	Her2-huMab4D5-D98W	168	4131	242	4205	316	4279
TSHR	TSHR-hu-3BD10	169	4132	243	4206	317	4280
PSMA	PSMA-83A12-HL-AM	170	4133	244	4207	318	4281
PSMA	PSMA-76-HL-AM	171	4134	245	4208	319	4282
PSMA	hu106mPSMA-4-HL	172	4135	246	4209	320	4283
MSLN	MSLN-3-HL-AM	173	4136	247	4210	321	4284
MSLN	MSLN-5-HL	174	4137	248	4211	322	4285

Antigen Target	Antigen binding domain	vL		vH		scFv	
		DNA SEQ ID	PRT SEQ ID	DNA SEQ ID	PRT SEQ ID	DNA SEQ ID	PRT SEQ ID
EGFRviii	EGFRviii-2-AM-HL	175	4138	249	4212	323	4286
EGFRviii	EGFRviii-H2M1863N2-HL	176	4139	250	4213	324	4287
EGFRviii	EGFRviii-H2M1915N-HL	177	4140	251	4214	325	4288
EGFRviii	EGFRviii-131-2	178	4141	252	4215	326	4289
DLL3	DLL3-AM6-HL	179	4142	253	4216	327	4290
DLL3	DLL3-AM14-HL	180	4143	254	4217	328	4291
Nectin4	Nectin4-66-HL	181	4144	255	4218	329	4292
MSLN	MSLN-237-HL	182	4145	256	4219	330	4293
MSLN	MSLN-HuAM15	183	4146	257	4220	331	4294
MSLN	MSLN76923-HL	184	4147	258	4221	332	4295
Prolactin Receptor	PRLR-CN	185	4148	259	4222	333	4296
Muc17	Muc17-11-CN	186	4149	260	4223	334	4297
CD19	CD19-AM1	187	4150	261	4224	335	4298
CD19	CD19-9B7	188	4151	262	4225	336	4299
CD20	CD20-HL	189	4152	263	4226	337	4300
CD70	CD70-HL-AM13	190	4153	264	4227	338	4301
CDH19	CDH19-USC1-HLv4	191	4154	265	4228	339	4302
CDH19	CDH19-USC2-HL	192	4155	266	4229	340	4303
CD16ORF54	C16ORF54-USC1-v4	193	4156	267	4230	341	4304
VISTA	huVISTA-USC1-v4	194	4157	268	4231	342	4305
VISTA	huVISTA-JJ-USC2-v4	195	4158	269	4232	343	4306
GPC3	GPC3-USC1-HL-V4	196	4159	270	4233	344	4307
GPC3	GPC3-USC2-HL-V4	197	4160	271	4234	345	4308
PRLR	PRLR-USC2-HL-V4	198	4161	272	4235	346	4309
Muc5Ac	Muc5Ac-USC1-HL-V4	199	4162	273	4236	347	4310
FCRH5	FCRH5-USC1-HL-V4	200	4163	274	4237	348	4311
LYPD1	LYPD1-HL-V4	201	4164	275	4238	349	4312
EMR2	EMR2-USC1-V4	202	4165	276	4239	350	4313
EMR2	EMR2-USC2-V4	203	4166	277	4240	351	4314
EMR2	mEMR2-USC3-V4	204	4167	278	4241	352	4315
gpNMB	m-gPNMB-USC1-HL-v4	205	4168	279	4242	353	4316

Antigen Target	Antigen binding domain	vL		vH		scFv	
		DNA SEQ ID	PRT SEQ ID	DNA SEQ ID	PRT SEQ ID	DNA SEQ ID	PRT SEQ ID
RNF43	RNF43-USC1-HL4	206	4169	280	4243	354	4317
RNF43	RNF43-USC2-HL4	207	4170	281	4244	355	4318
CD44v6	CD44v6-USC1-HL4	208	4171	282	4245	356	4319
Robo4	Robo4-USC1	209	4172	283	4246	357	4320
CEA	CEA-USC1-HL4	210	4173	284	4247	358	4321
Her3	Her3-USC1-HL4	211	4174	285	4248	359	4322
FOLR1	FOLR1-USC1-HL4	212	4175	286	4249	360	4323
FOLR1	FOLR1-USC2-HL4	213	4176	287	4250	361	4324
CLDN6	CLDN6-USC1-LH4	214	4177	288	4251	362	4325
CLDN6	CLDN6-USC2-LH4	215	4178	289	4252	363	4326
MMP16	hMMP16-USC1-LH4	216	4179	290	4253	364	4327
UPK1B	hUPK1B-USC1-LH4	217	4180	291	4254	365	4328
UPK1B	hUPK1B-USC2-LH4	218	4181	292	4255	366	4329
BMPR1B	hBMPR1B-USC1-LH4	219	4182	293	4256	367	4330
BMPR1B	hBMPR1B-USC2-LH4	220	4183	294	4257	368	4331
Ly6E	Ly6E-USC1-HL4	221	4184	295	4258	369	4332
STEAP1	STEAP1-USC1-HL4	222	4185	296	4259	370	4333
CD79b	CD79b-USC1-LH4	223	4186	297	4260	371	4334
WISP1	hu-UISP1-USC1-LH4	224	4187	298	4261	372	4335
WISP1	hu-UISP1-USC2-LH4	225	4188	299	4262	373	4336
SLC34A2	huMX35-LH4	226	4189	300	4263	374	4337
CD19	hu-CD19-USC1-LH4	227	4190	301	4264	375	4338
CD22	CD22-HA22	8000	9631	8031	9662	8062	9693
STEAP1	STEAP1-hu120	8001	9632	8032	9663	8063	9694
Liv1	hLiv1-mAb2	8002	9633	8033	9664	8064	9695
Nectin4	hu-Nectin4-mAb1	8003	9634	8034	9665	8065	9696
Cripto	hu-Cripto-L1H2	8004	9635	8035	9666	8066	9697
gpA33	hu-gpA33	8005	9636	8036	9667	8067	9698
ROR1	ROR1-DART4	8006	9637	8037	9668	8068	9699
BCMA	BCMA-FS	8007	9638	8038	9669	8069	9700

Antigen Target	Antigen binding domain	vL		vH		scFv	
		DNA SEQ ID	PRT SEQ ID	DNA SEQ ID	PRT SEQ ID	DNA SEQ ID	PRT SEQ ID
BCMA	BCMA-PC	8008	9639	8039	9670	8070	9701
BCMA	BCMA-AJ	8009	9640	8040	9671	8071	9702
BCMA	BCMA-NM	8010	9641	8041	9672	8072	9703
BCMA	BCMA-TS	8011	9642	8042	9673	8073	9704
BCMA	BCMA-PP	8012	9643	8043	9674	8074	9705
BCMA	BCMA-RD	8013	9644	8044	9675	8075	9706
BCMA	BCMA-BB-CAR02	8014	9645	8045	9676	8076	9707
CLL1	CLL1-24C8	8015	9646	8046	9677	8077	9708
CLL1	CLL1-24C1	8016	9647	8047	9678	8078	9709
FLT3	FLT3-10E3	8017	9648	8048	9679	8079	9710
FLT3	FLT3-8B5	8018	9649	8049	9680	8080	9711
IL1RAP	IL1RAP-IAPB57	8019	9650	8050	9681	8081	9712
IL1RAP	IL1RAP-IAPB63	8020	9651	8051	9682	8082	9713
IL1RAP	hu-IL1RAP-CANO4	8021	9652	8052	9683	8083	9714
MSLN	MSLN-7D9-v3	8022	9653	8053	9684	8084	9715
MSLN	MSLN-hu22A10	8023	9654	8054	9685	8085	9716
CD19	hu-Bu13	8024	9655	8055	9686	8086	9717
BST1	hu-BST1-A1	8025	9656	8056	9687	8087	9718
BST1	hu-BST1-A2	8026	9657	8057	9688	8088	9719
BST1	hu-BST1-A3	8027	9658	8058	9689	8089	9720
Her2	Her2-XMT-1519	8028	9659	8059	9690	8090	9721
Her2	Her2-XMT-1517	8029	9660	8060	9691	8091	9722
CD133	CD133-RW03	11300	11460	11304	11464	11308	11468
CD133	CD133-W6B3H10	11301	11461	11305	11465	11309	11469
CD133	CD133-293AC1C3B9	11302	11462	11306	11466	11310	11470
IL113Ra2	hu-IL13Ra2-mAb47	12642	14386	12673	14417	12704	14448
CD22	CD22-INO	12643	14387	12674	14418	12705	14449
CD22	CD22-CELL4	12644	14388	12675	14419	12706	14450
CD22	CD22-CELL13	12645	14389	12676	14420	12707	14451
CD22	CD22-CELL7	12646	14390	12677	14421	12708	14452
CD22	CD22-VM1011	12647	14391	12678	14422	12709	14453
CD22	CD22-RAB-4120	12648	14392	12679	14423	12710	14454
CD22	CD22-Med-12C5-HL	12649	14393	12680	14424	12711	14455
CD22	CD22-Med-19A3	12650	14394	12681	14425	12712	14456
CD22	CD22-Med-16F7	12651	14395	12682	14426	12713	14457
CD22	hu-RFB4	12652	14396	12683	14427	12714	14458
BCMA	BCMA-mJ22-9	12653	14397	12684	14428	12715	14459

Antigen Target	Antigen binding domain	vL		vH		scFv	
		DNA SEQ ID	PRT SEQ ID	DNA SEQ ID	PRT SEQ ID	DNA SEQ ID	PRT SEQ ID
BCMA	BCMA-huJ22-10	12654	14398	12685	14429	12716	14460
CD22	CD22-hu-HA22-2	12655	14399	12686	14430	12717	14461
CD19	huCD19-USC3	12656	14400	12687	14431	12718	14462
CD22	BCMA-hu72	12657	14401	12688	14432	12719	14463
MPL	hu-161-3	12658	14402	12689	14433	12720	14464
BAFF-R	hu-BAFFR-USC90	12659	14403	12690	14434	12721	14465
BAFF-R	hu-BAFFR-USC55	12660	14404	12691	14435	12722	14466
BAFF-R	hu-BAFFR-MOR6654	12661	14405	12692	14436	12723	14467
CD19	CD19-hu-mROO5-1	12662	14406	12693	14437	12724	14468
CD22	CD22-h10F4v2	12663	14407	12694	14438	12725	14469
CD22	CD22-HA22	12664	14408	12695	14439	12726	14470
MPL	hu-161-2	12665	14409	12696	14440	12727	14471
MSLN	MSLN-hu22A10	12666	14410	12697	14441	12728	14472
MSLN	MSLN-7D9-HL	12667	14411	12698	14442	12729	14473
MSLN	MSLN-5	12668	14412	12699	14443	12730	14474
BCMA	BCMA-huC13-F12	12669	14413	12700	14444	12731	14475
BCMA	BCMA-huC12A3-L3H3	12670	14414	12701	14445	12732	14476
BCMA	BCMA-J6M0	12671	14415	12702	14446	12733	14477

[00137] TABLE 4: SEQUENCE LISTING OF VARIOUS CDRs of vL and vH REGIONS BELONGING TO DIFFERENT ANTIGEN BINDING DOMAINS TARGETING DIFFERENT ANTIGENS

Antigen Target	Antigen binding domain	vL-CDR1	vL-CDR2	vL-CDR3	vH-CDR1	vH-CDR2	vH-CDR3
BCMA	BCMA-Am14-HL	11961	12068	12175	12282	12389	12497
BCMA	BCMA-Am08-HL	11962	12069	12176	12283	12390	12498
BCMA	BCMA-Am06-HL	11963	12070	12177	12284	12391	12499
CD19	hu-CAT18-1-HL	11964	12071	12178	12285	12392	12500
CD19	CAT17-HL	11965	12072	12179	12286	12393	12501
CD22	hu-HA22-1	11966	12073	12180	12287	12394	12502
CD19	CD19-DART1	11967	12074	12181	12288	12395	12503
CD20	hu-Ubli-1-v4	11968	12075	12182	12289	12396	12504
Integrin B7	Hu-IntB7-MMG49	11969	12076	12183	12290	12397	12505
BCMA	BCMA-BB-CAR02	11970	12077	12184	12291	12398	12506
Her2	Her2-169	11971	12078	12185	12292	12399	12507
Her2	Her2-XMT-1520	11972	12079	12186	12293	12400	12508
Her2	Her2-XMT-1518	11973	12080	12187	12294	12401	12509

Her2	Her2-huMab4D5-D98W	11974	12081	12188	12295	12402	12510
TSHR	TSHR-hu-3BD10	11975	12082	12189	12296	12403	12511
PSMA	PSMA-83A12-HL-AM	11976	12083	12190	12297	12404	12512
PSMA	PSMA-76-HL-AM	11977	12084	12191	12298	12405	12513
PSMA	hu106mPSMA-4-HL	11978	12085	12192	12299	12406	12514
MSLN	MSLN-3-HL-AM	11979	12086	12193	12300	12407	12515
MSLN	MSLN-5-HL	11980	12087	12194	12301	12408	12516
EGFRviii	EGFRviii-2-AM-HL	11981	12088	12195	12302	12409	12517
EGFRviii	EGFRviii-H2M1863N2-HL	11982	12089	12196	12303	12410	12518
EGFRviii	EGFRviii-H2M1915N-HL	11983	12090	12197	12304	12411	12519
EGFRviii	EGFRviii-131-2	11984	12091	12198	12305	12412	12520
DLL3	DLL3-AM6-HL	11985	12092	12199	12306	12413	12521
DLL3	DLL3-AM14-HL	11986	12093	12200	12307	12414	12522
Nectin4	Nectin4-66-HL	11987	12094	12201	12308	12415	12523
MSLN	MSLN-237-HL	11988	12095	12202	12309	12416	12524
MSLN	MSLN-HuAM15	11989	12096	12203	12310	12417	12525
MSLN	MSLN76923-HL	11990	12097	12204	12311	12418	12526
Prolactin Receptor	PRLR-CN	11991	12098	12205	12312	12419	12527
Muc17	Muc17-11-CN	11992	12099	12206	12313	12420	12528
CD19	CD19-AM1	11993	12100	12207	12314	12421	12529
CD19	CD19-9B7	11994	12101	12208	12315	12422	12530
CD20	CD20-HL	11995	12102	12209	12316	12423	12531
CD70	CD70-HL-AM13	11996	12103	12210	12317	12424	12532
CDH19	CDH19-USC1-HLv4	11997	12104	12211	12318	12425	12533
CDH19	CDH19-USC2-HL	11998	12105	12212	12319	12426	12534
CD16ORF54	C16ORF54-USC1-v4	11999	12106	12213	12320	12427	12535
VISTA	huVISTA-USC1-v4	12000	12107	12214	12321	12428	12536
VISTA	huVISTA-JJ-USC2-v4	12001	12108	12215	12322	12429	12537
GPC3	GPC3-USC1-HL-V4	12002	12109	12216	12323	12430	12538
GPC3	GPC3-USC2-HL-V4	12003	12110	12217	12324	12431	12539
PRLR	PRLR-USC2-HL-V4	12004	12111	12218	12325	12432	12540
Muc5Ac	Muc5Ac-USC1-HL-V4	12005	12112	12219	12326	12433	12541
FCRH5	FCRH5-USC1-HL-V4	12006	12113	12220	12327	12434	12542
LYPD1	LYPD1-HL-V4	12007	12114	12221	12328	12435	12543
EMR2	EMR2-USC1-V4	12008	12115	12222	12329	12436	12544
EMR2	EMR2-USC2-V4	12009	12116	12223	12330	12437	12545
EMR2	mEMR2-USC3-V4	12010	12117	12224	12331	12438	12546
gpNMB	m-gPNMB-USC1-HL-v4	12011	12118	12225	12332	12439	12547

RNF43	RNF43-USC1-HL4	12012	12119	12226	12333	12440	12548
RNF43	RNF43-USC2-HL4	12013	12120	12227	12334	12441	12549
CD44v6	CD44v6-USC1-HL4	12014	12121	12228	12335	12442	12550
Robo4	Robo4-USC1	12015	12122	12229	12336	12443	12551
CEA	CEA-USC1-HL4	12016	12123	12230	12337	12444	12552
Her3	Her3-USC1-HL4	12017	12124	12231	12338	12445	12553
FOLR1	FOLR1-USC1-HL4	12018	12125	12232	12339	12446	12554
FOLR1	FOLR1-USC2-HL4	12019	12126	12233	12340	12447	12555
CLDN6	CLDN6-USC1-LH4	12020	12127	12234	12341	12448	12556
CLDN6	CLDN6-USC2-LH4	12021	12128	12235	12342	12449	12557
MMP16	hMMP16-USC-1-LH4	12022	12129	12236	12343	12450	12558
UPK1B	hUPK1B-USC1-LH4	12023	12130	12237	12344	12451	12559
UPK1B	hUPK1B-USC2-LH4	12024	12131	12238	12345	12452	12560
BMPR1B	hBMPR1B-USC1-LH4	12025	12132	12239	12346	12453	12561
BMPR1B	hBMPR1B-USC2-LH4	12026	12133	12240	12347	12454	12562
Ly6E	Ly6E-USC1-HL4	12027	12134	12241	12348	12455	12563
STEAP1	STEAP1-USC1-HL4	12028	12135	12242	12349	12456	12564
CD79b	CD79b-USC1-LH4	12029	12136	12243	12350	12457	12565
WISP1	hu-WISP1-USC1-LH4	12030	12137	12244	12351	12458	12566
WISP1	hu-WISP1-USC2-LH4	12031	12138	12245	12352	12459	12567
SLC34A2	huMX35-LH4	12032	12139	12246	12353	12460	12568
CD19	hu-CD19-USC1-LH4	12033	12140	12247	12354	12461	12569
CD22	CD22-HA22	12034	12141	12248	12355	12462	12570
STEAP1	STEAP1-hu120	12035	12142	12249	12356	12463	12571
Liv1	hLiv1-mAb2	12036	12143	12250	12357	12464	12572
Nectin4	hu-Nectin4-mAb1	12037	12144	12251	12358	12465	12573
Cripto	hu-Cripto-L1H2	12038	12145	12252	12359	12466	12574
gpA33	hu-gpA33	12039	12146	12253	12360	12467	12575
ROR1	ROR1-DART4	12040	12147	12254	12361	12468	12576
BCMA	BCMA-FS	12041	12148	12255	12362	12469	12577
BCMA	BCMA-PC	12042	12149	12256	12363	12470	12578
BCMA	BCMA-AJ	12043	12150	12257	12364	12471	12579
BCMA	BCMA-NM	12044	12151	12258	12365	12472	12580
BCMA	BCMA-TS	12045	12152	12259	12366	12473	12581
BCMA	BCMA-PP	12046	12153	12260	12367	12474	12582
BCMA	BCMA-RD	12047	12154	12261	12368	12475	12583
BCMA	BCMA-BB-CAR02	12048	12155	12262	12369	12476	12584
CLL1	CLL1-24C8	12049	12156	12263	12370	12477	12585
CLL1	CLL1-24C1	12050	12157	12264	12371	12478	12586
FLT3	FLT3-10E3	12051	12158	12265	12372	12479	12587
FLT3	FLT3-8B5	12052	12159	12266	12373	12480	12588
IL1RAP	IL1RAP-IAPB57	12053	12160	12267	12374	12481	12589

IL1RAP	IL1RAP-IAPB63	12054	12161	12268	12375	12482	12590
IL1RAP	hu-IL1RAP-CANO4	12055	12162	12269	12376	12483	12591
MSLN	MSLN-7D9-v3	12056	12163	12270	12377	12484	12592
MSLN	MSLN-hu22A10	12057	12164	12271	12378	12485	12593
CD19	hu-Bu13	12058	12165	12272	12379	12486	12594
BST1	hu-BST1-A1	12059	12166	12273	12380	12487	12595
BST1	hu-BST1-A2	12060	12167	12274	12381	12488	12596
BST1	hu-BST1-A3	12061	12168	12275	12382	12489	12597
Her2	Her2-XMT-1519	12062	12169	12276	12383	12490	12598
Her2	Her2-XMT-1517	12063	12170	12277	12384	12491	12599
CD133	CD133-RW03	12064	12171	12278	12385	12492	12600
CD133	CD133-W6B3H10	12065	12172	12279	12386	12493	12601
CD133	CD133-293AC1C3B9	12066	12173	12280	12387	12494	12602
IL13Ra2	hu-IL13Ra2-mAb47	16126	16157	16188	16219	16250	16281
CD22	CD22-INO	16127	16158	16189	16220	16251	16282
CD22	CD22-CELL4	16128	16159	16190	16221	16252	16283
CD22	CD22-CELL13	16129	16160	16191	16222	16253	16284
CD22	CD22-CELL7	16130	16161	16192	16223	16254	16285
CD22	CD22-VM1011	16131	16162	16193	16224	16255	16286
CD22	CD22-RAB-4120	16132	16163	16194	16225	16256	16287
CD22	CD22-Med-12C5-HL	16133	16164	16195	16226	16257	16288
CD22	CD22-Med-19A3	16134	16165	16196	16227	16258	16289
CD22	CD22-Med-16F7	16135	16166	16197	16228	16259	16290
CD22	hu-RFB4	16136	16167	16198	16229	16260	16291
BCMA	BCMA-mJ22-9	16137	16168	16199	16230	16261	16292
BCMA	BCMA-huJ22-10	16138	16169	16200	16231	16262	16293
CD22	CD22-hu-HA22-2	16139	16170	16201	16232	16263	16294
CD19	huCD19-USC3	16140	16171	16202	16233	16264	16295
CD22	BCMA-hu72	16141	16172	16203	16234	16265	16296
MPL	hu-161-3	16142	16173	16204	16235	16266	16297
BAFF-R	hu-BAFFR-USC90	16143	16174	16205	16236	16267	16298
BAFF-R	hu-BAFFR-USC55	16144	16175	16206	16237	16268	16299
BAFF-R	hu-BAFFR-MOR6654	16145	16176	16207	16238	16269	16300
CD19	CD19-hu-mROO5-1	16146	16177	16208	16239	16270	16301
CD22	CD22-h10F4v2	16147	16178	16209	16240	16271	16302
CD22	CD22-HA22	16148	16179	16210	16241	16272	16303
MPL	hu-161-2	16149	16180	16211	16242	16273	16304
MSLN	MSLN-hu22A10	16150	16181	16212	16243	16274	16305
MSLN	MSLN-7D9-HL	16151	16182	16213	16244	16275	16306
MSLN	MSLN-5	16152	16183	16214	16245	16276	16307
BCMA	BCMA-huC13-F12	16153	16184	16215	16246	16277	16308
BCMA	BCMA-huC12A3-L3H3	16154	16185	16216	16247	16278	16309
BCMA	BCMA-J6M0	16155	16186	16217	16248	16279	16310

[00138] TABLE 5

CAR COMPONENT	DNA SEQ ID	PRT SEQ ID
CD8_Signal_Peptide	1	4000
CD8_Signal_Peptide	2	4001
IgH_Signal_Peptide	3	4002
IgH_Signal_Peptide	4	4003
(GGGGS)x3_LINKER	5	4004
DDAKK_linker	6	4005
GGGSG-Streptagx2-Tag	7	4006
PG4SP-linker	8	4007
PG4SP-v2-linker	9	4008
E-coil-linker	10	4009
K-coil-linker	11	4010
EAAAK-linker	12	4011
EAAAK-v2-linker	13	4012
Myc-(P)-TAG	14	4013
Myc-TAG	15	4014
MYC-TAG	16	4015
MYC2-TAG	17	4016
MYC4-TAG	18	4017
V5-TAG	19	4018
HA-TAG	20	4019
HIS-TAG	21	4020
AVI-TAG-delta-GSG	22	4021
G4Sx2-TAG	23	4022
G4Sx2-TAG	24	4023
StrepTagII	25	4024
StrepTagII	26	4025
FLAG-TAG	27	4026
IgCL	28	4027
IgG1-CHI	29	4028
IgG2-0C-CHI	30	4029
IgG2-IC-CHI	31	4030
IgG3-CHI	32	4031
IgG4-CHI	33	4032
IgA1-CHI	34	4033
IgA2-CHI	35	4034
IgD-CHI	36	4035
IgE-CHI	37	4036
IgM-CHI	38	4037
hTCR-alpha-constant_X02883.1	39	4038
hTCRa-WT	40	4039
hTCRa-CSDVP	41	4040
hTCRa-opt2	42	4041

hTCRa-T48C-opt	43	4042
hTCRa-T48C-opt1	44	4043
hTCRa-SDVP	45	4044
hTCRa-S61R	46	4045
hTCRa-SDVPR	47	4046
hTCRaECD-CD3zECDTMCP-opt2	48	4047
hTCR-b1-constant-region_X00437.1	49	4048
hTCR-b2-constant region_L34740	50	4049
hTCRb-WT	51	4050
hTCRb-S57C-opt1	52	4051
hTCRb-KACIAH	53	4052
hTCRb-opt2	54	4053
hTCRb-KAIAH	55	4054
hTCRb-R79G	56	4055
hTCRbECD-CD3zECDTMCP-opt	57	4056
preTCRa_gb_U38996.1	58	4057
preTCRa	59	4058
preTCRa-del48	60	4059
hTCR-gamma_M27331.1	61	4060
hTCR-Gamma-Opt	62	4061
hTCR-Delta	63	4062
hTCR-Delta-Opt	64	4063
CD3zECDTM-opt	65	4064
CD3zCP-opt	66	4065
CD3zECDTMCP-opt	67	4066
CD28-CP-opt	68	4067
41BB-CP-opt	69	4068
CD3e-CP-opt	70	4069
CD3zECDTM-opt2	71	4070
CD3zCP-opt2	72	4071
CD3zECDTMCP-opt2	73	4072
CD28-CP-opt2	74	4073
41BB-CP-opt2	75	4074
CD3zECDTM-28z-opt	76	4075
CD3zECDTM-BBz-opt	77	4076
CD3zECDTM-28z-opt2	78	4077
CD3zECDTM-BBz-opt2	79	4078
F2A	80	4079
T2A	81	4080
T2A	82	4081
P2A	83	4082
P2a-variant	84	4083
E2A	85	4084
SGSG	86	4085
SGSG	87	4086

FURINE-CLEAVAGE-SITE	88	4087
FURINE-CLEAVAGE-SITE	89	4088
FURINE-CLEAVAGE-SITE	90	4089
PuroR_Variant-(PAC)	91	4090
BlastR	92	4091
CNB30	93	4092
GMCSF-SP-tEGFR	94	4093
tEGFRviii	95	4094
tCD19	96	4095
tBCMA	97	4096
hCD8-Hinge-TM	98	4097
hCD8-Hinge-TM-BBz	99	4098
hCD8TM-Hinge-BB	100	4099
CD3z-cytosolic-domain	101	4100
CD3z-cytosolic-domain	102	4101
CD28-Hinge-TM-cytosolic-domain	103	4102
Myr-MYD88-CD40-Fv'-Fv	104	4103
IL12F	105	4104
41BB-L	106	4105
CD40L	107	4106
K13	108	4107
MC159	109	4108
cFLIP-L/MRIT-alpha	110	4109
cFLIP-p22	111	4110
FKBP-K13	112	4111
FKBPX2-K13	113	4112
HTLV1-TAX	114	4113
HTLV2-TAX	115	4114
HTLV2-TAX-RS	116	4115
icaspase-9	117	4116
HIV-1 Vif	118	4117

[00139] **TABLE 6: SEQUENCE LISTING OF DIFFERENT CAR classes based on the BCMA-Am06-HL antigen binding domain. The CAR type and accessory module(s) are also shown. CAR class 16 and 17 represent one chain of a double chain SIR and show biological activity only when co-expressed with their complementary chain (*i.e.*, CAR class 18 and 19, respectively). CAR classes 13-15 (single chain SIR) show only weak activity.**

CAR Class	SEQ ID (DNA)	SEQ ID (PRT)	NAME	CAR TYPE	Accessory Module
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CAR Class 1	377	4340	CD8SP-BCMA-Am06-HL-vL-V5-[hTCRb-KACIAH]-F-P2A-SP-BCMA-Am06-HL-vH-Myc-[hTCRa-CSDVP]-F-F2A-PAC	Double chain SIR	PAC
CAR Class 2	378	4341	CD8SP-BCMA-Am06-HL-vL-V5-[hTCRb-KACIAH]-F-P2A-SP-BCMA-Am06-HL-vH-Myc-[preTCRa-Del48]-F-F2A-PAC	Double chain SIR	PAC
CAR Class 3	379	4342	CD8SP-V5-[hTCRb-KACIAH]-F-P2A-CD8SP-BCMA-Am06-HL-vL-Gly-Ser-Linker-BCMA-Am06-HL-vH-Myc-[hTCRa-CSDVP]-F-F2A-PAC	One and half chain SIR	PAC
CAR Class 4	380	4343	CD8SP-V5-[hTCRb-KACIAH]-F-P2A-CD8SP-BCMA-Am06-HL-vL-Gly-Ser-Linker-BCMA-Am06-HL-vH-Myc4-[preTCRa-Del48]-F-F2A-PAC	One and half chain SIR	PAC
CAR Class 5	381	4344	CD8SP-MYC-[hTCRa-T48C-opt1]-F-F2A-SP-BCMA-Am06-HL-vL-Gly-Ser-Linker-BCMA-Am06-HL-vH-V5-[hTCRb-S57C-opt1]-F-F2A-PAC	Double chain SIR	PAC
CAR Class 6	382	4345	CD8SP-BCMA-Am06-HL-vL-V5-[hTCRb-S57C-opt]-F-P2A-SP-BCMA-Am06-HL-vH-Myc-[hTCRa-T48C-opt]-F-F2A-PAC	Double chain SIR	PAC
CAR Class 7	383	4346	CD8SP-BCMA-Am06-HL-vL-[hTCRb-opt2]-F-P2A-SP-BCMA-Am06-HL-vH-[hTCRa-opt2]-F-F2A-PAC	Double chain SIR	PAC
CAR Class 8	384	4347	CD8SP-BCMA-Am06-HL-vL-[hTCRb-opt2]-F-P2A-SP-BCMA-Am06-HL-vH-Myc-[preTCRa-Del48]-F-F2A-PAC	Double chain SIR	PAC
CAR Class 9	385	4348	CD8SP-[hTCRb-opt2]-F-P2A-CD8SP-BCMA-Am06-HL-vL-Gly-Ser-Linker-BCMA-Am06-HL-vH-Myc4-[preTCRa-Del48]-F-F2A-PAC	One and half chain SIR	PAC
CAR Class 10	386	4349	CD8SP-BCMA-Am06-HL-vL-V5-[hTCRg1-opt]-F-P2A-SP-BCMA-Am06-HL-vH-Myc-[hTCRd-opt]-F-F2A-PAC	Double chain SIR	PAC
CAR Class 11	387	4350	CD8SP-V5-[hTCRg1-opt]-F-P2A-CD8SP-BCMA-Am06-HL-vL-Gly-Ser-Linker-BCMA-Am06-HL-vH-Myc-[hTCRd-opt]-F-F2A-PAC	One and half chain SIR	PAC
CAR Class 12	388	4351	CD8SP-G4Sx2-[hTCRa-S61R-opt]-F-F2A-SP-BCMA-Am06-HL-vL-Gly-Ser-Linker-BCMA-Am06-HL-vH-G4Sx2-[hTCRb-R79G-opt]-F-F2A-PAC	One and half chain SIR	PAC
CAR Class 13	389	4352	CD8SP-BCMA-Am06-HL-vL-Gly-Ser-Linker-BCMA-Am06-HL-vH-[hTCRa-SDVP]-F-F2A-PAC	Single Chain SIR	PAC
CAR Class 14	390	4353	CD8SP-BCMA-Am06-HL-vL-Gly-Ser-Linker-BCMA-Am06-HL-vH-[hTCRb-KAIAH]-F-P2A-PAC	Single Chain SIR	PAC

CAR Class 15	391	4354	CD8SP-BCMA-Am06-HL-vL-Gly-Ser-Linker-BCMA-Am06-HL-vH-Myc4-[preTCRa-Del48]-F-F2A-PAC	Single Chain SIR	PAC
CAR Class 16	392	4355	CD8SP-BCMA-Am06-HL-vL-V5-[hTCRb-S57C-opt]-F-P2A-PAC	One chain of a double chain SIR	PAC
CAR Class 17	393	4356	CD8SP-BCMA-Am06-HL-vL-V5-[hTCRb-S57C-opt]	One chain of a double chain SIR	
CAR Class 18	394	4357	IgHSP-BCMA-Am06-HL-vH-Myc-[hTCRa-T48C-opt]-F-F2A-BlastR	One chain of a double chain SIR	
CAR Class 19	395	4358	IgHSP-BCMA-Am06-HL-vH-Myc-[hTCRa-T48C-opt]	One chain of a double chain SIR	
CAR Class 20	396	4359	CD8SP-BCMA-Am06-HL-vL-Gly-Ser-Linker-BCMA-Am06-HL-vH-Myc-CD8TM-BBz	CAR II	
CAR Class 21	397	4360	CD8SP-BCMA-Am06-HL-vH-Gly-Ser-Linker-vL-Myc-CD8TM-BBz	CAR II	
CAR Class 22	398	4361	CD8SP-BCMA-Am06-HL-vL-Gly-Ser-Linker-BCMA-Am06-HL-vH-Myc-CD8TM-z-P2A-K13-FLAG-T2A-PAC	CAR 1	K13 and PAC
CAR Class 23	399	4362	CD8SP-BCMA-Am06-HL-vL-[hTCRa-CSDVP]-F-F2A-SP-BCMA-Am06-HL-vH-[hTCRb-KACIAH]-F-P2A-Xba-PAC	Double chain SIR	PAC
CAR Class 24	400	4363	CD8SP-BCMA-Am06-HL-vL-PG4SP-v2-[hTCRb-KACIAH]-F-P2A-SP-BCMA-Am06-HL-vH-PG4SP-[hTCRa-CSDVP]-F-F2A-PAC	Double chain SIR	PAC
CAR Class 25	401	4364	CD8SP-BCMA-Am06-HL-vL-E-Coil-[hTCRb-KACIAH]-F-P2A-SP-BCMA-Am06-HL-vH-K-Coil-[hTCRa-CSDVP]-F-F2A-PAC	Double chain SIR	PAC
CAR Class 26	402	4365	CD8SP-BCMA-Am06-HL-vL-EAAAK-[hTCRb-KACIAH]-F-P2A-SP-BCMA-Am06-HL-vH-EAAAK-v2-[hTCRa-CSDVP]-F-F2A-PAC	Double chain SIR	PAC
CAR Class 27	403	4366	CD8SP-BCMA-Am06-HL-vL-V5-[hTCRb-KACIAH]-F-P2A-SP-BCMA-Am06-HL-vH-Myc4-[hTCRa-CSDVP]-F-F2A-PAC	Double chain SIR	PAC
CAR Class 28	404	4367	CD8SP-BCMA-Am06-HL-vL-Myc2-[hTCRb-KACIAH]-F-P2A-SP-BCMA-	Double chain SIR	PAC

			Am06-HL-vH-Myc4-[hTCRa-CSDVP]-F-F2A-PAC		
CAR Class 29	405	4368	CD8SP-BCMA-Am06-HL-vL-[hTCRb-KACIAH]-F-P2A-SP-BCMA-Am06-HL-vH-[hTCRa-CSDVP]-F-F2A-PAC	Double chain SIR	PAC
CAR Class 30	406	4369	CD8-BCMA-Am06-HL-vL-IgCL-CD3zECDTMCP-opt-F-P2A-SP-Bst-BCMA-Am06-HL-vH-IgG1-CH1-CD3zECDTMCP-opt2-F-F2A-PAC	zSIR	PAC
CAR Class 31	407	4370	CD8SP-BCMA-Am06-HL-vL-[hTCRbECD-Bam-CD3zECDTMCP-opt]-F-P2A-SP-BCMA-Am06-HL-vH-[hTCRaECD-Kpn-CD3zECDTMCP-opt2]	zSIR	
CAR Class 32	408	4371	CD8SP-BCMA-Am06-HL-vL-[hTCRb-KACECD-Bam-CD3zECDTMCP-opt]-F-P2A-SP-BCMA-Am06-HL-vH-[hTCRa-CSDVP-ECD-Kpn-CD3zECDTMCP-opt2]	SIR	
CAR Class 33	409	4372	CD8SP-BCMA-Am06-HL-vL-V5-[hTCRbECD-Bam-CD3zECDTMCP-opt]-F-P2A-SP-BCMA-Am06-HL-vH-Myc-[hTCRaECD-Kpn-CD3zECDTM-28z-opt2]	SIR	PAC
CAR Class 34	410	4373	CD8SP-BCMA-Am06-HL-vL-V5-[hTCRbECD-Bam-CD3zECDTM-28z-opt]-F-P2A-SP-BCMA-Am06-HL-vH-Myc-[hTCRaECD-Kpn-CD3zECDTM-28z-opt2]	SIR	
CAR Class 35	411	4374	CD8SP-BCMA-Am06-HL-vL-V5-[hTCRbECD-Bam-CD3zECDTMCP-opt]-F-P2A-SP-BCMA-Am06-HL-vH-Myc4-[hTCRaECD-Kpn-CD3zECDTM-BBz-opt2]	SIR	
CAR Class 36	412	4375	CD8SP-BCMA-Am06-HL-vL-V5-[hTCRbECD-Bam-CD3zECDTM-BBz-opt]-F-P2A-SP-BCMA-Am06-HL-vH-Myc4-[hTCRaECD-Kpn-CD3zECDTM-BBz-opt2]	SIR	
CAR Class 37	413	4376	CD8-BCMA-Am06-HL-vL-IgCL-Xho-CD3zECDTMCP-opt-F-P2A-Spe-SP-Bst-BCMA-Am06-HL-vH-IgG1-CH1-Mlu-CD3zECDTMCP-opt2-F-F2A-PAC-DeltaWPRE	zSIR	
CAR Class 38	414	4377	CD8SP-BCMA-Am06-HL-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-PAC	CAR I	hNEMO-K277A-Flag and PAC
CAR Class 39	415	4378	CD8SP-BCMA-Am06-HL-(vL-vH)-CD3e-ECDTMCP-opt2-P2A-hNEMO-K277A-Flag-T2A-PAC	TFP	hNEMO-K277A-Flag and PAC

CAR Class 40	416	4379	CD8SP-BCMA-Am06-HL-(vL-vH)-CD3d-ECDTMCP-opt2-P2A-hNEMO-K277A-Flag-T2A-PAC	TFP	hNEMO-K277A-Flag and PAC
CAR Class 41	417	4380	CD8SP-BCMA-Am06-HL-(vL-vH)-CD3g-ECDTMCP-opt2-P2A-hNEMO-K277A-Flag-T2A-PAC	TFP	hNEMO-K277A-Flag and PAC
CAR Class 42	418	4381	CD8SP-BCMA-Am06-HL-(vL-vH)-CD3z-ECDTMCP-opt2-P2A-hNEMO-K277A-Flag-T2A-PAC	TFP	hNEMO-K277A-Flag and PAC
CAR Class 43	419	4382	CD8SP-BCMA-Am06-HL-vL-[IgCL-TCRg-6MD]-F-P2A-SP-BCMA-Am06-HL-vH-[IgG1-CH1-TCRd-6MD]-F-F2A-hNEMO-K277A	Ab-TCR	hNEMO-K277A-Flag
CAR Class 44	420	4383	CD8SP-BCMA-Am06-HL-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-BCMA-Am06-HL-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-hNEMO-K277A	Ab-TCR	hNEMO-K277A-Flag
CAR Class 45	421	4384	CD8SP-BCMA-Am06-HL-(vH-vL)-CD3e-ECDTMCP-opt2-P2A-hNEMO-K277A-Flag-T2A-PAC	TFP	hNEMO-K277A-Flag and PAC
CAR Class 46	422	4385	CD8SP-BCMA-Am06-HL-(vH-vL)-CD3d-ECDTMCP-opt2-P2A-hNEMO-K277A-Flag-T2A-PAC	TFP	hNEMO-K277A-Flag and PAC
CAR Class 47	423	4386	CD8SP-BCMA-Am06-HL-(vH-vL)-CD3g-ECDTMCP-opt2-P2A-hNEMO-K277A-Flag-T2A-PAC	TFP	hNEMO-K277A-Flag and PAC
CAR Class 48	424	4387	CD8SP-BCMA-Am06-HL-(vH-vL)-CD3z-ECDTMCP-opt2-P2A-hNEMO-K277A-Flag-T2A-PAC	TFP	hNEMO-K277A-Flag and PAC
CAR Class 49	425	4388	CD8SP-BCMA-Am06-HL-vL-Xho-CD3zECDTMCP-opt-F-P2A-Spe-SP-BCMA-Am06-HL-vH-Mlu-CD3zECDTMCP-opt2-F-F2A-PAC	zSIR	PAC
CAR Class 50	12784	14528	CD8SP-BCMA-Am06-vL-[hTCRb-S57C]-F-P2A-SP-BCMA-Am06-vH-[hTCRa-T48C]	Double chain SIR	
CAR Class 51	12785	14529	CD8SP-BCMA-Am06-vL-[hTCRb-S57C]-F-P2A-SP-BCMA-Am06-vH-[hTCRa-T48C]-F-F2A-K13-opt	Double chain SIR	K13-vFLIP

CAR Class 52	12786	14530	CD8SP-BCMA-Am06-vL-[hTCRa-T48C]-F-P2A-SP-BCMA-Am06-vH-[hTCRa-S57C]	Double chain SIR	
CAR Class 53	12787	14531	CD8SP-BCMA-Am06-vL-[hTCRa-T48C]-F-P2A-SP-BCMA-Am06-vH-[hTCRa-S57C]-F-P2A-K13-opt	Double chain SIR	K13-vFLIP

[00140] TABLE 7: SEQUENCE LISTING OF VARIOUS CAR CONSTRUCTS CONTAINING DIFFERENT ANTIGEN BINDING DOMAINS. THE ORDER OF DIFFERENT CAR CONSTRUCTS IS AS SHOWN IN TABLE 6 FOR BCMA-Am06-HL BASED CARs.

			CARs	
	Antigen Target	Antigen Binding Domain	SEQ ID NO (DNA)	SEQ ID NO (PRT)
1	BCMA	BCMA-Am14-HL	475-523	4438-4486
2	BCMA	BCMA-Am08-HL	426-474	4389-4437
3	BCMA	BCMA-Am06-HL	377-425;12784-12787	4340-4388; 14528-14531
4	CD19	hu-CAT18-1-HL	867-915	4830-4871
5	CD19	CAT17-HL	818-866	4781-4829
6	CD22	hu-HA22-1	1112-1160	5068-5115
7	CD19	CD19-DART1	916-964	4872-4920
8	CD20	hu-Ubli-1-v4	1063-1111	5019-5067
9	Hu	Hu-IntB7-MMG49	2533-2581	6489-6537
10	BCMA	BCMA-BB-CAR02	524-572	4487-4535
11	Her2	Her2-169	2288-2336	6244-6292
12	Her2	Her2-XMT-1520	2435-2483	6391-6439
13	Her2	Her2-XMT-1518	2386-2434	6342-6390
14	Her2	Her2-huMab4D5-D98W	2337-2385	6293-6341
15	TSHR	TSHR-hu-3BD10	3611-3659	7567-7615
16	PSMA	PSMA-83A12-HL-AM	3317-3365	7273-7321
17	PSMA	PSMA-76-HL-AM	3268-3316	7224-7272
18	PSMA	hu106mPSMA-4-HL	3219-3267	7175-7223
19	MSLN	MSLN-3-HL-AM	2729-2777	6685-6733
20	MSLN	MSLN-5-HL	2778-2826	6734-6782
21	EGFRviii	EGFRviii-2-AM-HL	1651-1699	5607-5655
22	EGFRviii	EGFRviii-H2M1863N2-HL	1749-1797	5705-5753
23	EGFRviii	EGFRviii-H2M1915N-HL	1798-1846	5754-5802
24	EGFRviii	EGFRviii-131-2	1700-1748	5656-5704
25	DLL3	DLL3-AM6-HL	1553-1601	5509-5557
26	DLL3	DLL3-AM14-HL	1602-1650	5558-5606
27	Nectin 4	Nectin4-66-HL	3072-3120	7028-7076
28	MSLN	MSLN-237-HL	2827-2875	6783-6831

29	MSLN	MSLN-HuAM15	2925-2973	6881-6929
30	MSLN	MSLN76923-HL	2876-2924	6832-6880
31	PRLR	PRLR-CN	3121-3169	7077-7125
32	Muc17	Muc17-11-CN	3023-3071	6979-7027
33	CD19	CD19-AM1	769-817	4732-4780
34	CD19	CD19-9B7	720-768	4683-4731
35	CD20	CD20-HL	1014-1062	4970-5018
36	CD70	CD70-HL-AM13	1210-1258	5166-5214
37	CDH19	CDH19-USC1-HLv4	1308-1356	5264-5312
38	CDH19	CDH19-USC2-HL	1357-1405	5313-5361
39	C16ORF54	C16ORF54-USC1-v4	671-719	4634-4682
40	VISTA	huVISTA-USC1-v4	3807-3855	7763-7811
41	VISTA	huVISTA-JJ-USC2-v4	3758-3806	7714-7762
42	GPC3	GPC3-USC1-HL-V4	2141-2189	6097-6145
43	GPC3	GPC3-USC2-HL-V4	2190-2238	6146-6194
44	PRLR	PRLR-USC2-HL-V4	3170-3218	7126-7174
45	Muc5Ac	Muc5Ac-USC1-HL-V4	2974-3022	6930-6978
46	FCRH5	FCRH5-USC1-HL-V4	1994-2042	5950-5998
47	LYPD1	LYPD1-HL-V4	2631-2679	6587-6635
48	EMR2	EMR2-USC1-V4	1847-1895	5803-5851
49	EMR2	EMR2-USC2-V4	1896-1944	5852-5900
50	EMR2	mEMR2-USC3-V4	1945-1993	5901-5949
51	gPNMB	m-gPNMB-USC1-HL-v4	2239-2287	6195-6243
52	RNF43	RNF43-USC1-HL4	3366-3414	7322-7370
53	RNF43	RNF43-USC2-HL4	3415-3463	7371-7419
54	CD44v6	CD44v6-USC1-HL4	1161-1209	5117-5165
55	Robo4	Robo4-USC1	3464-3512	7420-7468
56	CEA	CEA-USC1-HL4	1406-1454	5362-5410
57	Her3	Her3-USC1-HL4	2484-2532	6440-6488
58	FOLR1	FOLR1-USC1-HL4	2043-2091	5999-6047
59	FOLR1	FOLR1-USC2-HL4	2092-2140	6048-6096
60	CLDN6	CLDN6-USC1-LH4	1455-1503	5411-5459
61	CLDN6	CLDN6-USC2-LH4	1504-1552	5460-5508
62	MMP16	hMMP16-USC-1-LH4	2680-2728	6636-6684
63	UPK1B	hUPK1B-USC1-LH4	3660-3708	7616-7664
64	UPK1B	hUPK1B-USC2-LH4	3709-3757	7665-7713
65	BMPR1B	hBMPR1B-USC1-LH4	573-621	4536-4584
66	BMPR1B	hBMPR1B-USC2-LH4	622-670	4585-4633
67	Ly6E	Ly6E-USC1-HL4	2582-2630	6538-6586
68	STEAP1	STEAP1-USC1-HL4	3513-3561	7469-7517
69	CD79b	CD79b-USC1-LH4	1259-1307	5215-5263
70	WISP1	hu-UISP1-USC1-LH4	3856-3904	7812-7860
71	WISP1	hu-UISP1-USC2-LH4	3905-3953	7861-7909
72	SLC34A2	huMX35-LH4	3562-3610	7518-7566
73	CD19	hu-CD19-USC1-LH4	965-1013	4921-4969

74	CD22	CD22-HA22	8730-8778	10361-10409
75	STEAP1	STEAP1-hu120	9563-9611	11194-11242
76	Liv1	hLiv1-mAb2	9318-9366	10949-10997
77	Nectin 4	hu-Nectin4-mAb1	9465-9513	11096-11144
78	Cripto	hu-Cripto-L1H2	8877-8925	10508-10556
79	gpA33	hu-gpA33	9024-9072	10655-10703
80	ROR1	ROR1-DART4	9514-9562	11145-11193
81	BCMA	BCMA-FS	8191-8239	9822-9870
82	BCMA	BCMA-PC	8289-8337	9920-9968
83	BCMA	BCMA-AJ	8093-8141	9724-9772
84	BCMA	BCMA-NM	8240-8288	9871-9919
85	BCMA	BCMA-TS	8436-8484	10067-10115
86	BCMA	BCMA-PP	8338-8386	9969-10017
87	BCMA	BCMA-RD	8387-8435	10018-10066
88	BCMA	BCMA-BB-CAR02	8142-8190	9773-9821
89	CLL1	CLL1-24C8	8828-8876	10459-10507
90	CLL1	CLL1-24C1	8779-8827	10410-10458
91	FLT3	FLT3-10E3	8975-9023	10606-10654
92	FLT3	FLT3-8B5	8926-8974	10557-10605
93	IL1RAP	IL1RAP-IAPB57	9171-9219	10802-10850
94	IL1RAP	IL1RAP-IAPB63	9220-9268	10851-10899
95	IL1RAP	hu-IL1RAP-CANO4	9269-9317	10900-10948
96	MSLN	MSLN-7D9-v3	9367-9415	10998-11046
97	MSLN	MSLN-hu22A10	9416-9464	11047-11095
98	CD19	hu-Bu13	8632-8680	10263-10311
99	BST1	hu-BST1-A1	8485-8533	10116-10164
100	BST1	hu-BST1-A2	8534-8582	10165-10212
101	BST1	hu-BST1-A3	8583-8631	10213-10262
102	Her2	Her2-XMT-1519	9122-9170	10753-10801
103	Her2	Her2-XMT-1517	9073-9121	10704-10752
104	CD133	CD133-RW03	11312-11360	11472-11520
105	CD133	CD133-W6B3H10	11361-11409	11521-11569
106	CD133	CD133-293AC1C3B9	11410-11458	11570-11618
107	IL113Ra2	hu-IL13Ra2-mAb47	14113-14165	15857-15909
108	CD22	CD22-INO	13424-13476	15168-15220
109	CD22	CD22-CELL4	13106-13158	14850-14902
110	CD22	CD22-CELL13	13212-13264	14956-15008
111	CD22	CD22-CELL7	13159-13211	14903-14955
112	CD22	CD22-VM1011	13689-13741	15433-15485
113	CD22	CD22-RAB-4120	13636-13688	15380-15432
114	CD22	CD22-Med-12C5-HL	13477-13529	15221-15273
115	CD22	CD22-Med-19A3	13583-13635	15327-15379
116	CD22	CD22-Med-16F7	13530-13582	15274-15326
117	CD22	hu-RFB4	14166-14218	15910-15962
118	BCMA	BCMA-mJ22-9	13053-13105	14797-14849

119	BCMA	BCMA-huJ22-10	12947-12999	14691-14743
120	CD22	CD22-hu-HA22-2	13371-13423	15115-15167
121	CD19	huCD19-USC3	14060-14112	15804-15856
122	CD22	BCMA-hu72	12788-12840	14532-14584
123	MPL	hu-161-3	13795-13847	15539-15591
124	BAFF-R	hu-BAFFR-USC90	13954-14006	15698-15750
125	BAFF-R	hu-BAFFR-USC55	13901-13953	15645-15697
126	BAFF-R	hu-BAFFR-MOR6654	13848-13900	15592-15644
127	CD19	CD19-hu-mROO5-1	14007-14059	15751-15803
128	CD22	CD22-h10F4v2	13265-13317	15009-15061
129	CD22	CD22-HA22	13318-13370	15062-15114
130	MPL	hu-161-2	13742-13794	15486-15538
131	MSLN	MSLN-hu22A10	14325-14377	16069-16021
132	MSLN	MSLN-7D9-HL	14272-14324	16016-16068
133	MSLN	MSLN-5	14219-14271	15963-16015
134	BCMA	BCMA-huC13-F12	12894-12946	14638-14690
135	BCMA	BCMA-huC12A3-L3H3	12841-12893	14585-14637
136	BCMA	BCMA-J6M0	13000-13052	14744-14796

[00141] Table 8. Exemplary zSIR, SIR and miscellaneous constructs

SEQ ID (DNA)	SEQ ID (PRT)	NAME
3955	7955	CD8SP-hCD19-EUK5-13-vL-IgCL-Bam-CD3zECDTMCP-opt-F-P2A-SP-Bst-hCD19-EUK5-13-vH-IgG1-CH1-KPN-CD3zECDTMCP-opt2-F-F2A-PAC
3956	7956	CD8-hCD19-EUK5-13-vL-IgCL-Xho-CD3zECDTMCP-opt-F-P2A-Spe-SP-Bst-hCD19-EUK5-13-vH-IgG1-CH1-Mlu-CD3zECDTMCP-opt2-F-F2A-PAC
3957	7957	CD8SP-hCD19-EUK5-13-vL-Xho-CD3zECDTMCP-opt-F-P2A-Spe-SP-hCD19-EUK5-13-vH-Mlu-CD3zECDTMCP-opt2-F-F2A-PAC
3958	7958	
3959	7959	CD8SP-FMC63-vL-Xho-CD3zECDTMCP-opt-F-P2A-Spe-SP-FMC63-vH-Mlu-CD3zECDTMCP-opt2-F-F2A-PAC
3960	7960	hCD19-Bu12-Xho-CD3zECDTMCP-opt-F-P2A-Pac
3961	7961	CD8SP-CD19Bu12-scFv-Xho-CD3zECDTMCP-opt-F-P2A-SP-CD19MM-scFv-Mlu-CD3zECDTMCP-opt2-F-F2A-PAC
3962	7962	CD8SP-CD19Bu12-scFv-Xho-CD3zECDTMCP-opt-F-P2A-SP-CD123-DART2-scFv-Mlu-CD3zECDTMCP-opt2-F-F2A-PAC
3963	7963	CD8SP-CD19Bu12-scFv-Xho-CD3zECDTMCP-opt-F-P2A-SP-CD20-2F2-scFv-Mlu-CD3zECDTMCP-opt2-F-F2A-PAC
3964	7964	CD8SP-CD19Bu12-scFv-Xho-CD3zECDTMCP-opt-F-P2A-SP-AFP-61-scFv-Mlu-CD3zECDTMCP-opt2-F-F2A-PAC
3965	7965	CD8SP-CD19Bu12-scFv-Xho-CD3zECDTMCP-opt-F-P2A-SP-CD22-h10F4v2-scFv-Mlu-CD3zECDTMCP-opt2-F-F2A-PAC
3966	7966	CD8SP-CD19Bu12-scFv-Xho-CD3zECDTMCP-opt-F-P2A-SP-hSC22-10-HL-scFv-Mlu-CD3zECDTMCP-opt2-F-F2A-PAC

SEQ ID (DNA)	SEQ ID (PRT)	NAME
3967	7967	CD8SP-CD19Bu12-scFv-Xho-CD3zECDTMCP-opt-F-P2A-SP-CD123-DART1-scFv-Mlu-CD3zECDTMCP-opt2-F-F2A-PAC
3968	7968	CD8SP-CD19Bu12-scFv-Xho-CD3zECDTMCP-opt-F-P2A-SP-WT1-Ab5-scFv-Mlu-CD3zECDTMCP-opt2-F-F2A-PAC
3969	7969	CD8SP-CD19-USC2-vL-[hTCRb-KACIAH]-F-P2A-SP-CD19-USC2-vH-[hTCRa-CSDVP]-F-F2A-PAC
3971	7971	CD8SP-BCMA-Am06-HL-vL-[CD3zECDTM-28z-opt]-F-P2A-SP-BCMA-Am06-HL-vH-[CD3zECDTM-28z-opt2]
3972	7972	CD8SP-BCMA-Am06-HL-vL-[CD3zECDTM-BBz-opt]-F-P2A-SP-BCMA-Am06-HL-vH-[CD3zECDTM-BBz-opt2]
16311	16335	CD8SP-FMC63-BBz
16312	16336	CD8SP-MSLN-hu22A10-BBz
16313	16337	CD8SP-MSLN-7D9-HL-BBz
16314	16338	CD8SP-MSLN-5-HL-BBz
16315	16339	CD8SP-MPL-hu-161-2-BBz
16316	16340	CD8SP-BCMA-huC13-F12-BBz
16317	16341	CD8SP-huCD19-mROO5-1-BBz
16318	16342	CD8SP-huCD19-mROO5-1-vL-[hTCRb-KACIAH]-F-P2A-SP-huCD19-mROO5-1-vH-[hTCRa-CSDVP]-F-F2A-K13-opt
16319	16343	CD8SP-CD22-INO-vL-[hTCRb-S57C]-F-P2A-SP-CD22-INO-vH-[hTCRa-T48C]-F-F2A-PAC
16320	16344	CD8SP-CD22-hu-HA22-2-vL-[hTCRa-T48C]-F-P2A-SP-CD22-hu-HA22-2-vH-[hTCRa-S57C]-F-F2A-Pac
16321	16345	CD8SP-CD22-Med-12C5-HL-vH-[hTCRb-S57C]-F-P2A-SP-CD22-Med-12C5-HL-vL-[hTCRa-T48C]-F-F2A-PAC
16322	16346	CD8SP-hu-RFB4-vL-[hTCRb-S57C]-F-P2A-SP-hu-RFB4-vH-[hTCRa-T48C]-F-F2A-PAC
16323	16347	CD8SP-CD22-CELL7-vH-[hTCRb-S57C]-F-P2A-SP-CD22-CELL7-vL-[hTCRa-T48C]-F-F2A-PAC
16324	16348	CD8SP-CD22-HA22-vL-[hTCRb-S57C]-F-P2A-SP-CD22-HA22-vH-[hTCRa-T48C]-F-F2A-PAC
16325	16349	CD8SP-MSLN-7D9-HL-vH-[hTCRa-T48C]-F-P2A-SP-MSLN-7D9-HL-vL-[hTCRa-S57C]
16326	16350	CD8SP-MSLN-7D9-HL-vH-[hTCRb-S57C]-F-P2A-SP-MSLN-7D9-HL-vL-[hTCRa-T48C]
16328	16351	CD8SP-huCD19-mROO5-1-(vL-vH)-CD3c-ECDTMCP-opt2-T2A-PAC
16329	16352	CD8SP-huCD19-mROO5-1-(vL-vH)-CD3d-ECDTMCP-opt2-T2A-PAC
16330	16353	CD8SP-huCD19-mROO5-1-vL-[hTCRb-KACIAH]-F-P2A-SP-huCD19-mROO5-1-vH-[hTCRa-CSDVP]
16331	16354	CD8SP-MSLN-hu22A10-vL-[hTCRb-KACIAH]-F-P2A-SP-MSLN-hu22A10-vH-[hTCRa-CSDVP]-F-F2A-K13-Opt
16332	16355	CD8SP-MSLN-hu22A10-vL-[hTCRb-KACIAH]-F-P2A-SP-MSLN-hu22A10-vH-[hTCRa-CSDVP]

SEQ ID (DNA)	SEQ ID (PRT)	NAME
16333	16356	CD8SP-MSLN-7D9-HL-vH-[hTCRb-KACIAH]-F-P2A-SP-MSLN-7D9-HL-vL-[hTCRa-CSDVP]-F-F2A-K13-opt
16334	16357	CD8SP-MSLN-7D9-HL-vH-[hTCRb-KACIAH]-F-P2A-SP-MSLN-7D9-HL-vL-[hTCRa-CSDVP]
16361	16358	CD8SP-MSLN-5-HL-vH-[hTCRa-CSDVP]-F-F2A-SP-MSLN-5-HL-vL-[hTCRb-KACIAH]
16362	16359	CD8SP-MSLN-7D9-HL-vH-[hTCRa-CSDVP]-F-F2A-SP-MSLN-7D9-HL-vL-[hTCRb-KACIAH]
16363	16360	CD8SP-MSLN-hu22A10-vL-[hTCRa-CSDVP]-F-F2A-SP-MSLN-hu22A10-vH-[hTCRb-KACIAH]

[00142] Table 9: Exemplary Vif constructs

SEQ ID (DNA)	SEQ ID (PRT)	NAME
11243	11270	HIV1-Vif
11244	11271	CD8SP-FMC63-(vL-vH)-Myc-BBz-T2A-PAC
11245	11272	CD8SP-FMC63-(vL-vH)-Myc-BBz-F-P2A-Vif-F-P2A-PAC
11246	11273	CD8SP-hu-CD19-USC1-LH4-vL-V5-[hTCRb-KACIAH]-F-P2A-SP-hu-CD19-USC1-LH4-vH-Myc-[hTCRa-CSDVP]-F-F2A-Vif
11247	11274	CD8SP-hu-CD19-USC1-LH4-vL-V5-[hTCRb-KACIAH]-F-P2A-SP-hu-CD19-USC1-LH4-vH-Myc-[preTCRa-Del48]-F-F2A-Vif
11248	11275	CD8SP-V5-[hTCRb-KACIAH]-F-P2A-CD8SP-hu-CD19-USC1-LH4-vL-Gly-Ser-Linker-hu-CD19-USC1-LH4-vH-Myc-[hTCRa-CSDVP]-F-F2A-Vif
11249	11276	CD8SP-hu-CD19-USC1-LH4-vL-V5-[hTCRg1-opt]-F-P2A-SP-hu-CD19-USC1-LH4-vH-Myc-[hTCRd-opt]-F-F2A-Vif
11250	11277	CD8SP-V5-[hTCRg1-opt]-F-P2A-CD8SP-hu-CD19-USC1-LH4-vL-Gly-Ser-Linker-hu-CD19-USC1-LH4-vH-Myc-[hTCRd-opt]-F-F2A-Vif
11251	11278	CD8SP-hu-CD19-USC1-LH4-vL-Gly-Ser-Linker-hu-CD19-USC1-LH4-vH-Myc-CD8TM-z-P2A-K13-FLAG-T2A-Vif
11252	11279	CD8SP-hu-CD19-USC1-LH4-vL-[hTCRa-CSDVP]-F-F2A-SP-hu-CD19-USC1-LH4-vH-[hTCRb-KACIAH]-F-P2A-Vif
11253	11280	CD8-hu-CD19-USC1-LH4-vL-IgCL-Bam-CD3zECDTMCP-opt-F-P2A-Spe-SP-Bst-hu-CD19-USC1-LH4-vH-IgG1-CH1-KPN-CD3zECDTMCP-opt2-F-F2A-Xba-Vif
11254	11281	CD8-hu-CD19-USC1-LH4-vL-IgCL-Xho-CD3zECDTMCP-opt-F-P2A-Spe-SP-Bst-hu-CD19-USC1-LH4-vH-IgG1-CH1-Mlu-CD3zECDTMCP-opt2-F-F2A-Vif
11255	11282	CD8SP-hu-CD19-USC1-LH4-(vL-vH)-Myc-z-P2A-hNEMO-K277A-Flag-T2A-Vif
11256	11283	CD8SP-hu-CD19-USC1-LH4-(vL-vH)-CD3e-ECDTMCP-opt2-P2A-hNEMO-K277A-Flag-T2A-Vif
11257	11284	CD8SP-hu-CD19-USC1-LH4-(vL-vH)-CD3d-ECDTMCP-opt2-P2A-hNEMO-K277A-Flag-T2A-Vif

SEQ ID (DNA)	SEQ ID (PRT)	NAME
11258	11285	CD8SP-hu-CD19-USC1-LH4-(vL-vH)-CD3g-ECDTMCP-opt2-P2A-hNEMO-K277A-Flag-T2A-Vif
11259	11286	CD8SP-hu-CD19-USC1-LH4-(vL-vH)-CD3z-ECDTMCP-opt2-P2A-hNEMO-K277A-Flag-T2A-Vif
11260	11287	CD8SP-hu-CD19-USC1-LH4-vL-[IgCL-TCRg-6MD]-F-P2A-SP-hu-CD19-USC1-LH4-vH-[IgG1-CH1-TCRd-6MD]-F-F2A-Vif
11261	11288	CD8SP-hu-CD19-USC1-LH4-vL-[IgCL-TCRb-IAH-6MD]-F-P2A-SP-hu-CD19-USC1-LH4-vH-[IgG1-CH1-TCRa-SDVP-6MD]-F-F2A-Vif
11262	11289	CD8SP-hu-CD19-USC1-LH4-(vH-vL)-CD3e-ECDTMCP-opt2-P2A-Vif
11263	11290	CD8SP-hu-CD19-USC1-LH4-(vH-vL)-CD3d-ECDTMCP-opt2-P2A-hNEMO-K277A-Flag-T2A-Vif
11264	11291	CD8SP-hu-CD19-USC1-LH4-(vH-vL)-CD3g-ECDTMCP-opt2-P2A-hNEMO-K277A-Flag-T2A-Vif
11265	11292	CD8SP-hu-CD19-USC1-LH4-(vH-vL)-CD3z-ECDTMCP-opt2-P2A-hNEMO-K277A-Flag-T2A-Vif
11266	11293	CD8SP-hu-CD19-USC1-LH4-vL-Xho-CD3zECDTMCP-opt-F-P2A-Spe-SP-hu-CD19-USC1-LH4-vH-Mlu-CD3zECDTMCP-opt2-F-F2A-Vif
11267	11294	CD8SP-hu-CD19-USC1-LH4-vH-Gly-Ser-Linker-vL-Myc-CD8TM-BBz-2A-Vif
11268		pLenti-EF1a-CD8SP-hu-CD19-USC1-LH4-vH-Gly-Ser-Linker-vL-Myc-CD8TM-BBz-2A-Vif
11269		pCDNA3-Vif

[00143] Table 10. Exemplary Bispecific Antibodies targeting different antigens

Antigen (Ag)	Antigen Binding Domain	Ag x CD3		Ag x CD28		Ag x 41BB	
		SEQ ID NO (DNA)	SEQ ID NO (PRT)	SEQ ID NO (DNA)	SEQ ID NO (PRT)	SEQ ID NO (DNA)	SEQ ID NO (PRT)
CD19	FMC63	11620	11790	11676	11846	11732	11902
CD19	huFMC63-11	11621	11791	11677	11847	11733	11903
CD19	huFMC63-11-N203Q	11622	11792	11678	11848	11734	11904
CD19	CD19Bu12	11623	11793	11679	11849	11735	11905
CD19	CD19MM	11624	11794	11680	11850	11736	11906
CD19	Ritx-CD19-MOR0028	11625	11795	11681	11851	11737	11907
CD19	CD19-hu-mROO5-1	11626	11796	11682	11852	11738	11908
BCMA	BCMA-J6M0	11627	11797	11683	11853	11739	11909
BCMA	BCMA-huC12A3-L3H3	11628	11798	11684	11854	11740	11910

		Ag x CD3			Ag x CD28			Ag x 41BB	
BCMA	BCMA-huC11.D5.3L1 H3	11629	11799		11685	11855		11741	11911
BCMA	BCMA-huC13-F12	11630	11800		11686	11856		11742	11912
CD20	CD20-2F2	11631	11801		11687	11857		11743	11913
CD20	CD20-GA101	11632	11802		11688	11858		11744	11914
CD20	CD20-2H7	11633	11803		11689	11859		11745	11915
CD20	CD20-Ubli-v4	11634	11804		11690	11860		11746	11916
CD20	CD20-2H7	11635	11805		11691	11861		11747	11917
CD20	CD20-7D8	11636	11806		11692	11862		11748	11918
CD22	CD22-h10F4v2	11637	11807		11693	11863		11749	11919
CD22	CD22-H22Rhov2A	11638	11808		11694	11864		11750	11920
CD22	CD22-m971-HL	11639	11809		11695	11865		11751	11921
CD22	CD22-5-HL	11640	11810		11696	11866		11752	11922
CD22	CD22-10-HL	11641	11811		11697	11867		11753	11923
CD22	CD22-HA22	11642	11812		11698	11868		11754	11924
CD30	CD30-5F11	11643	11813		11699	11869		11755	11925
CD30	CD30-Ac10	11644	11814		11700	11870		11756	11926
CD32	CD32-Med9	11645	11815		11701	11871		11757	11927
CD33	CD33-AF5	11646	11816		11702	11872		11758	11928
CD33	CD33-huMyc9	11647	11817		11703	11873		11759	11929
CD33	CD33-Him3-4	11648	11818		11704	11874		11760	11930
CD33	CD33-SGNh2H12	11649	11819		11705	11875		11761	11931
CD33	CD33-15G15-33	11650	11820		11706	11876		11762	11932
CD33	CD33-33H4	11651	11821		11707	11877		11763	11933
CD123	CD123-CSL362	11652	11822		11708	11878		11764	11934
CD123	CD123-1172	11653	11823		11709	11879		11765	11935
CD123	CD123-DART-1	11654	11824		11710	11880		11766	11936
CD123	CD123-DART-2	11655	11825		11711	11881		11767	11937
CD123	CD123-9D7	11656	11826		11712	11882		11768	11938
CD123	CD123-3B10	11657	11827		11713	11883		11769	11939
CD138	CD138	11658	11828		11714	11884		11770	11940
CS1	CS1-HuLuc64	11659	11829		11715	11885		11771	11941
CS1	CS1-huLuc90	11660	11830		11716	11886		11772	11942
FLT3	FLT3-NC7	11661	11831		11717	11887		11773	11943

		Ag x CD3			Ag x CD28			Ag x 41BB	
MPL	MPL-175	11662	11832		11718	11888		11774	11944
MPL	MPL-161	11663	11833		11719	11889		11775	11945
MPL	MPL-111	11664	11834		11720	11890		11776	11946
MPL	Hu-161-2	11665	11835		11721	11891		11777	11947
MPL	MPL-hu-175-2	11666	11836		11722	11892		11778	11948
MPL	MPL-hu-111-2	11667	11837		11723	11893		11779	11949
Lym1	Lym1	11668	11838		11724	11894		11780	11950
Lym2	Lym2	11669	11839		11725	11895		11781	11951
CD70	CD70-h1F6	11670	11840		11726	11896		11782	11952
CD79b	CD79b-2F2	11671	11841		11727	11897		11783	11953
CD179b	CD179b	11672	11842		11728	11898		11784	11954
GPRC5D	GPRC5D-ET150-5	11673	11843		11729	11899		11785	11955
GPRC5D	GPRC5D-ET150-18	11674	11844		11730	11900		11786	11956

[00144] Table 11:

CAR "X" TARGET	EXEMPLARY DISEASE TARGETED BY CARs (<i>i.e.</i> conventional CARs and next generation CARs. <i>E.g.</i> , SIR, Ab-TCR, TFP and zSIR)
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 macroglobulinemia
CD5	Blood cancer, T cell leukemia, T cell lymphoma
BAFF-R	Blood cancer, chronic lymphocytic leukemia, B-ALL
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
CD34	Blood cancers, AML, MDS
CD44v6	Blood cancers, AML, MDS
CD70	Blood cancers, lymphoma, myeloma, waldenstrom's macroglobulinemia
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

CAR “X” TARGET	EXEMPLARY DISEASE TARGETED BY CARs (<i>i.e.</i> conventional CARs and next generation CARs. <i>E.g.</i>, SIR, Ab-TCR, TFP and zSIR)
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
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

CAR “X” TARGET	EXEMPLARY DISEASE TARGETED BY CARs (<i>i.e.</i> conventional CARs and next generation CARs. <i>E.g.</i>, SIR, Ab-TCR, TFP and zSIR)
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/HAVER1	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
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

CAR “X” TARGET	EXEMPLARY DISEASE TARGETED BY CARs (<i>i.e.</i> conventional CARs and next generation CARs. <i>E.g.</i>, SIR, Ab-TCR, TFP and zSIR)
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	HIV1/AIDS and related conditions
HIV1-gag	HIV1/AIDS and related conditions
Influenza A HA	Influenza A infection
Integrin B7	Plasma cell neoplasms, primary effusion lymphoma
Muc17	Pancreatic cancer, colon cancer
CD16ORF54	Blood cancers
VISTA	Blood cancers
Muc5Ac	Pancreatic cancer, stomach cancer, colon cancer
FCRH5	Plasma cell neoplasm
LYPD1	Ovarian cancer, endometrial cancer, melanoma
EMR2	Acute Leukemia, Lymphoma, breast cancer, colon cancer
gpNMB	Melanoma, brain cancer, breast cancer, solid tumors

CAR "X" TARGET	EXEMPLARY DISEASE TARGETED BY CARs (<i>i.e.</i> conventional CARs and next generation CARs. <i>E.g.</i>, SIR, Ab-TCR, TFP and zSIR)
RNF43	Colorectal cancer, breast cancer, endometrial cancer
CD44v6	Epithelial cancers
Robo4	Renal, colon, breast cancer, solid tumors
GPC3	Liver cancer, lung cancer, breast cancer
FOLR1	Ovarian cancer, lung cancer, kidney cancer, solid tumors
CLDN6	Ovarian cancer, liver cancer
MMP16	Melanoma, brain cancer, small lung cancer, neuroblastoma
BMPR1B	Prostat cancer, breast cancer, ovarian cancer
Ly6E	Breast, ovarian, pancreatic, lung
WISP1	Glioblastoma, breast cancer
SLC34A2	Lung cancer, ovarian cancer, endometrial cancer
CD133	Lung cancer, brain cancer

[00145] Table 12:

Antigen (Ag)	Antigen Binding Domain	Ag x CD3		Ag x CD28		Ag x 41BB	
		SEQ ID NO (DNA)	SEQ ID NO (PRT)	SEQ ID NO (DNA)	SEQ ID NO (PRT)	SEQ ID NO (DNA)	SEQ ID NO (PRT)
CD19	FMC63	11620	11790	11676	11846	11732	11902
CD19	huFMC63-11	11621	11791	11677	11847	11733	11903
CD19	huFMC63-11-N203Q	11622	11792	11678	11848	11734	11904
CD19	CD19Bu12	11623	11793	11679	11849	11735	11905
CD19	CD19MM	11624	11794	11680	11850	11736	11906
CD19	Ritx-CD19-MOR0028	11625	11795	11681	11851	11737	11907
CD19	CD19-hu-mROO5-1	11626	11796	11682	11852	11738	11908
BCMA	BCMA-J6M0	11627	11797	11683	11853	11739	11909
BCMA	BCMA-huC12A3-L3H3	11628	11798	11684	11854	11740	11910
BCMA	BCMA-huC11.D5.3L1H3	11629	11799	11685	11855	11741	11911
BCMA	BCMA-huC13-F12	11630	11800	11686	11856	11742	11912
CD20	CD20-2F2	11631	11801	11687	11857	11743	11913
CD20	CD20-GA101	11632	11802	11688	11858	11744	11914
CD20	CD20-2H7	11633	11803	11689	11859	11745	11915
CD20	CD20-Ubli-v4	11634	11804	11690	11860	11746	11916
CD20	CD20-2H7	11635	11805	11691	11861	11747	11917
CD20	CD20-7D8	11636	11806	11692	11862	11748	11918
CD22	CD22-h10F4v2	11637	11807	11693	11863	11749	11919

CD22	CD22-H22Rhov2A	11638	11808		11694	11864		11750	11920
CD22	CD22-m971-HL	11639	11809		11695	11865		11751	11921
CD22	CD22-5-HL	11640	11810		11696	11866		11752	11922
CD22	CD22-10-HL	11641	11811		11697	11867		11753	11923
CD22	CD22-HA22	11642	11812		11698	11868		11754	11924
CD30	CD30-5F11	11643	11813		11699	11869		11755	11925
CD30	CD30-Ac10	11644	11814		11700	11870		11756	11926
CD32	CD32-Med9	11645	11815		11701	11871		11757	11927
CD33	CD33-AF5	11646	11816		11702	11872		11758	11928
CD33	CD33-huMyc9	11647	11817		11703	11873		11759	11929
CD33	CD33-Him3-4	11648	11818		11704	11874		11760	11930
CD33	CD33-SGNh2H12	11649	11819		11705	11875		11761	11931
CD33	CD33-15G15-33	11650	11820		11706	11876		11762	11932
CD33	CD33-33H4	11651	11821		11707	11877		11763	11933
CD123	CD123-CSL362	11652	11822		11708	11878		11764	11934
CD123	CD123-1172	11653	11823		11709	11879		11765	11935
CD123	CD123-DART-1	11654	11824		11710	11880		11766	11936
CD123	CD123-DART-2	11655	11825		11711	11881		11767	11937
CD123	CD123-9D7	11656	11826		11712	11882		11768	11938
CD123	CD123-3B10	11657	11827		11713	11883		11769	11939
CD138	CD138	11658	11828		11714	11884		11770	11940
CS1	CS1-HuLuc64	11659	11829		11715	11885		11771	11941
CS1	CS1-huLuc90	11660	11830		11716	11886		11772	11942
FLT3	FLT3-NC7	11661	11831		11717	11887		11773	11943
MPL	MPL-175	11662	11832		11718	11888		11774	11944
MPL	MPL-161	11663	11833		11719	11889		11775	11945
MPL	MPL-111	11664	11834		11720	11890		11776	11946
MPL	Hu-161-2	11665	11835		11721	11891		11777	11947
MPL	MPL-hu-175-2	11666	11836		11722	11892		11778	11948
MPL	MPL-hu-111-2	11667	11837		11723	11893		11779	11949
Lym1	Lym1	11668	11838		11724	11894		11780	11950
Lym2	Lym2	11669	11839		11725	11895		11781	11951
CD70	CD70-h1F6	11670	11840		11726	11896		11782	11952
CD79b	CD79b-2F2	11671	11841		11727	11897		11783	11953
CD179b	CD179b	11672	11842		11728	11898		11784	11954
GPRC5D	GPRC5D-ET150-5	11673	11843		11729	11899		11785	11955
GPRC5D	GPRC5D-ET150-18	11674	11844		11730	11900		11786	11956

[00146] **Table 13:** TCR chains useful in various embodiments:

SEQ ID NO (PRT)	TCR CHAIN
4038	hTCR-alpha-constant_X02883.1
4039	hTCRa-WT
4040	hTCRa-CSDVP
4041	hTCRa-opt2
4042	hTCRa-T48C-opt
4043	hTCRa-T48C-opt1
4044	hTCRa-SDVP
4045	hTCRa-S61R
4046	hTCRa-SDVPR
4047	hTCRaECD-CD3zECDTMCP-opt2
4048	hTCR-b1-constant-region_X00437.1
4049	hTCR-b2-constant
4050	hTCRb-WT
4051	hTCRb-S57C-opt1
4052	hTCRb-KACIAH
4053	hTCRb-opt2
4054	hTCRb-KAIAH
4055	hTCRb-R79G
4056	hTCRbECD-CD3zECDTMCP-opt
4057	preTCRa_gb_U38996.1
4058	preTCRa
4059	preTCRa-del48
4060	hTCR-gamma_M27331.1
4061	hTCR-Gamma-Opt
4062	hTCR-Delta
4063	hTCR-Delta-Opt
12602	TCRa transmembrane domain
12603	TCRb transmembrane domain
12604	TCRd transmembrane domain
12605	TCRg transmembrane domain
12606	TCRa connecting peptide
12607	TCRb connecting peptide
12608	TCRd connecting peptide
12609	TCRy connecting peptide
12610	TCRa connecting peptide MD
12611	TCRb connecting peptide MD
12612	TCRd connecting peptide MD
12613	TCRy connecting peptide MD
12614	TCRb intracellular domain
12615	TCRy intracellular domain
12616	TCRD alpha
12617	TCRD beta

12618	TCRD alpha MD
12619	TCRD beta MD
12620	TCRD delta
12621	TCRD gamma
12622	TCRD delta MD
12623	TCRD gamma MD
12624	CD3e-ECDTMCP
12625	CD3e-ECD
12626	CD3e-TM
12627	CD3e-CP
12628	CD3d-ECDTMCP
12629	CD3d-ECD
12630	CD3d-TM
12631	CD3d-CP
12632	CD3g-ECDTMCP
12633	CD3g-ECD
12634	CD3g-TM
12635	CD3g-CP
12636	CD3zECDTMCP
12637	CD3z-TM
12638	CD3z-CP

[00147] In some embodiments, the compositions comprise nucleic acids encoding CARs 1-15 (**Table 1**), wherein the antigen specific domain of the CAR targets one or more specific antigens as described in **Tables 3** 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 1-60 (**Table 2**) where the antigen specific domain of the encoded CAR targets one or more specific antigens as described herein and in **Table 3** 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 and in **Table 3** or **Tables 5-6** in PCT/US2017/064379. In some embodiments, the compositions comprise nucleic acids encoding backbone-8, wherein the antigen specific domain of the CAR in backbone-1 targets one or more cancer specific antigens as described herein and in **Table 3** or **Tables 5-6** in PCT/US2017/064379.

[00148] In various embodiments, the isolated nucleic acid molecules encoding the CAR components of the backbones described herein, encode one, two, three or more antigen specific domains (ASD).

[00149] In various embodiments, the isolated nucleic acid molecules encoding the CAR components of the backbones described herein, encodes zero, one, two, three or more co-stimulatory domains.

[00150] In various embodiments, the isolated nucleic acid molecules encoding the CAR components of the backbones described herein, encode zero, one, two, three or more intracellular signaling domain.

[00151] In various embodiments, the isolated nucleic acid molecules encoding the CAR and the backbones described herein, encode zero, one, two, three or more accessory modules.

[00152] The nucleic acid sequences encoding for the desired components of the CARs and accessory modules described herein can be obtained using recombinant methods known in the art. Alternatively, the nucleic acid of interest can be produced synthetically, rather than cloned.

[00153] In some embodiments, the genetically modified cells described herein that express the CARs and accessory components described herein also express agents that reduce toxicity of CARs.

[00154] In some embodiments, the genetically modified cells described herein that express the CARs and accessory components described herein also express agents that enhance the activity of CARs.

[00155] In some embodiments, the genetically modified cells described herein that express the CARs and accessory components described herein also express agents that enhance the persistence of CARs.

[00156] In some embodiments, the genetically modified cells described herein that express the CARs and accessory components described herein also express agents that prevent the exhaustion of CARs.

[00157] The compositions comprising various backbones as described herein comprise CARs which comprise one or more ASD that binds specifically to a cancer associated antigen as described herein. 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.

[00158] The polynucleotides, 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, PCT/US2016/058305, WO 2015/117229 A1 and PCT/US17/64379, which are incorporated herein by reference in their entirety.

[00159] The disclosure provides several antigen binding domains that can be used in the generation of CARs (*e.g.*, CAR 1-15 and backbones 1-60) for applications in adoptive cellular therapy. In some embodiments, these antigen binding domains are derived from antibodies and target antigens that are expressed in cancer, non-cancer proliferative disorders (*e.g.*, endometrioses) and/or immune disorders. The target antigens, SEQ IDs (DNA) and SEQ IDs (PRT) of vL, vH and scFv fragments of these antigen binding domains are shown in **Table 3**. The CDRs of the vL and vH fragments of the antigen binding domains targeting different antigens are shown in **Table 4**.

[00160] In some embodiments, the encoded antigen binding domain(s) of the CARs polypeptide targeting a specific antigen comprise any one or more of light chain variable domain (vL or VL) amino acid sequences of SEQ ID NO 4118 to 4190, 9631 to 9660 and 11460 to 11462, 14386 to 14415 targeting that antigen as listed in **Table 3** wherein up to 9 amino acid residues but no more than 10 amino acids are replaced by any other amino acid residues, or sequences with 80-100% identity to amino acid sequences as set forth in any one of SEQ ID NO 4118 to 4190, 9631 to 9660, or 11460 to 11462 and 14386 to 14415, or sequences with 85-100% identity to the complementarity determining regions (CDR's) of any one of SEQ ID NO 4118 to 4190, 9631 to 9660, or 11460 to 11462 and 14386 to 14415. The CDR1, CDR2 and CDR3 of vL fragments with the SEQ ID NO: 4118 to 4190, 9631 to 9660, or 11460 to 11462 are represented by SEQ ID NOs: 11961 to 12066, 12068 to 12173, 12175 to 12280, respectively (**Table 4**).

[00161] In some embodiments, the encoded one or more antigen binding domains of the CARs (conventional CARs and next generation CARs, *e.g.*, SIRs, zSIRs, Ab-TCRs, Tri-Tac and TFP) polypeptide comprise any one or more of heavy chain variable domain (vH or VH) amino acid sequences of SEQ ID NO 4192 to 4264, 9662 to 9691, 11464 to 11466 and 14417 to 14446 targeting that antigen as listed in **Table 3** wherein up to 9 amino acid residues but no more than 10 amino acids are replaced by any other amino acid residues or sequences with 80-100% identity to amino acid sequences of SEQ ID NO 4192 to 4264, 9662 to 9691, 11464 to 11466, and 14417 to 14446 or sequences with 85-100% identity to the complementarity determining regions (CDR's) of any one of SEQ ID NO 4192 to 4264, 9662 to 9691, 11464 to 11466, and 14417 to 14446. The CDR1, CDR2 and CDR3 of vH fragments with the SEQ ID NO: 4192 to 4264, 9662 to 9691, 11464 to 11466, and 14417 to 14446 are represented by SEQ ID NOs: 12282 to 12387, 12389 to 12494, 12497 to 12602, 16219-16310 respectively (**Table 4**).

[00162] In some embodiments, the encoded one or more antigen binding domains of the CARs 1-15 and backbones 1-60 polypeptide comprise any one or more of single chain variable fragments (scFv) amino acid sequences of SEQ ID NO 4266 to 4338, 9693 to 9722, 11468 to 11470, and 14448-14477 wherein up to 9 amino acid residues but no more than 10 amino acids are replaced by any other amino acid residues or sequences with 80-100% identity to amino acid sequences of SEQ ID NO 4266 to 4338, 9693 to 9722, 11468 to 11470, and 14448-14477 or sequences with 85-100% identity to the complementarity determining regions (CDR's) of SEQ ID NO 4266 to 4338, 9693 to 9722, 11468 to 11470 and 14448-14477. The CDR1, CDR2 and CDR3 of the vL regions of the scFv fragments with the SEQ ID NO: 4266 to 4338, 9693 to 9722, 11468 to 11470, and and 14448-14477 are represented by SEQ ID NOs: 11961 to 12066, 12068 to 12173, 12175 to 12280, 16126-16217 respectively (**Table 4**). The CDR1, CDR2 and CDR3 of the vH regions of the scFv fragments with the SEQ ID NO: 4266 to 4338, 9693 to 9722, 11468 to 11470, and 14448-14477 are represented by SEQ ID NOs: 12282 to 12387, 12389 to 12494 and 12497 to 12602 and 16219-16310 respectively (**Table 4**).

[00163] It is to be understood that the order of vL and vH fragments in a scFv fragment can be either vL-vH or vH-vL. Thus, even though the exemplary scFv fragments shown in **Table 3** represent either vL-vH or vH-vL orientation, the scFv fragments with the complementary orientation (*i.e.*, vH-vL and vL-vH) can be also used in the methods or compositions of the disclosure.

[00164] The DNA and PRT SEQ IDs of exemplary elements that can be used in the construction of different CARs 1-15 and backbones 1-60 are listed in **Table 5**. The nucleic acid and amino acid SEQ IDs of exemplary conventional CARs (*e.g.*, 2nd generation CARs containing 41BB costimulatory domains), and next generation CARs (*e.g.*, SIRs, zSIRs, Ab-TCRs and TFP) based on the vL and vH fragments derived from BCMA-AM06-HL scFv are provided in **Tables 6**. The nucleic acid and amino acid SEQ IDs of exemplary conventional CARs (*e.g.*, 2nd generation CARs containing 41BB costimulatory domains), and next generation CARs (*e.g.*, SIRs, zSIRs, Ab-TCRs and TFP) based on the vL and vH fragments derived from other scFv fragments can be derived by replacing the vL and vH fragments of BCMA-AM06-HL scFv with the vL and vH fragments of scFv fragments listed in **Table 3**. The sequence of exemplary CAR constructs containing different antigen binding domains are referenced in **Table 7**. The order of the different CAR constructs in **Table 7** is as shown in **Table 6** for BCMA-Am06-HL based CARs. Thus, the CAR construct represented by SEQ ID NO: 475 resembles the CAR construct represented by SEQ ID NO: 377 with the

exception that the vL and vH fragments corresponding to the antigen binding domain BCMA-Am06-HL are replaced with the vL and vH fragments corresponding to the antigen binding domain BCMA-Am14-HL. Similarly, the CAR construct represented by SEQ ID NO: 476 resembles the CAR construct represented by SEQ ID NO: 378 with the exception that the vL and vH fragments corresponding to the antigen binding domain BCMA-Am06-HL are replaced with the vL and vH fragments corresponding to the antigen binding domain BCMA-Am14-HL.

[00165] In various embodiments, the antigen binding domains of the disclosure show superior *in vitro* and *in vivo* properties, such as binding affinity to the target antigens, cytokine secretion, proliferation, cytotoxicity, exhaustion, and long term persistence, when used in the construction of CARs (*i.e.* conventional CARs and next generation CARs). In various embodiments, 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, when used in the construction of CARs (*i.e.* conventional CARs and next generation CARs). In various embodiments, the CARs containing these target antigens can be used to generate a diverse immune response.

[00166] The disclosure further contemplates CARs that target the same antigen but with different antigen binding domains and may possess diverse biological properties depending in part on the epitope of the antigen targeted by them. Thus, the two groups of Her2 targeting CARs represented by SEQ ID NOs: 2435-2483 and SEQ ID NOs: 2386-2434, see rows 12-13 of **Table 7**, show different biological properties, such as T cell activation, cytokine secretion and cytotoxicity.

[00167] In some embodiments, the antigen specific domain of the encoded 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 CAR is a scFv antibody fragment that is humanized compared to the murine sequence of the scFv from which it is derived.

[00168] 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. The linker length can greatly affect how the variable regions of a scFv fold and interact. For example, if a short polypeptide linker is employed (for example, between 5-10 amino acids) intrachain

folding is prevented. Interchain folding is may be useful to bring the two variable regions together to form a functional epitope binding site. For examples of linker orientation and size see, *e.g.*, Hollinger *et al.* 1993 Proc Natl Acad. Sci. U.S.A. 90:6444-6448, U.S. Patent Application Publication Nos. 2005/0100543, 2005/0175606, 2007/0014794, and PCT publication Nos. WO2006/020258 and WO2007/024715, the disclosure of which are incorporated herein by reference.

[00169] 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. The linker sequence may comprise any naturally occurring amino acid. 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 such as (Gly4Ser)_n, where n is a positive integer equal to or greater than 1. In one embodiment, the linker can be (Gly4Ser)₃ or (Gly4Ser)₃ or Whitlow linker. Variation in the linker length may retain or enhance activity, giving rise to superior efficacy in activity studies.

[00170] In one embodiment, the antigen specific domain of a CAR targeting a specific antigen comprises one, two or all three vH (heavy chain) CDRs (*i.e.*, vH-CDR1, vH-CDR2 and vH-CDR3) of an antigen binding domain listed herein (**Table 4**), and/or one, two or all three vL (light chain) CDRs (*i.e.*, vL-CDR1, vL-CDR2 and vL-CDR3) of an antigen binding domain listed herein (**Table 4**).

[00171] In another embodiment, the antigen specific domain comprises a humanized antibody or an antibody fragment.

[00172] In some embodiments, the antigen specific domain of a CAR described herein is a scFv antibody fragment. 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 K_D 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.

[00173] In some embodiments, antigen specific domain of a CAR described herein binds to a MHC presented peptide. 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. For example, TCR-like antibody can be identified from screening a library, such as a human scFv phage displayed library.

[00174] In some embodiments, when the CARs comprising functional fragments of antibodies (including scFv fragments), as described herein, bind the target antigen, a

biological response is induced such as activation of an immune response, cytokine production, cytotoxicity, and the like, as will be understood by a skilled artisan.

[00175] In some embodiments, the antigens specific for disease that may be targeted by conventional CARs (*e.g.*, second generation CARs), next generation CARs, (*e.g.*, zSIR, SIR, Ab-TCR, Tri-TAC, TFP *etc.*) and rTCR 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; CS-1 (also referred to as CD2 subset 1, CRACC, SLAMF7, CD319, and 19A24); BAFF-R, C-type lectin-like molecule-1 (CLL-1 or CLECL1); CD33; MPL; 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)bDC1alp(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 (GPC5D); 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-ES0-1); 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 (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, MPL, GPA33, BST1/CD157, low conductance chloride channel, Integrin B7, Muc17, C16ORF54, VISTA, Muc5Ac, FCRH5, CLDN6, MMP16, UPK1B, BMPR1B, Ly6E, WISP1 and SLC34A2.

[00176] In some embodiments, the antigens associated with or specific for a disease that may be targeted by the CARs, 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 and 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.

[00177] In some embodiments, the antigens associated with or specific for cancer that may be targeted by the CARs, when expressed alone or with the accessory modules as described herein, include, but are not limited to, any one or more of BCMA, FLT3, CD19, CD20 (MS4A1), CD22, STEAP1, CD79b, Integrin B7, Her2, Her3, Liv1, TSHR (Thyroid Stimulating Hormone Receptor), PSMA, MSLN (Mesothelin), EGFRviii, Nectin 4, Prolactin Receptor (PRLR), Muc17, Muc5Ac, CD70, CD179b, CDH19, CD16ORF54, VISTA (V-set immunoregulatory receptor or VSIR), GPC3 (glypican 3), DLL3 (delta like canonical Notch ligand 3), PTK7, FCRH5 (Fc receptor like 5), LYPD1 (LY6/PLAUR domain containing 1), EMR2 (adhesion G protein-coupled receptor E2 or ADGRE2), gpNMB (glycoprotein nmb), ring finger protein 43 (RNF43), Robo4, CEA, Her3, Folate Receptor 1 (FOLR1), CLDN6 (Claudin 6), MMP16 (matrix metalloproteinase 16), uroplakin 1B (UPK1B), bone morphogenetic protein receptor type 1B (BMPRI1B), Ly6E, WISP1, SLC34A2, Cripto, gpA33, ROR1, CLL1, IL1RAP, BST1, CD133 and combinations thereof. In some embodiments, the antigen specific domains of the CARs are specific for BCMA, FLT3, CD19, CD20 (MS4A1), CD22, STEAP1, CD79b, Integrin B7, Her2, Her3, Liv1, TSHR (Thyroid Stimulating Hormone Receptor), PSMA, MSLN (Mesothelin), EGFRviii, Nectin 4, Prolactin Receptor (PRLR), Muc17, Muc5Ac, CD70, CD179b, CDH19, CD16ORF54, VISTA (V-set immunoregulatory receptor or VSIR), GPC3 (glypican 3), DLL3 (delta like canonical Notch ligand 3), PTK7, FCRH5 (Fc receptor like 5), LYPD1 (LY6/PLAUR domain containing 1), EMR2 (adhesion G protein-coupled receptor E2 or ADGRE2), gpNMB (glycoprotein nmb), ring finger protein 43 (RNF43), Robo4, CEA, Her3, Folate Receptor 1 (FOLR1), CLDN6 (Claudin 6), MMP16 (matrix metalloproteinase 16), uroplakin 1B (UPK1B), bone morphogenetic protein receptor type 1B (BMPRI1B), Ly6E, WISP1, SLC34A2, Cripto, gpA33, ROR1, CLL1, IL1RAP, BST1 and CD133. In some embodiments, the antigen specific domains of the CARs comprise scFv sequences whose SEQ IDs are set forth in **Table 3**. In some embodiments, the antigen specific domains of the CARs comprise CDR sequences whose SEQ IDs are set forth in **Table 4**.

[00178] In various embodiments, the immune cells expressing the CARs, both conventional and next generation CARs (*e.g.*, SIR, zSIR, Ab-TCR, TFP and the like), 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 PCT/US2017/024843, WO 2014/160030 A2, WO 2016/187349 A1, PCT/US2016/058305 and PCT/US17/64379, which are incorporated herein by reference in their entirety.

[00179] A CAR (*e.g.*, CAR II, SIR, zSIR, Ab-TCR, Tri-TAC, TFP and the like) 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 (*e.g.*, CARII, SIR, zSIR, Ab-TCR, TFP and the like) expressing cells destroy the disease-supporting cells, thereby indirectly blocking growth or survival of disease causing cells.

[00180] In some embodiments, the stromal cell antigen is selected from one or more of: bone marrow stromal cell antigen 2 (BST2), fibroblast activation protein (FAP) and tenascin. 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.

[00181] In another embodiment, each antigen specific region of the CAR (*e.g.*, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) may comprise a divalent (or bivalent) single-chain variable fragment (di-scFvs, bi-scFvs). In some embodiments, CARs (*e.g.*, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) 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. An exemplary CAR (a zSIR) targeting two antigens is represented by SEQ ID NO: 3962 and targets CD19 and CD123.

[00182] In an additional embodiment, each ASD of the CAR comprises a diabody.

[00183] In some embodiments, the ASD of the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) comprises V_L fragments whose SEQ IDs and target antigens are listed in **Table 3**.

[00184] In some embodiments, the ASD of the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) comprises V_H fragments whose SEQ IDs and target antigens are listed in **Table 3**.

[00185] In some embodiments, the ASD of the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) comprises scFvs whose SEQ IDs and target antigens are listed in **Table 3**.

[00186] In one embodiment, an antigen specific domain of a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) against a target antigen is an antigen binding portion, *e.g.*, CDRs, of vL and vH fragments targeting this antigen whose SEQ IDs are listed in **Table 4**.

[00187] In one embodiment, an antigen specific domain of a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) against a target antigen is an antigen binding portion, *e.g.*, CDRs, of the vL and vH fragments of scFvs targeting this antigen whose SEQ IDs are listed in **Tables 4**.

[00188] In some embodiments, the ASD of the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) comprises V_{HH} fragments (nanobodies).

[00189] In one embodiment, an antigen specific domain of a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) against a target antigen is an antigen binding portion of a non-immunoglobulin scaffold targeting this antigen.

[00190] In one embodiment, an antigen specific domain of a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) against a target antigen is an antigen binding portion of a receptor known to bind this target antigen.

[00191] In one embodiment, an antigen binding specific domain of a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) against a target antigen is an antigen binding portion of a ligand known to bind this target antigen.

[00192] The disclosure demonstrates that CARs targeting the same antigen may have different biological properties depending on the particular epitope of the antigen to which they bind. Thus, two CD19-targeted CARs (*e.g.*, SEQ ID NO: 916 and 818) may have different biological properties (*e.g.*, cytotoxicity, proliferation or cytokine secretion *etc.*) depending on the different CD19-epitopes to which they bind. In an embodiment, the disclosure provides CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) that bind to the same epitope on the different targets listed in **Tables 3** as any of the CARs of the disclosure (*i.e.*, CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) 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 CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) 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 CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are scFvs targeting that antigen and having sequences as shown in SEQ ID NOs: 4266-4338, 9693-9722 and 11468-11470 (**Table 3**). In an exemplary embodiment, the reference scFv BCMA-Am14-HL represented by SEQ ID NO: 4266 can be used in cross-competition studies to determine the target-epitope recognized by BCMA-Am14-HL-based CARs and backbones of the disclosure. In some embodiments, the reference CARs for cross-competition studies against different targets are CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) whose SEQ IDs are shown in **Table 7**.

[00193] In an exemplary embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the CD19-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are scFvs having sequences as shown in SEQ ID NOs: 4269-4270, 4272, 4298, 4299, 4338, 14462 (**Table 3**).

[00194] In an exemplary embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the CD19-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are CARs having sequences as shown in SEQ ID NOs: 4830-4871, 4781-4829, 4872-4920, 4732-4780, 4683-4731, 4970-5018, and 4921-4969 (**Table 7**).

[00195] In an embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the CD20-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are scFvs targeting CD20 and having SEQ IDs as listed in **Table 3**.

[00196] In an embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the CD20-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are CARs targeting CD20 and having SEQ IDs as listed in **Table 7**.

[00197] In an embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the CD22-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are scFvs targeting CD20 and having SEQ IDs 14449-14458, 14460, 14469-70 as listed in **Table 3**.

[00198] In an embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the CD22-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are CARs targeting CD22 and having SEQ IDs as listed in **Table 7**.

[00199] In an embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the BAFF-R-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are scFvs targeting BAFF-R and having SEQ IDs: 14465-14467 as listed in **Table 3**.

[00200] In an embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the BAFF-R-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are CARs targeting BAFF-R and having SEQ IDs as listed in **Table 7**.

[00201] In an embodiment, the reference scFvs for cross-competition studies against DLL3-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are scFvs targeting DLL3 and having SEQ IDs as listed in **Table 3**.

[00202] In an embodiment, the reference CARs for cross-competition studies against DLL3-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are CARs targeting DLL3 and having SEQ IDs as listed in **Table 7**.

[00203] In an embodiment, the reference scFvs for cross-competition studies against PTK7-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are scFvs targeting PTK7 and having SEQ IDs as listed in **Table 3**.

[00204] In an embodiment, the reference CARs for cross-competition studies against PTK7-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are CARs targeting PTK7 and having SEQ IDs as listed in **Table 7**.

[00205] In an embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by MSLN (Mesothelin)-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are scFvs targeting MSLN and having SEQ IDs as listed in **Table 3**.

[00206] In one embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the MSLN-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are represented by SEQ ID NOs: 4284-4285, 4293-4295, 9715 and 9716.

[00207] In another embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the MSLN-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are CARs targeting MSLN and having SEQ IDs as listed in **Table 7**.

[00208] In an embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by Her2-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-

TCR, TFP and the like) of the disclosure are scFvs targeting Her2 and having SEQ IDs as listed in **Table 3**.

[00209] In one embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the Her2-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are represented by SEQ ID NOs: 4276-4279.

[00210] In another embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the Her2-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are Her2-CARs with SEQ ID NOs: 6244-6292, 6391-6439, 6342-6390, and 6293-6341 (**Table 7**).

[00211] In an embodiment, the reference scFv for cross-competition studies to determine the target-epitopes recognized by TSHR-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure is scFv targeting TSHR and having SEQ ID: 4280 as listed in **Table 3**.

[00212] In another embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the TSHR -targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are TSHR-CARs with SEQ ID NOs: 7567-7615 (**Table 7**).

[00213] In an embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by EGFRviii-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are scFvs targeting EGFRviii and having SEQ IDs as listed in **Table 3**.

[00214] In another embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the EGFRviii -targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are EGFRviii -CARs with SEQ ID NOs: 5607-5655, 5705-5753, 5754-5802 and 5656-5704.

[00215] In an embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by PRLR (Prolactin Receptor)-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are scFvs targeting PRLR (Prolactin Receptor) and having SEQ IDs as listed in **Table 3**. In one embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the PRLR-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are represented by SEQ ID NOs: 4296 and 4309.

[00216] In another embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the PRLR-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are PRLR CARs with SEQ ID Nos: 7077-7125 and 7126-7174 as listed in **Table 7**.

[00217] In another embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the PSMA (Prostate Specific Membrane Antigen)-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure (*e.g.*, SEQ ID NOs: 7273-7321, 7224-7272 and 7175-7223) are the scFvs targeting PSMA listed in **Table 3** (*e.g.*, SEQ ID NOs: 4281-4283). In one embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the PSMA-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are represented by SEQ ID NOs: 4281-4283.

[00218] In another embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the PSMA-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are PSMA CARs listed in **Table 7** (*e.g.*, SEQ ID NOs: 7273-7321, 7224-7272 and 7175-7223).

[00219] In another embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the DLL3-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are the DLL3-targeting scFvs listed in **Table 3** (*e.g.*, SEQ ID NOs: 4290-4291).

[00220] In another embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the FOLR1-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure (*e.g.*, SEQ ID NOs: 5999-6047 and 6048-6096) are the FOLR1-targeting scFvs listed in **Table 3** (*e.g.*, SEQ ID NOs: 4323-4324). In one embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the FOLR1-targeting CARs of the disclosure are represented by SEQ ID NOs: 5999-6047 and 6048-6096).

[00221] In another embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the GPC3-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure (*e.g.*, SEQ ID NOs: 6097-6145 and 6146-6194) are the GPC3-targeting scFvs listed in **Table 3** (*e.g.*, SEQ ID NOs: 4307-4308). In one embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the GPC3-targeting CARs of the disclosure are represented by SEQ ID NOs: 6097-6145 and 6146-6194).

[00222] In another embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the WISP1-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure (*e.g.*, SEQ ID NOs: 7812-7860 and 7861-7909) are the WISP1-targeting scFvs listed in **Table 3** (*e.g.*, SEQ ID NOs: 4335 and 4336). In one embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the WISP1-targeting CARs of the disclosure are represented by SEQ ID NOs: 7812-7860 and 7861-7909.

[00223] In another embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the EMR2-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure (*e.g.*, SEQ ID NOs: 5803-5851, 5852-5900 and 5901-5949) are the EMR2-targeting scFvs listed in **Table 3** (*e.g.*, SEQ ID NOs: 4313, 4314 and 4315). In one embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the EMR2-targeting CARs of the disclosure are represented by SEQ ID NOs: 4803-5851, 585-5900, 5901-5949

[00224] In another embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the UPK1B -targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure (*e.g.*, SEQ ID NOs: 7616-7664, 7665-7713) are the UPK1B-targeting scFvs listed in **Table 3** (*e.g.*, SEQ ID NOs: 4328 and 4329). In one embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the UPK1B-targeting CARs of the disclosure are represented by SEQ ID NOs: 7616-7664, 7665-7713.

[00225] In another embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the BMPR1B-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure (*e.g.*, SEQ ID NOs: 4536-4584, 4585-4633) are the BMPR1B-targeting scFvs listed in **Table 3** (*e.g.*, SEQ ID NOs: 4330 and 4331). In one embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the BMPR1B-targeting CARs of the disclosure are represented by SEQ ID NOs: 4536-4584, 4585-4633.

[00226] In another embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the BMPR1B-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are BMPR1B CARs listed in **Table 7** (*e.g.*, SEQ ID NOs: 4536-4584, 4585-4633).

[00227] In another embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the CDH19-targeting CARs (*e.g.*, CAR I, CAR

II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure (*e.g.*, SEQ ID NOs: 5264-5312, 5313-5361) are the CDH19-targeting scFvs listed in **Table 3** (*e.g.*, SEQ ID NOs: 4302 and 4303). In one embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the CDH19-targeting CARs of the disclosure are represented by SEQ ID NOs: 5264-5312, 5313-5361.

[00228] In another embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the CDH19-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are the CDH19 CARs listed in **Table 7** (*e.g.*, SEQ ID NOs: 5264-5312, 5313-5361).

[00229] In another embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the VISTA-targeting CARs of the disclosure (*e.g.*, SEQ ID NOs: 7763-7811, 7714-7762) are the VISTA-targeting scFvs listed in **Table 3** (*e.g.*, SEQ ID NOs: 4305 and 4306). In one embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the VISTA-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are represented by SEQ ID NOs: 7763-7811, 7714-7762.

[00230] In another embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the VISTA-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are VISTA CARs listed in **Table 7** (*e.g.*, SEQ ID NOs: 7763-7811, 7714-7762).

[00231] In another embodiment, the reference scFv for cross-competition studies to determine the target-epitopes recognized by the IL13Ra2-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are IL13Ra2 scFv listed in **Table 3** (*e.g.*, SEQ ID NO: 14448).

[00232] In another embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the IL13Ra2-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are IL13Ra2 CARs listed in **Table 7** (*e.g.*, SEQ ID NO: 15857-15909).

[00233] In another embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the FLT3 -targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure (*e.g.*, SEQ ID NOs: 10606-10654, 10557-10605) are the FLT3-targeting scFvs listed in **Table 3** (*e.g.*, SEQ ID NOs: 9710 and 9711). In one embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the FLT3-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR,

Ab-TCR, TFP and the like) of the disclosure are represented by SEQ ID NOs: 10557-10605, 10606-10654.

[00234] In another embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the FLT3-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are FLT3 CARs listed in **Table 7** (*e.g.*, SEQ ID NOs: 10557-10605, 10606-10654).

[00235] In another embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the CLDN6 -targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure (*e.g.*, SEQ ID NOs: 5411-5459, 5460-5508) are the CLDN6-targeting scFvs listed in **Table 3** (*e.g.*, SEQ ID NOs: 4325 and 4326). In one embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the CLDN6-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are represented by SEQ ID NOs: 5411-5459, 5460-5508.

[00236] In another embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the CLDN6-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are CLDN6 CARs listed in **Table 7** (*e.g.*, SEQ ID NOs: 5411-5459, 5460-5508).

[00237] In another embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the ROBO4 -targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure (*e.g.*, SEQ ID NOs: 7420-7468) are the ROBO4-targeting scFvs listed in **Table 3** (*e.g.*, SEQ ID NOs: 4320). In one embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the ROBO4-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are represented by SEQ ID NOs: 7420-7468.

[00238] In another embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the ROBO4-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are ROBO4 CARs listed in **Table 7** (*e.g.*, SEQ ID NOs: 7420-7468).

[00239] In another embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the IL1RAP -targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure (*e.g.*, SEQ ID NOs: 10802-10850, 10851-10899, 10900-10948) are the IL1RAP-targeting scFvs listed in **Table 3** (*e.g.*, SEQ ID NOs: 9712, 9713 and 9714). In one embodiment, the reference scFvs for cross-competition

studies to determine the target-epitopes recognized by the IL1RAP-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are represented by SEQ ID NOs: 10802-10850, 10851-10899, 10900-10948.

[00240] In another embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the IL1RAP-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are IL1RAP CARs listed in **Table 7** (*e.g.*, SEQ ID NOs: 10802-10850, 10851-10899, 10900-10948).

[00241] In another embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the CD22 -targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure (*e.g.*, SEQ ID NOs: 5068-5115, 10361-10409) are the CD22-targeting scFvs listed in **Table 3** (*e.g.*, SEQ ID NOs: 4271, 9693, 12502). In one embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the CD22-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are represented by SEQ ID NOs: 5068-5115, 10361-10409.

[00242] In another embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the CD22-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are CD22 CARs listed in **Table 7** (*e.g.*, SEQ ID NOs: 5068-5115, 10361-10409).

[00243] In another embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the CLL1-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure (*e.g.*, SEQ ID NOs: 10459-10507, 10410-10458) are the CLL1-targeting scFvs listed in **Table 3** (*e.g.*, SEQ ID NOs: 9708 and 9703). In one embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the CLL1-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are represented by SEQ ID NOs: 10410-10458, 10459-10507.

[00244] In another embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the CLL1-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are CLL1 CARs listed in **Table 7** (*e.g.*, SEQ ID NOs: 10410-10458, 10459-10507).

[00245] In another embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the BST1-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure (*e.g.*, SEQ ID NOs: 10116-10164,

10165-10212, 10213-10262) are the BST1-targeting scFvs listed in **Table 3** (*e.g.*, SEQ ID NOs: 9718, 9719, and 9720). In one embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the BST1-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are represented by SEQ ID NOs: 10116-10164, 10165-10212, 10213-10262.

[00246] In another embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the BST1-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are BST1 CARs listed in **Table 7** (*e.g.*, SEQ ID NOs: 10116-10164, 10165-10212, 10213-10262).

[00247] In another embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the NECTIN-4 -targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure (*e.g.*, SEQ ID NOs: 7028-7076, 11096-11242) are the NECTIN-4 -targeting scFvs listed in **Table 3** (*e.g.*, SEQ ID NOs: 4292, 9696). In one embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the NECTIN-4-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are represented by SEQ ID NOs: 7028-7076, 11096-11242.

[00248] In another embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the NECTIN-4-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are NECTIN-4 CARs listed in **Table 7** (*e.g.*, SEQ ID NOs: 7028-7076, 11096-11242).

[00249] In another embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the GPA33-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure (*e.g.*, SEQ ID NOs: 10655-10703) are the GPA33-targeting scFvs listed in **Table 3** (*e.g.*, SEQ ID NOs: 9698). In one embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the GPA33-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are represented by SEQ ID NOs: 10655-10703.

[00250] In another embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the GPA33-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are GPA33 CARs listed in **Table 7** (*e.g.*, SEQ ID NOs: 10655-10703).

[00251] In another embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the ROR1-targeting CARs (*e.g.*, CAR I, CAR II,

SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure (*e.g.*, SEQ ID NOs: 11145-11193) are the ROR1-targeting scFvs listed in **Table 3** (*e.g.*, SEQ ID NOs: 9699). In one embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the ROR1-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are represented by SEQ ID NOs: 11145-11193.

[00252] In another embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the ROR1-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are ROR1 CARs listed in **Table 7** (*e.g.*, SEQ ID NOs: 11145-11193).

[00253] In another embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the CRIPTO-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure (*e.g.*, SEQ ID NOs: 10508-10556) are the CRIPTO-targeting scFvs listed in **Table 3** (*e.g.*, SEQ ID NOs: 9697). In one embodiment, the reference scFvs for cross-competition studies to determine the target-epitopes recognized by the CRIPTO-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are represented by SEQ ID NOs: 10508-10556.

[00254] In another embodiment, the reference CARs for cross-competition studies to determine the target-epitopes recognized by the CRIPTO-targeting CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure are CRIPTO CARs listed in **Table 7** (*e.g.*, SEQ ID NOs: 10508-10556).

[00255] In some embodiments, two or more functional domains of the CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) 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 CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) 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 (or any integer there between). 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. The SEQ ID Nos of several exemplary linkers are listed in **Table 5** (see, *e.g.*, SEQ ID Nos: 4007 to 4012).

[00256] In some embodiments, the CARs (which form part of the backbones) described herein comprise a hinge 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) combinations thereof.

[00257] As described herein, the CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) (which form part of the backbones) 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 (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure may also comprise an artificial hydrophobic sequence. The transmembrane domains of the CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) described herein may be selected so that the transmembrane domain do not dimerize. In some embodiments, the TMD encoded CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) 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 ϵ , CD3 ζ , CD3 γ , 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 α , 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.

[00258] A transmembrane domain can include one or more additional amino acids adjacent to the transmembrane region, *e.g.*, one or more amino acid associated with the extracellular region of the protein from which the transmembrane was derived (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 up to 15 amino acids of the extracellular region) and/or one or more additional amino acids associated with the intracellular region of the protein from which the transmembrane protein is derived (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 up to 15 amino acids of the intracellular region). In one aspect, the transmembrane domain is contiguous with one of the other domains of the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like). 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 (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) is derived from.

[00259] As described herein, the CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) (which form part of the backbones) described herein 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, Fc ϵ R1v and β chains, MB1 (Iga) chain, B29 (IgP) 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. 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 disclosure. 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.

[00260] As described herein, the CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) (which form part of the backbones) described herein comprise a co-stimulatory domain. 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.

[00261] Cleavable linkers as described herein 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 other embodiments, the linker sequences may comprise Asp-Val/Ile-Glu-X-Asn-Pro-Gly^(2A)-Pro^(2B) motif, which results in cleavage between the 2A glycine and the 2B proline. The nucleic sequences of several exemplary cleavable linkers are provided in SEQ ID NO: 80 to SEQ ID NO: 85 and amino acid sequences of several exemplary linkers are provided in SEQ ID NO: 4079 to SEQ ID NO: 4084. Other linkers will be apparent to those of skill in the art and may be used in connection with alternate embodiments of the disclosure. In an embodiment, a Ser-Gly-Ser-Gly (SGSG) motif (SEQ ID NOs: 931-932 and SEQ ID NO: 4844-4845) 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: 88-90 and 4087-4089) is added upstream of the SGSG motifs to facilitate cleavage of the residual 2A peptide following translation. In an embodiment, cleavable linkers are placed between the polypeptide encoding the CAR and the polypeptide encoding the accessory modules. The cleavage at the site of cleavable linker results in separation of the two polypeptides.

[00262] “Accessory modules” as used herein refer to agents that enhance, reduce or modify the activity of T cells expressing the CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) or reduce toxicity associated with CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) so that the therapeutic response of the CARs is enhanced. An accessory module may also enhance the gene transfer into and/or expression of CAR encoding cassette in the target cells, *e.g.*, an immune effector cell.

[00263] In some embodiments, vectors comprising polynucleotides encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) can further comprise polynucleotides encoding viral and cellular signaling proteins which (i) extend the life span of T cells expressing the CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like), (ii) stimulate T cell proliferation and/or (iii) protect T cells expressing the CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) from apoptosis; (iv) enhance packaging, gene transfer and/or expression of CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) constructs. In exemplary embodiments, such proteins include but are not limited to

vFLIP-K13 (SEQ ID NO (DNA): 108; SEQ ID NO (PRT): 4107) from Kaposi's sarcoma associated herpes virus and HIV-1 Vif (SEQ ID NO: 118 and 4117).

[00264] In one embodiment, vectors encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) further encode vFLIP-K13. In one embodiment, vFLIP-K13 nucleotide sequence is codon optimized. An exemplary CAR (*i.e.*, SIR) co-expressing codon optimized vFLIP K13 is represented by SEQ ID NO: 14057. In one embodiment, vectors encoding CARs further encode HIV-1 Vif. In an alternate embodiment, vectors encoding CARs further encode both vFLIP K13 and HIV-1 Vif.

[00265] In some embodiments, the accessory molecules are encoded by vectors that are distinct from the vectors encoding by the CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) described herein. In some embodiments, effector cells comprising vectors encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) also comprise vectors encoding accessory molecules. In some embodiments, the accessory molecules are encoded by modifying the genomic locus encoding the corresponding endogenous protein.

[00266] In some embodiments, vectors comprising polynucleotides encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) further comprise polynucleotides encoding siRNA or scFv specific for cytokines. In exemplary embodiments, the cytokines are any one or more of IL-10, IL-6, IFN or combinations thereof. In some embodiment, the CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) are co-expressed with a secreted bispecific antibody fragment that binds to IL6 receptor α and human serum albumin. In some embodiment, the CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) are co-expressed with a secreted scFv fragment that binds to IL6. In some embodiments, the CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) are coexpressed with the peptide FX06 so as to mitigate capillary leak associated with CAR therapy.

[00267] In further embodiments, vectors comprising polynucleotides encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) further comprise polynucleotides encoding siRNA or a nuclease targeting the endogenous TCR- α , TCR- β , TCR- γ , TCR- δ , CD3gamma, CD3zeta, CD3epsilon, CD3- δ . In further embodiments, polynucleotides encoding siRNA or a nuclease targeting the endogenous TCR- α , TCR- β , TCR- γ , TCR- δ , CD3gamma, CD3zeta, CD3epsilon, CD3- δ are encoded by vectors others than the vectors encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like).

[00268] In further embodiments, vectors comprising polynucleotides encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) further comprise polynucleotides encoding a selectable marker. In exemplary embodiment, the selectable marker can encode a

drug resistance gene, such as gene that confers resistance to puromycin or calcineurin inhibitors (*e.g.* CNB30). In some embodiment, the selectable marker may encode for extracellular and transmembrane domains of human CD30, CD20, CD19 (SEQ ID NO: 96 and 4095), BCMA (SEQ ID NO: 97 and 4096), EGFR (SEQ ID NO: 95 and 4094), CD34, or any protein or protein fragment that is expressed on cell surface and can be recognized by an antibody that can be used to eliminate cells expressing its target antigen. In an exemplary embodiment, cetuximab, an anti-EGFR monoclonal is used to eliminate CAR-expressing cells of the disclosure which coexpress a truncated EGFR. The selectable marker(s) can be used to enrich for cells expressing the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like), to select for cells that express high levels of CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) and/or to reduce the clonal diversity of the cells expressing the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like). In further embodiments, polynucleotides encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) may encode for epitope tags (*e.g.*, Myc tag) that are expressed on the extracellular domain of the CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) and can be used to enrich for cells expressing the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like), to select for cells that express high levels of CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) and/or to reduce the clonal diversity of the cells expressing the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like). Reducing the clonal diversity of allogeneic T cells expressing the CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) will in turn lead to reduced incidence of Graft versus Host Disease (GVHD), thereby allowing the use of allogeneic T cells for CAR-T cell therapy.

[00269] It is to be noted that the accessory modules are optional for the activity of a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like). The polypeptide and polynucleotides of a number of exemplary CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) constructs (*i.e.*, backbones) in **Table 6 and Table 7** contain accessory modules such as PAC, K13, and/or hNEMO-K277A-Flag. In alternate embodiments of the disclosure, these CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) constructs can be used without the presence of the accessory modules and the intervening cleavable liner (*e.g.*, P2A or F2A or T2A).

[00270] In certain embodiments, the disclosure provides a novel platform of synthetic immune receptors, designated zSIRs, containing two CD3z chains. The nucleic acid sequences of the CD3z chains that can be used in the construction of zSIR are provided in

SEQ ID NO: 67 and 71. The corresponding amino acid sequences are provided in SEQ ID NO: 4066 and 4070, respectively. The disclosure provides that the vL fragment of an antibody can be joined to one of the two CD3z chains and the vH fragment can be joined to the other CD3z chain. When the two such chains (*e.g.* vL-CD3z and vH-CD3z) are co-expressed in the same cell, the vL and vH fragments can bind their cognate antigen and transmit a T cell signal. In particular, T cells expressing such zSIR when exposed to a cell line expressing the cognate target antigen can activate NFAT signaling, induce IL2 production, promote T cell proliferation, promote T cell activation and exert cytotoxicity. The expression and activity of the zSIR can be further increased by incorporation of a linker between the vL/vH and the CD3z fragments. In particular, the IgCL (SEQ ID NO (DNA): 28 and SEQ ID NO (PRT): 4027) and IgCH domains (SEQ ID NO (DNA): 29 and SEQ ID NO (PRT): 4028) derived from antibodies serve as useful linkers between the vL/vH and CD3z fragments.

[00271] In another embodiment, a costimulatory domain is also incorporated in the the CD3z chain(s) of zSIR. Exemplary costimulatory domains include costimulatory domains of 41BB and CD28. CD3z chains containing 41BB and CD28 costimulatory domains are presented in SEQ ID NO: 4076, 4078 and 4075, 4077, respectively (**Table 5**). Collectively, the above results provide a novel platform for adoptive cellular therapy that overcomes some of the design limitations of SIR and also provide a complementary approach to SIRs

[00272] The two chains of zSIRs described herein may be encoded by a single polynucleotide chain and translated into a single polypeptide chain, which is subsequently cleaved into different proteins. The two chains of zSIRs described herein may be expressed using two distinct promoters and encoded by two separate polynucleotide chains. The two chains of zSIRs described herein may be encoded by a single vector. The two chains of zSIRs described herein may be encoded by a two different vectors. The nucleic acid molecule encoding a zSIR can comprise one or more leader sequences (also known as a signal peptide). In one embodiment, each functional unit (*e.g.*, an antigen binding domain joined to a CD3z chain plus Furine-SGSG-cleavable linker) of a zSIR can be preceded by a leader sequence which directs the zSIR to the cell surface as a type I transmembrane protein. In one embodiment, the antigen-binding domain of zSIR is extracellular-facing. In some embodiments, the leader sequence comprises the nucleic acid sequence of any of SEQ ID NO: 1 to 4 and amino acid sequences of SEQ ID NO: 4000 to SEQ ID NO: 4003. In some embodiments, short nucleic acid sequences (3-9 nucleic acids) comprising restriction enzyme

sites are located between the different subunits of a zSIR, *e.g.*, between a signal sequence and the antigen binding domain of the zSIR or between the antigen binding and the CD3z chain.

[00273] Provided herein are one or more polypeptides encoded by one or more nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) 1 to 15 (**Table 1**) or any one or more of backbones 1-60 described herein (**Table 2**).

[00274] In some embodiments, the antigen-specific domain of the CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) is specific to one, two, three or more antigens on target cells, such as cancer cells. As described herein, in some embodiments, each component of the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) is contiguous and in the same reading frame with each other components of the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like). In some embodiments, if the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) 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 (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like).

[00275] Also provided herein are one or more polypeptides encoded by one or more nucleic acid molecules encoding backbone-1 comprising CAR I and K13-vFLIP as described herein. In some embodiments, the antigen-specific domain of the CAR comprising backbone-1 is specific to one, two, three or more antigens on target cells, such as cancer cells. As described herein, in some embodiments, each component of the CAR is contiguous and in the same reading frame with each other components of the CAR comprising backbone-1. In some embodiments, the CAR comprising backbone-1 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.

[00276] Also provided herein are one or more polypeptides encoded by one or more nucleic acid molecules encoding backbone-8 which comprises CAR II (CAR 2) and HIV-1 Vif as described herein. In some embodiments, the antigen-specific domain of the CAR comprising backbone-8 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-8 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.

[00277] In various embodiments, the polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of CARs 1 to 15 (see, **Table 1**) or part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, comprise two, three or more antigen specific domains.

[00278] In various embodiments, the polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of CARs 1 to 15 (see, **Table 1**) or part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, comprise two, three or more co-stimulatory domains.

[00279] In various embodiments, the polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of CARs 1 to 15 (see, **Table 1**) or part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, comprise zero, one, two, three or more intracellular signaling domain.

[00280] In various embodiments, the polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, comprise one, two, three or more viral and/or cellular signaling proteins.

[00281] The nucleic acid sequences encoding for the desired components of the CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) 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.

[00282] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) is specific to target as described in **Table 3**.

[00283] In one embodiment, an antigen specific domain of a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) against a target antigen is an antigen binding portion,

e.g., CDRs, of vL and vH fragments targeting this antigen whose SEQ ID is shown in **Tables 3 and 4**.

[00284] In one embodiment, an antigen specific domain of a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) against a target antigen is an antigen binding portion, *e.g.*, CDRs, of vHH fragments targeting this antigen.

[00285] In one embodiment, an antigen specific domain of a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) against a target antigen is an antigen binding portion of a non-immunoglobulin scaffold targeting this antigen.

[00286] In one embodiment, an antigen specific domain of a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) against a target antigen is an antigen binding portion of a receptor known to bind this target antigen.

[00287] In one embodiment, an antigen binding specific domain of a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) against a target antigen is an antigen binding portion of a ligand known to bind this target antigen.

[00288] In one embodiment, an antigen specific domain of a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) against a target antigen is an antigen binding portion, *e.g.*, CDRs, of vL and vH fragments of a scFV targeting this antigen whose SEQ ID is shown in **Table 3**. The SEQ ID NOs of the CDRs are shown in **Table 4**.

[00289] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to the targets shown in **Table 3**.

[00290] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to CD19.

[00291] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to CD20.

[00292] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to CD22.

[00293] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to BCMA.

[00294] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the conventional CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to Integrin B7.

[00295] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to Her2.

[00296] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to TSHR.

[00297] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to PSMA.

[00298] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described

herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to MSLN.

[00299] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to EGFR viii.

[00300] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to DLL3.

[00301] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to Nectin-4.

[00302] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to Prolactin Receptor (PRLR).

[00303] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to Muc17.

[00304] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to CD70.

[00305] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like)

which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to Prolactin Receptor CDH19.

[00306] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to CD16ORF54.

[00307] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to VISTA.

[00308] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to GPC3.

[00309] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to Muc5Ac.

[00310] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to FCRH5.

[00311] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to LYPD1

[00312] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to EMR2.

[00313] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to gpNMB.

[00314] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to RNF43.

[00315] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to CD44v6.

[00316] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to Robo4.

[00317] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to CEA.

[00318] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described

herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to Her3.

[00319] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to FOLR1.

[00320] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to CLDN6.

[00321] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to MMP16.

[00322] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to UPK1B.

[00323] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to BMPR1B.

[00324] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to Ly6E.

[00325] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like)

which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to CD79b.

[00326] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to WISP1.

[00327] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to SLC34A2.

[00328] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to Liv1.

[00329] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to Cripto.

[00330] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to gpA33.

[00331] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to ROR1.

[00332] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to CLL1.

[00333] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to FLT3.

[00334] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to IL1RAP.

[00335] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to BST1.

[00336] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) which are part of the CARs 1 to 15 (see, *e.g.*, **Table 1**) or are part of backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domains of the CARs are specific to CD133.

[00337] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to CD200R.

[00338] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to CD276.

[00339] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to CD324.

[00340] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to CS1.

[00341] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to ALK1.

[00342] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to ROR1.

[00343] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to CDH6

[00344] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to CDH16.

[00345] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to CDH17.

[00346] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to Folate Receptor beta.

[00347] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to CLEC5A.

[00348] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to NY-ESO/MHC class I complex.

[00349] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to WT1/MHC class I complex.

[00350] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to AFP/MHC class I complex.

[00351] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to HPV16-E7/MHC class I complex.

[00352] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to gp100/MHC class I complex.

[00353] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to hTERT/MHC class I complex.

[00354] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to MART1/MHC class I complex.

[00355] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to HTLV1-Tax/MHC class I complex.

[00356] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to PR1/MHC class I complex.

[00357] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to HIV1-gag/MHC class I complex.

[00358] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to HIV1-envelop gp120.

[00359] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to PTK7.

[00360] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to TROP2.

[00361] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to BAFF-R.

[00362] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to LAMP1.

[00363] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to Tim1.

[00364] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to TCR gamma-delta.

[00365] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to TCR beta1 constant chain.

[00366] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to TCR beta2 constant chain.

[00367] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to GCC.

[00368] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to B7H4.

[00369] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to LHR.

[00370] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to Tn-Mucl.

[00371] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to TSLPR.

[00372] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to Tissue Factor.

[00373] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to SSEA-4.

[00374] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to SLea.

[00375] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to Muc1/MHC class I complex.

[00376] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to Muc16.

[00377] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to NYBR-1.

[00378] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to IL13Ra2.

[00379] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to IL11Ra.

[00380] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to L1CAM.

[00381] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to EpCAM1.

[00382] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to gpNMB.

[00383] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to GRP78.

[00384] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to GPC3.

[00385] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to GRPC5D.

[00386] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to GFRa4.

[00387] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to FITC.

[00388] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to CD79b.

[00389] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to Lym1.

[00390] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to Lym2.

[00391] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to CLD18A2.

[00392] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to CD43 epitope expressed on leukemia cells.

[00393] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to CD179a.

[00394] In some embodiments, provided herein are polypeptides encoded by the nucleic acid molecules encoding CARs 1-6 (see, *e.g.*, **Table 1**) or are part of backbones described herein such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen specific domain is as described in **Table 3**.

[00395] In some embodiments, the nucleic acid molecule encoding the CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) and/or accessory molecules described herein is provided as a messenger RNA (mRNA) transcript. In another embodiment, the nucleic acid molecule encoding the CARs and/or accessory molecules described herein is provided as a DNA construct.

[00396] Also provided are vectors comprising the polynucleotides described herein. In some embodiments, the vectors are viral vectors. Examples of viral vectors include but are not limited to retrovirus, an adenovirus, an adeno-associated virus, a lentivirus, a pox virus, a herpes virus vector or a sleeping beauty transposon vector. In various embodiments, the disclosure includes retroviral and lentiviral vector constructs expressing the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) and the accessory molecules that can be directly transduced into a cell.

[00397] 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 polyA 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 polyA tail, typically 50-2000 bases in length. RNA so produced can efficiently transfect different kinds of cells. In one embodiment, the template includes sequences for the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like). In an embodiment, an RNA CAR or next generation CAR vector is transduced into a cell, *e.g.*, a T cell or a NK cell, by electroporation. In another embodiment, an RNA CAR or next generation 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). The polynucleotide sequences coding for the desired molecules can be obtained using recombinant methods known in the art, for example by screening libraries from cells expressing the gene, by deriving the gene from a vector known to include the same, or by isolating directly from cells and tissues containing the same, using standard techniques. Alternatively, the gene of interest can be produced synthetically, rather than cloned.

[00398] The disclosure also provides vectors in which a DNA encoding the CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure is inserted. Vectors derived from retroviruses such as the lentivirus are suitable tools to achieve long-term gene

transfer since they allow long-term, stable integration of a transgene and its propagation in daughter cells. Lentiviral vectors have the added advantage over vectors derived from onco-retroviruses such as murine leukemia viruses in that they can transduce non-proliferating cells, such as hepatocytes. Exemplary lentiviral vectors are provided in SEQ ID NOs: 129-130 and 12639. 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 CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like). Exemplary gammaretroviral vectors include Murine Leukemia Virus (MLV), Spleen-Focus Forming Virus (SFFV), and Myeloproliferative Sarcoma Virus (MPSV), and vectors derived therefrom. In another embodiment, the vector comprising the nucleic acid encoding the desired CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure is an adenoviral vector (A5/35).

[00399] The expression of natural or synthetic nucleic acids encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) is typically achieved by operably linking a nucleic acid encoding the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) polypeptide or portions thereof to a promoter, and incorporating the construct into an expression vector. Exemplary lentiviral vector encoding CAR of the disclosure are provided in SEQ ID Nos: 12640-41 and 14378, 14380-85. The vectors can be suitable for replication and integration in eukaryotes. Typical cloning vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the desired nucleic acid sequence. The vector may contain a single promoter or more than one promoter. In some embodiments, the two or more functional units of a CAR (*e.g.*, nucleotides encoding two functional polypeptide units of a SIR or a zSIR or an Ab-TCR) are under the control of separate promoters. The expression constructs of the disclosure may also be used for nucleic acid immunization and gene therapy, using standard gene delivery protocols. Methods for gene delivery are known in the art. See, *e.g.*, U.S. Pat. Nos. 5,399,346, 5,580,859, 5,589,466, incorporated by reference herein in their entireties.

[00400] Cloning and expression methods will be apparent to a person of skill in the art.

[00401] Physical methods for introducing polynucleotides 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 another embodiment, a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) 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.

[00402] In various embodiments, the cells for modifications with CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) 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, placenta, 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 (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like).

[00403] In one aspect, the disclosure provides a number of CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) 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 aspect, the disclosure provides an immune effector cell (*e.g.*, T cell, NKT cell) engineered to express a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like), wherein the engineered immune effector cell exhibits a therapeutic property. In one aspect, the disclosure provides an immune effector cell (*e.g.*, T cell, NKT cell) engineered to express a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like), wherein the engineered immune effector cell exhibits an anticancer property. In one embodiment, a cell is transformed with the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) and the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) is expressed on the cell surface. In some embodiments, the cell (*e.g.*, T cell, NKT cell) is transduced with a viral vector encoding a CAR (*e.g.*, SIR, zSIR, Ab-TCR, TFP and the like). 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 CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like). In another embodiment, the cell (*e.g.*, T cell, NKT cell) is transfected with a nucleic acid, *e.g.*, mRNA, cDNA, DNA, encoding a CAR or next generation CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like). In some such embodiments, the cell may transiently express the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like).

[00404] The disclosure provides immune effector cells (*e.g.*, T cells, NKT or NK cells) that are engineered to contain one or more CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR,

TFP and the like) 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 CAR (*e.g.*, SIR, zSIR, Ab-TCR, Tri-Tac, TFP and the like) 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 (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, Tri-Tac, TFP and the like) 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).

[00405] Furthermore, the disclosure provides CAR- (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) -expressing cells 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.

[00406] In one aspect, the disclosure provides an immune effector cell (*e.g.*, T cell, NKT, or NK cell) engineered to express a CAR or next generation CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like), wherein the engineered immune effector cell exhibits an anti-disease property, such as antitumor property. In one embodiment, the antigen is a cancer associated antigen (*i.e.*, tumor antigen) described herein. In one aspect, the antigen binding domain of the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) comprises a partially humanized antibody fragment. In one aspect, the antigen binding domain of the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) comprises a partially humanized scFv. Accordingly, the disclosure provides CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) that comprises a humanized antigen binding domain and is engineered into a cell, *e.g.*, a T cell or a NK cell, and methods of their use for adoptive therapy.

[00407] Further provided herein are genetically engineered cells, comprising the polynucleotides and/or the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) 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 CARs of the disclosure 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 disclosure 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 disclosure may produce IL-2, IFN, TNF 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 disclosure 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 CAR (*e.g.*, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure.

[00408] The genetically engineered cells may be engineered to knock-out the expression of the endogenous TCR chains, *e.g.*, TCR α , TCR β , TCR γ , TCR δ or pre-TCR α chains. The knock-out of the endogenous TCR α , TCR β , TCR γ , TCR δ or pre-TCR α chains can be achieved using a number of techniques known in the art, such as the use of CRISP/Cas9 and Zn finger nucleases. In an exemplary embodiment, gRNAs targeting TCR α and TCR β loci can be introduced into T cells or iPSC or stem cell along with Cas9 mRNA to knock out the expression of endogenous TCR α and TCR β chains. Such TCR α / β knock-out cells can be used to introduce the CARs of the disclosure. A T cell lacking a functional endogenous TCR can be engineered such that it does not express any functional endogenous TCR on its surface, *e.g.*, 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 one embodiment, the allogeneic T cell or allogeneic NKT cell lacks expression or has low expression of a functional TCR and/or a functional HLA.

[00409] Various methods produce stable transfectants which express the CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure. In one embodiment, a method of stably transfecting and re-directing cells is by electroporation using naked DNA. By using naked DNA, the time required to produce redirected cells may be significantly reduced. Additional methods to genetically engineer cells using naked DNA encoding the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure include, but are not limited to, chemical transformation methods (*e.g.*, using calcium phosphate, dendrimers, liposomes and/or cationic polymers), non-chemical transformation methods (*e.g.*, electroporation, optical transformation, gene electrotransfer, transient perturbation in cell membranes and/or hydrodynamic delivery) and/or particle-based methods (*e.g.*, impalefection, using a gene gun and/or magnetofection). The transfected cells demonstrating presence of a single integrated un-rearranged vector and expression of the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) 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.

[00410] Viral transduction methods may also be used to generate redirected cells which express the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure. Cell types that may be used to generate genetically modified cells expressing the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) of the disclosure include but are not limited to T-lymphocytes (T-cells), natural killer cells, hematopoietic stem cells and/or pluripotent embryonic/induced stem cells capable of giving rise to therapeutically relevant progeny.

[00411] 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 CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) 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 CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like). The disclosure also provides a method of making and expanding cells expressing a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like). The method comprises transfecting or transducing the cells with the vector expressing the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) 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) T cells, hematopoietic stem cells

(HSCs) or pluripotent embryonic/induced stem cells capable of giving rise to therapeutically relevant progeny.

[00412] In some embodiments, genetically engineered cells described herein express the various backbones described herein, wherein the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) component of the backbone determines target specificity based on the antigen specific domain of the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like).

[00413] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 (see, *e.g.*, **Table 1**) which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to an antigen target in **Table 3** and/or **7** and comprises the antigen binding domain sequences set forth in **Table 3** and/or **7**.

[00414] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to MPL.

[00415] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to MPL.

[00416] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to CD19.

[00417] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to CD19.

[00418] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to CD20.

[00419] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to CD20.

[00420] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to BCMA.

[00421] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to BCMA.

[00422] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to CD22.

[00423] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to CD22.

[00424] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to BAFF-R.

[00425] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to BAFF-R.

[00426] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to Integrin B7.

[00427] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to Nectin 4.

[00428] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to Prolactin Receptor.

[00429] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to Muc17.

[00430] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to CD70.

[00431] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to CD70.

[00432] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to VISTA.

[00433] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to GPC3.

[00434] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to GPC3.

[00435] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to EMR2.

[00436] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to EMR2.

[00437] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to gpNMB.

[00438] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as

backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to RNF43.

[00439] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to STEAP1.

[00440] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to Robo4.

[00441] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to CLDN6.

[00442] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to CD44v6.

[00443] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to MMP16.

[00444] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to UPK1B.

[00445] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to BMPRI1B.

[00446] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to Ly6E.

[00447] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to CD79b.

[00448] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to CD79b.

[00449] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to WISP1.

[00450] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to Cripto.

[00451] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to gpA33.

[00452] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to IL1RAP.

[00453] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to BST1.

[00454] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to CD133.

[00455] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to CD123.

[00456] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to CD123.

[00457] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to CD138.

[00458] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to CD138.

[00459] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to CLL1.

[00460] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding CARs 1 to 15 which are part of the backbones described herein, such as backbone-1, backbone-2, backbone-32 or backbone-60, wherein the antigen-specific domain of the CARs is specific to CLL1.

[00461] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to TCR-beta1 constant chain.

[00462] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to TCR-beta2 constant chain.

[00463] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to ALK.

[00464] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to PTK7.

[00465] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to DLL3.

[00466] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to TROP2.

[00467] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to Tim1.

[00468] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to LAMP1.

[00469] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to CS1.

[00470] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to Lym1.

[00471] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to Lym2.

[00472] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to TSHR.

[00473] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to NY-ESO/MHC class I complex.

[00474] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to WT1/ MHC class I complex.

[00475] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to Ras/MHC class I complex.

[00476] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to CD179a.

[00477] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to CLD18A2.

[00478] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to CD43 epitope expressed on leukemia cells.

[00479] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to HIV1 envelop glycoprotein gp120.

[00480] In one embodiment, the genetically engineered cells comprise nucleic acid molecules encoding zSIRs which are part of CARs 7-15 (see, *e.g.*, **Table 1**), wherein the antigen-specific domains are specific to Fc region of an immunoglobulin.

[00481] In one embodiment, the CAR-expressing effector cell described herein can further comprise a second CAR that may include a different antigen binding domain to the same or a different target. In some embodiments, the second CAR may target the same or a different cell type from the first CAR. In some embodiment, the second CAR may be of the same class (*i.e.*, CAR 1 to CAR 15) as the first CAR. In some embodiment, the second CAR is of a different class as the first CAR. In some embodiment, the second CAR has the same backbone as the first CAR. In some embodiment, the second CAR has a different backbone as the first CAR.

[00482] In one embodiment, the CAR (*e.g.*, a CAR 7-15)-expressing effector cell described herein can further comprise a CAR of a different class (*e.g.*, CAR 1 or CAR 2 *etc.*) with the same or a different antigen binding domain, optionally the same or a different target. In some embodiments, the second CAR (*e.g.*, a CAR 1, CAR 2 *etc.*) may target the same or a different cell type from the first CAR (*e.g.*, *e.g.*, a CAR 7-15). In one embodiment, the CAR includes an antigen binding domain to a target expressed on the same disease cell type (*e.g.* cancer) as the disease associated antigen. In one embodiment, the CAR (*e.g.*, a CAR 7-15, *e.g.*, a zSIR) expressing cell comprises a CAR that targets a first antigen, and a second antigen specific receptor (*e.g.*, a CAR) that targets a second, different, antigen and includes an intracellular signaling domain having no primary signaling domain but a costimulatory signaling domain. While not wishing to be bound by theory, placement of a costimulatory signaling domain, *e.g.*, 4-1BB, CD28, CD27 or OX-40, onto an antigen specific receptor, can modulate the CAR (*e.g.*, a CAR 7-15, *e.g.*, a zSIR) activity on cells where both targets are expressed. In one embodiment, the CAR (*e.g.*, a CAR 7-15, *e.g.*, a zSIR) expressing cell

comprises i) a first disease associated antigen CAR that includes one or more antigen binding domains that bind a target antigen described herein, and ii) a CAR that targets a different target antigen (*e.g.*, an antigen expressed on that same disease associated (*e.g.* cancer) cell type as the first target antigen) and includes an antigen binding domain, a transmembrane domain and a primary signaling domain and a costimulatory domain. The nucleic acid and amino acid sequences of an exemplary construct with this configuration are presented in SEQ ID NO: 14380 and SEQ ID NO: 16124, respectively. The antigen binding domains of the SIR in this construct are comprised of the vL and vH fragments derived from BCMAAm06 monoclonal antibody that targets BCMA, while the antigen binding domain of the CAR is comprised of the extracellular domain of PD1. The primary signaling domain of the CAR in this construct comprises of CD3z cytosolic domain while the costimulatory domain comprises of the 4-1BB cytosolic domain. In another embodiment, the CAR (*e.g.*, a CAR 7-15, *e.g.*, a zSIR) expressing cell comprises i) a first disease associated antigen CAR that includes one or more antigen binding domains that bind a target antigen described herein, and ii) a CAR that targets a different target antigen (*e.g.*, an antigen expressed on that same disease associated (*e.g.* cancer) cell type as the first target antigen) and includes an antigen binding domain, a transmembrane domain and a costimulatory domain but without a primary signaling or activation domain. The nucleic acid and amino acid sequences of an exemplary construct with this configuration are presented in SEQ ID NO: 14379 and SEQ ID NO: 16123, respectively. This construct is similar to the construct shown in SEQ ID NO: 14380 with the exception that the CAR lacks the CD3z domain. In yet another embodiment, the CAR (*e.g.*, a CAR 7-15, *e.g.*, a zSIR) expressing cell comprises i) a first disease associated antigen CAR that includes one or more antigen binding domains that bind a target antigen described herein, and ii) a CAR that targets a different target antigen (*e.g.*, an antigen expressed on that same disease associated (*e.g.* cancer) cell type as the first target antigen) and includes an antigen binding domain, a transmembrane domain and a primary signaling domain but without a costimulatory domain.

[00483] In one embodiment, the CAR comprises the antigen binding domain, a transmembrane domain and an intracellular signaling domain (such as but not limited to one or more intracellular signaling domain from 41BB, CD27, OX40, CD28, Dap10, CD2, CD5, ICAM-1, LFA-1, Lck, TNFR-1, TNFR-II, Fas, CD30, CD40 or combinations thereof) and/or a primary signaling domain (such as but not limited to a CD3 zeta signaling domain). Exemplary SIRs co-expressing a CAR are presented in SEQ ID NO: 3217 to 3219 and SEQ ID NO: 3221 and 3222.

[00484] Immune effector cells such as T cells and NK cells comprising CARs 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.

[00485] Provided herein are methods for treating a disease associated with expression of a disease-associated antigen or a cancer associated antigen.

[00486] In one embodiment, provided herein are methods for treating a disease in a subject in need thereof by administering to the subject a therapeutically effective amount of genetically modified cells described herein (such as T cells, NK cells) that are engineered to express an antigen-specific CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) alone or an antigen specific CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) and an accessory molecule, wherein the antigen is a disease specific antigen as described herein, and wherein the disease causing or disease-associated cells express the said disease-specific antigen.

[00487] In one embodiment, provided herein are methods for treating cancer in a subject in need thereof by administering to the subject a therapeutically effective amount of genetically modified cells described herein (such as T cells, NK cells) that are engineered to express an antigen-specific CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Tri-Tac, Ab-TCR, TFP and the like) alone or an antigen specific CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) and an accessory molecule, wherein the antigen is a cancer specific antigen as described herein, and wherein the cancer cells express the said tumor antigen.

[00488] In one embodiment, the cancer specific antigen is expressed on both normal cells and cancers cells, but is expressed at lower levels on normal cells. In one embodiment, the method further comprises selecting a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) that binds the cancer specific antigen of interest with an affinity that allows the antigen specific CAR to bind and kill the cancer cells. In some embodiments, the antigen specific CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) kills cancer cells but kills less than 30%, 25%, 20%, 15%, 10%, 5% or less of the normal cells expressing the cancer antigen. In exemplary embodiments, the percentage of cells killed by the antigen specific CARs may be determined using the cell death assays (*e.g.*, Matador assay) described herein.

[00489] In some embodiment, the disclosure provides methods of treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective

amount of the genetically modified cells (*e.g.*, T cells, NK cells) that are engineered to express conventional CARs 1 to 15, wherein the ASD of the CARs is specific to the antigen that is expressed on cancer cells (for example, the antigen is expressed at lower levels on normal cells relative to cancer cells) and whose SEQ ID NO is listed in **Table 3**.

[00490] In some embodiment, the disclosure provides methods of treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of the genetically modified cells (*e.g.*, T cells, NK cells) that are engineered to express backbone-1 comprising the conventional CARs I and the accessory module K13-vFLIP, wherein the ASD of the CARs is specific to the antigen that is expressed on cancer cells (for example, the antigen is expressed at lower levels on normal cells relative to cancer cells) and whose SEQ ID NO is listed in **Table 3**.

[00491] In some embodiment, the disclosure provides methods of treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of the genetically modified cells (*e.g.*, T cells, NK cells) that are engineered to comprising backbone-12 comprising the conventional CARs II and the accessory module HIV1-Vif, wherein the ASD of the CARs is specific to the antigen that is expressed on disease causing or disease associated cells (for example, the antigen is expressed at lower levels on normal cells relative to cancer cells).

[00492] In some embodiment, the disclosure provides methods of treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of the genetically modified cells (*e.g.*, T cells, NK cells) that are engineered to comprising backbone-32 comprising the conventional CAR II and the accessory module K13-vFLIP, wherein the ASD of the CARs is specific to the antigen that is expressed on cancer cells (for example, the antigen is expressed at lower levels on normal cells relative to cancer cells).

[00493] In exemplary embodiments, the antigens that may be targeted for the therapeutic methods described herein include but are not limited to any one, two, three, four or more of: CD19; CD5, CD123; CD22; CD30; CD171; CS1 (also referred to as CD2 subset 1, CRACC, SLAMF7, CD319, and 19A24); C-type lectin-like molecule-1 (CLL-1 or CLECL1); BAFF-R; 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-ES0-1); 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-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), CLDN6, P-

glycoprotein, STEAP1, Liv1, Nectin-4, Cripto, MPL, gpA33, BST1/CD157, low conductance chloride channel, and the antigen recognized by TNT antibody.

[00494] In some embodiments, the antigen specific domains of the CARs comprise scFv sequences whose SEQ ID is set forth in **Table 3**.

[00495] In exemplary embodiments, the antigens that may be targeted for the therapeutic methods described herein include but are not limited to any one, two, three, four or more of the targets described in **Table 3**.

[00496] The disclosure also provides a method comprising administering a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) molecule, a cell expressing a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) molecule or a cell comprising a nucleic acid encoding a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) molecule to a subject. In one embodiment, the subject has a disorder described herein, *e.g.*, the subject has cancer, infectious disease, allergic disease, degenerative disease or autoimmune disease, which expresses a target antigen described herein. In yet one embodiment, the subject has increased risk of a disorder described herein, *e.g.*, the subject has increased risk of cancer, infectious disease, allergic disease, degenerative disease or autoimmune disease, which expresses a target antigen described herein. In one embodiment, the subject is a human. In another embodiment, the subject is an animal. In yet another embodiment, the subject is a companion animal such as a dog.

[00497] The disclosure provides methods for treating or preventing a disease associated with expression of a disease-associated antigen described herein.

[00498] 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, wherein X represents a disease associated antigen as described herein, and wherein the disease causing or disease-associated cells express said X antigen. **Table 11** provides a list of different antigens and the exemplary diseases that can be prevented, inhibited or treated using immune effector cells expressing CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) targeting these antigens.

[00499] In one embodiment, the disclosure provides methods of treating cancer, autoimmune or allergic disease by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60) (see,

e.g., **Table 2**) specific to different antigens shown in **Table 3** wherein the ASD of CARs is comprised of vL and vH fragments whose SEQ ID Nos are listed in **Table 3**.

[00500] In one embodiment, the disclosure provides methods of treating cancer, autoimmune or allergic disease by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(See, *e.g.*, **Table 2**) specific to CD19, wherein the disease causing or disease associated cells express CD19 and wherein the ASD of CD19-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the cancer to be treated is acute lymphoblastic leukemia, chronic lymphocytic leukemia, B cell malignancy, non-Hodgkins lymphoma, diffuse large B-cell lymphoma, mantle cell lymphoma, or, multiple myeloma. In one embodiment, the disease to be treated is an immune (*e.g.*, lupus, SLE, ITP *etc.*) or allergy disease.

[00501] In one embodiment, the disclosure provides methods of treating cancer, autoimmune or allergic disease by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to CD20, wherein the disease causing or disease associated cells express CD20 and wherein the ASD of CD20-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the cancer to be treated is acute lymphoblastic leukemia, chronic lymphocytic leukemia, B cell malignancy, non-Hodgkins lymphoma, diffuse large B-cell lymphoma, or mantle cell lymphoma. In one embodiment, the disease to be treated is an immune (*e.g.*, lupus, SLE, ITP *etc.*) or allergy disease.

[00502] In one embodiment, the disclosure provides methods of treating cancer, autoimmune or allergic disease by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to CD22, wherein the disease causing or disease associated cells express CD22 and wherein the ASD of CD22-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the cancer to be treated is acute lymphoblastic leukemia, chronic lymphocytic leukemia, B cell malignancy, non-Hodgkins lymphoma, diffuse large B-cell lymphoma, or mantle cell lymphoma. In one

embodiment, the disease to be treated is an immune (*e.g.*, lupus, SLE, ITP *etc.*) or allergy disease.

[00503] In one embodiment, the disclosure provides methods of treating cancer, autoimmune or allergic disease by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60) (see, *e.g.*, **Table 2**) specific to BCMA, wherein the disease causing or disease associated cells express BCMA and wherein the ASD of BCMA-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer or immune or allergic disease. In one embodiment, the cancer to be treated or prevented is a plasma cell malignancy or multiple myeloma or primary effusion lymphoma or diffuse large cell lymphoma. In one embodiment, the disease to be treated is an immune (*e.g.*, lupus, SLE, ITP *etc.*) or allergy disease.

[00504] In one embodiment, the disclosure provides methods of treating cancer, autoimmune or allergic disease by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to MPL, wherein the disease causing or disease associated cells express MPL and wherein the ASD of MPL-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the cancer to be treated is acute myeloid leukemia, chronic myeloid leukemia, myelodysplastic syndrome.

[00505] In one embodiment, the disclosure provides methods of treating cancer, autoimmune or allergic disease by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to BAFF-R, wherein the disease causing or disease associated cells express BAFF-R and wherein the ASD of BAFF-R-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the cancer to be treated is chronic lymphocytic leukemia, mantle cell lymphoma, B cell lymphoma and acute leukemia.

[00506] In one embodiment, the disclosure provides methods of treating cancer, autoimmune or allergic disease by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see,

e.g., **Table 2**) specific to IL13Ra2, wherein the disease causing or disease associated cells express IL13Ra2 and wherein the ASD of IL13Ra2-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the cancer to be treated is a brain tumor.

[00507] In one embodiment, the disclosure provides methods of treating cancer, autoimmune or allergic disease by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to CD79b, wherein the disease causing or disease associated cells express CD79b and wherein the ASD of CD79b-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the cancer to be treated is acute lymphoblastic leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, diffuse large B-cell lymphoma, mantle cell lymphoma, myelodysplastic syndrome or multiple myeloma. In one embodiment, the disease to be treated is an immune (*e.g.*, lupus, SLE, ITP *etc.*) or allergy disease.

[00508] In one embodiment, the disclosure provides methods of treating cancer, autoimmune or allergic disease by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to Her2, wherein the disease causing or disease associated cells express Her2 and wherein the ASD of Her2-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the cancer to be treated is breast cancer or gastric cancer.

[00509] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to Mesothelin (MSLN), wherein the disease causing or disease associated cells express MSLN and wherein the ASD of MSLN-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the cancer to be treated is mesothelioma, lung cancer, pancreatic cancer, gastro-intestinal cancer, or ovarian cancer.

[00510] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example,

backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to TSHR, wherein the disease causing or disease associated cells express TSHR and wherein the ASD of TSHR-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the cancer to be treated is thyroid cancer or T cell leukemia/lymphoma.

[00511] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to Prolactin Receptor (PRLR), wherein the disease causing or disease associated cells express PRLR and wherein the ASD of PRLR-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the cancer to be treated is breast cancer or chromophobe renal cell carcinoma.

[00512] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to Folate Receptor 1 (FOLR1), wherein the disease causing or disease associated cells express FOLR1 and wherein the ASD of FOLR1-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the cancer to be treated is ovarian cancer, lung cancer, endometrial cancer or other solid tumors.

[00513] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to PTK7, wherein the disease causing or disease associated cells express PTK7 and wherein the ASD of PTK7-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented is melanoma, lung cancer or ovarian cancer.

[00514] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to DLL3, wherein the disease causing or disease associated cells express DLL3 and wherein the ASD

of DLL3-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented is melanoma, lung cancer or ovarian cancer.

[00515] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to EGFRviii, wherein the disease causing or disease associated cells express EGFRviii and wherein the ASD of EGFRviii -CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented is brain cancer or lung cancer or other solid tumors.

[00516] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to PSMA, wherein the disease causing or disease associated cells express PSMA and wherein the ASD of PSMA-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented is prostate cancer.

[00517] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to UPK1B, wherein the disease causing or disease associated cells express UPK1B and wherein the ASD of UPK1B-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented is bladder cancer.

[00518] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60) (see, *e.g.*, **Table 2**) specific to WISP1, wherein the disease causing or disease associated cells express WISP1. In one embodiment the ASD of WISP1-CAR is comprised of vL and vH fragments whose SEQ ID

NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented is glioblastoma or breast cancer.

[00519] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to MMP16, wherein the disease causing or disease associated cells express MMP16. In one embodiment, the ASD of MMP16-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented is glioblastoma, melanoma, small cell lung cancer or neuroblastoma.

[00520] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60) (see, *e.g.*, **Table 2**) specific to BMPR1B, wherein the disease causing or disease associated cells express BMPR1B. In one embodiment, the ASD of BMPR1B-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented is prostate cancer, breast cancer or ovarian cancer.

[00521] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60) (see, *e.g.*, **Table 2**) specific to SLC34A2, wherein the disease causing or disease associated cells express SLC34A2. In one embodiment, ASD of SLC34A2-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented is lung cancer, ovarian cancer or endometrial cancer.

[00522] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to gpA33,

wherein the disease causing or disease associated cells express gpA33 and wherein the ASD of gpA33-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented is colorectal cancer, ovarian cancer or endometrial cancer.

[00523] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to BST1, wherein the disease causing or disease associated cells express BST1 and wherein the ASD of BST1-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented is blood cancer.

[00524] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to CD133, wherein the disease causing or disease associated cells express CD133 and wherein the ASD of CD133-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented is lung cancer or brain cancer.

[00525] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to EMR2, wherein the disease causing or disease associated cells express EMR2 and wherein the ASD of EMR2-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented is acute leukemia, lymphoma, breast cancer and colon cancer.

[00526] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to GPC3,

wherein the disease causing or disease associated cells express GPC3 and wherein the ASD of GPC3-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented is liver cancer, breast cancer and lung cancer.

[00527] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to gpNMB, wherein the disease causing or disease associated cells express gpNMB and wherein the ASD of gpNMB-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented is melanoma, brain cancer, breast cancer, lung cancer and other solid tumors.

[00528] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to IL1RAP, wherein the disease causing or disease associated cells express IL1RAP and wherein the ASD of IL1RAP -CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer or endometrioses. In one embodiment, the cancer to be treated or prevented is liver cancer, cervical cancer, colon cancer, ovarian cancer and other solid tumors.

[00529] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to Nectin-4, wherein the disease causing or disease associated cells express Nectin-4 and wherein the ASD of Nectin-4-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer or endometriosis. In one embodiment, the cancer to be treated or prevented is bladder cancer, renal cancer, head and neck cancer, ovarian cancer, breast cancer, lung cancer and other solid tumors.

[00530] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that

are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to Cripto, wherein the disease causing or disease associated cells express Cripto and wherein the ASD of Cripto-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented is, colorectal cancer, ovarian cancer, endometrial cancer and other solid tumors.

[00531] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to RNF43, wherein the disease causing or disease associated cells express RNF43 and wherein the ASD of RNF43-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented is, colorectal cancer, breast cancer, endometrial cancer and other solid tumors.

[00532] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to ROR1, wherein the disease causing or disease associated cells express ROR1 and wherein the ASD of ROR1-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented is, blood cancer, CLL and lymphoma.

[00533] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to FLT3, wherein the disease causing or disease associated cells express FLT3 and wherein the ASD of FLT3-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented is blood cancer.

[00534] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that

are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to CLL-1, wherein the disease causing or disease associated cells express CLL-1 and wherein the ASD of CLL-1-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented is blood cancer.

[00535] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to Robo4, wherein the disease causing or disease associated cells express Robo4. In one embodiment, the ASD of Robo4-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented is renal cancer, colon cancer, breast cancer or other solid tumor.

[00536] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to CLDN6, wherein the disease causing or disease associated cells express CLDN6. In one embodiment, the ASD of CLDN6-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented is ovarian cancer, liver cancer or other solid tumor.

[00537] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60) (see, *e.g.*, **Table 2**) specific to Muc5Ac, wherein the disease causing or disease associated cells express Muc5Ac. In one embodiment, the ASD of Muc5Ac-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented is pancreatic cancer, stomach cancer, colon cancer or other solid tumor.

[00538] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to Muc17, wherein the disease causing or disease associated cells express Muc17. In one embodiment, the ASD of Muc17-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented is pancreatic cancer, stomach cancer, colon cancer or other solid tumor.

[00539] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60) (see, *e.g.*, **Table 2**) specific to Ly6E, wherein the disease causing or disease associated cells express Ly6E. In one embodiment, the ASD of Ly6E-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented is pancreatic cancer, breast cancer, ovarian cancer, pancreatic cancer or other solid tumor.

[00540] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to Integrin B7, wherein the disease causing or disease associated cells express Integrin B7. In one embodiment, the ASD of Integrin B7-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented is plasma cell neoplasm or primary effusion lymphoma.

[00541] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60)(see, *e.g.*, **Table 2**) specific to STEAP1, wherein the disease causing or disease associated cells express STEAP1. In one embodiment, the ASD of STEAP1-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a

cancer. In one embodiment, the cancer to be treated or prevented gastric cancer, prostate cancer or lymphoma.

[00542] In one embodiment, the disclosure provides methods of treating cancer by providing to the subject in need thereof immune effector cells (*e.g.*, T cells, NKT cells) that are engineered to express CARs 1 to 15 (see, *e.g.*, **Table 1**) or backbones (for example, backbone-1, backbone-2, backbone-32 or backbone-60) (see, *e.g.*, **Table 2**) specific to Liv1, wherein the disease causing or disease associated cells express Liv1. In one embodiment, the ASD of Liv1-CAR is comprised of vL and vH fragments whose SEQ ID NOs are listed in **Table 3**. In one embodiment, the disease to be treated or prevented is a cancer. In one embodiment, the cancer to be treated or prevented breast cancer, prostate cancer or solid tumor.

[00543] Exemplary cancers whose growth can be inhibited include cancers typically responsive to immunotherapy. Non-limiting examples of cancers for treatment include melanoma (*e.g.*, metastatic malignant melanoma), renal cancer (*e.g.* clear cell carcinoma), prostate cancer (*e.g.* hormone refractory prostate adenocarcinoma), breast cancer, colon cancer and lung cancer (*e.g.* non-small cell lung cancer). Additionally, refractory or recurrent malignancies can be treated using the molecules described herein. Finally, non-malignant diseases, such as endometriosis, can be treated using the CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) described herein.

[00544] In exemplary embodiments, cancers treated by the methods described herein include solid tumors such as sarcomas, adenocarcinomas, and carcinomas, of the various organ systems, such as those affecting liver, lung, breast, lymphoid, gastrointestinal (*e.g.*, colon), genitourinary tract (*e.g.*, renal, urothelial cells), prostate and pharynx.

Adenocarcinomas include malignancies 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.

[00545] Examples of other cancers that can be treated include 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 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 CAR molecules described herein. 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 associated with expression of a cancer associate antigen as described herein. Finally, non-malignant diseases, such as endometriosis, can be treated using the CARs described herein. In some embodiments, a CAR-expressing T cell or NKT cell as described herein reduces the quantity, number, amount or percentage of cells and/or cancer cells by at least 25%, at least 30%, at least 40%, at least 50%, at least 65%, at least 75%, at least 85%, at least 95%, or at least 99% in a subject with hematological cancer or another cancer associated with a cancer associated antigen as described herein, expressing cells relative to a negative control. In one embodiment, the subject is a human.

[00546] In one aspect, the disclosure pertains to a method of inhibiting growth of a disease (*e.g.*, cancer, autoimmune disease, infectious disease or allergic disease or a degenerative disease), comprising contacting the disease causing or disease associated cell with a genetically modified cell of the disclosure expressing a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) or a CAR with accessory modules (*i.e.*, backbones 1-60; see **Table 2**) such that the CAR-T is activated in response to the antigen and targets the disease causing or disease associated cell, wherein the growth of the disease causing or disease associated cell is inhibited. In one aspect, the disclosure pertains to a method of preventing a disease, comprising administering to a patient at risk of disease a CAR- or next generation CAR- (*e.g.*, SIR, zSIR, Ab-TCR, TFP and the like) expressing cell or a cell that is capable of generating a CAR-expressing cell of the disclosure such that the CAR-T is activated in response to the antigen and targets the disease causing or disease associated cell,

wherein the growth of the disease causing or disease associated cell is prevented. In one aspect the disease is a cancer, an infectious disease, an immune disease, an allergic disease, or a degenerative disease.

[00547] In one aspect, the disease is an autoimmune disease. In one embodiment, the autoimmune disease is selected from the group consisting of Acquired Immunodeficiency Syndrome (AIDS), alopecia areata, ankylosing spondylitis, antiphospholipid syndrome, autoimmune Addison's disease, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune inner ear disease (AIED), autoimmune lymphoproliferative syndrome (ALPS), autoimmune thrombocytopenic purpura (ATP), Behcet's disease, cardiomyopathy, celiac sprue-dermatitis hepetiformis; chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy (CIPD), cicatricial pemphigoid, cold agglutinin disease, crest syndrome, Crohn's disease, Degos' disease, dermatomyositis-juvenile, discoid lupus, essential mixed cryoglobulinemia, fibromyalgia-fibromyositis. Graves' disease, Guillain-Barre syndrome, Hashimoto's thyroiditis, idiopathic pulmonary fibrosis, idiopathic thrombocytopenia u ura (Γ P), IgA nephropathy, insulin-dependent diabetes mellitus, juvenile chronic arthritis (Still's disease), juvenile rheumatoid arthritis, Meniere's disease, mixed connective tissue disease, multiple sclerosis, myasthenia gravis, pernicious anemia, polyarteritis nodosa, polychondritis, polyglandular syndromes, polymyalgia rheumatica, polymyositis and dermatomvositis, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, psoriatic arthritis, Raynaud's phenomena, Reiter's syndrome, rheumatic fever, rheumatoid arthritis, sarcoidosis, scleroderma (progressive systemic sclerosis (PSS), also known as systemic sclerosis (SS)), Sjogren's syndrome, stiff-man syndrome, systemic lupus erythematosus (SLE), Takayasu arteritis, temporal arteritis/giant cell arteritis, ulcerative colitis, uveitis, vitiligo, Wegener's granulomatosis, and any combination thereof.

[00548] Embodiments of the disclosure include a type of cellular therapy where effector cells (such as T cells and NK cells) or stem cells that can give rise to effector cells are genetically modified to express a CAR as described herein and the CAR- or next generation CAR- (*e.g.*, SIR, zSIR, Ab-TCR, TFP and the like)-expressing T cell or NKT cell is infused to a recipient in need thereof. The infused cell is able to kill tumor cells in the recipient. In various aspects, the immune effector cells (*e.g.*, T cells, NKT 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, twenty-three months, two years, three years, four years, or five years after administration of the T cell or NK cell to the patient.

[00549] The disclosure also includes a type of cellular therapy where immune effector cells (*e.g.*, T cells, NK cells) are modified, *e.g.*, by *in vitro* transcribed RNA, to transiently express a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) or a CAR with accessory modules (*e.g.*, backbones 1-60). T cells or NKT 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. Thus, in various aspects, the CAR- or next generation CAR-expressing immune effector cells (*e.g.*, T cells, NKT cells) persist for less than one month, *e.g.*, three weeks, two weeks, one week, after administration of the T cell or NK cell to the patient.

[00550] 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 or NK cells) are modified to express a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) or a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) with accessory modules (*e.g.*, backbones 1-60; see **Table 2**) and are administered to a recipient in need thereof. The administered stem cells give rise to immune effector cells (*e.g.*, T cells or NKT 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, NKT cells) that are produced in the patient after administration of CAR- or next generation CAR-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 genetically modified 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 or NKT cells) are modified to express a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) or a CAR with accessory modules (*e.g.* backbones 1-60; see **Table 2**) 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 or NKT

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, NK 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, twenty three months, two years, three years, four years, five years, ten years or twenty years.

[00551] The disclosure also includes a type of cellular therapy where regulatory immune effector cells (*e.g.*, TREG, or CD25+ T Cells) are modified to express a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) or a CAR with accessory modules (*e.g.*, backbones 1-60) targeting a specific antigen. Such CAR-TREG are administered to a patient to suppress immune response against the specific antigen. The CAR-TREG can be used to prevent and treat autoimmune diseases and to enhance immune tolerance.

[00552] The anti-tumor immunity response elicited by the CAR- or next generation CAR-modified immune effector cells (*e.g.*, T cells, NKT cells) may be an active or a passive immune response, or alternatively may be due to a direct vs indirect immune response. In one aspect, the CAR- or next generation CAR-transduced immune effector cells (*e.g.*, T cells, NK cells) exhibit specific pro-inflammatory cytokine secretion and potent cytolytic activity in response to human diseased cells (*e.g.*, cancer or infected cells) expressing the disease associate antigen as described herein, resist soluble disease associate antigen as described herein, mediate bystander killing and mediate regression of an established human disease, including cancer.

[00553] The disclosure also includes a type of cellular therapy where immune effector cells (*e.g.*, T cells and NKT cells) or stem cells that are capable of giving rise to immune effector cells (*e.g.*, T cells or NK cells) are modified to express a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) or a CAR with accessory modules (*e.g.*, backbones 1-60) and are used *ex vivo* to purge the bone marrow or peripheral blood hematopoietic stem cells of disease-associated cells (*e.g.* cancer cells). As an example, T cells expressing a CD19-specific CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) are cocultured with bone marrow or peripheral blood stem cell sample taken from a patient with acute lymphocytic leukemia or non-Hodgkin lymphoma so as to kill off any leukemia or lymphoma cells present in the bone marrow or peripheral blood stem cell preparation. After a

suitable duration of culture *in vitro* (ex vivo), which may range from a 6 hours to several days, the purged bone marrow and peripheral blood sample is used for autologous transplant in the patient.

[00554] *Ex vivo* expansion of hematopoietic stem and progenitor cells has been described in U.S. Pat. No. 5,199,942, and is incorporated herein by reference, and can be applied to the cells of the disclosure. However, the disclosure is not limited to any particular method of ex vivo expansion of the cells and other suitable methods known in the art can be utilized.

Briefly, ex vivo culture and expansion of hematopoietic stem 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.

[00555] In addition to using a cell-based vaccine in terms of ex vivo immunization, the disclosure also provides compositions and methods for *in vivo* immunization to elicit an immune response directed against an antigen in a patient.

[00556] In some embodiments, the fully-human CAR- or next generation CAR-modified genetically modified cells (such as T cells, NKT cells) of the disclosure may be a type of vaccine for ex vivo immunization and/or *in vivo* therapy in a mammal (for example, human). 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 CAR to the cells or iii) cryopreservation of the cells. *Ex vivo* procedures are well known in the art, for example, as described in U.S. Pat. No. 5,199,942, incorporated herein by reference.

[00557] In addition to using a cell-based vaccine in terms of ex vivo immunization, the disclosure also provides compositions and methods for *in vivo* immunization to elicit an immune response directed against an antigen in a patient.

[00558] Further described herein are methods for controlling the activity of CAR-T cells when administered to the patients. In some embodiment these methods can be used to control the side effects of CAR-T cells, such as cytokine release syndrome, capillary leak syndrome and neurological complications. In some embodiment the method involves administration of inhibitors of tyrosine kinases, particularly Src family kinase, and in particular Lck kinase. In one embodiment, the method involves administration of Dasatinib, an oral small molecule inhibitor of Abl and Src family tyrosine kinases (SFK), including p56Lck (Lck) (Lee KC et al, Leukemia (2010) 24, 896–900). In one embodiment, the Src kinase inhibitor is

administered to the patient after the administration of CAR-expressing cells to control or terminate the activity of CAR-expressing cells. In one embodiment, an Lck inhibitor is administered to the patient after the administration of CAR-expressing cells to control or terminate the activity of CAR-expressing cells. In one embodiment, Lck inhibitor is A-770041.

[00559] In one embodiment, Dasatinib is administered to the patient after the administration of CAR-expressing cells to control or terminate the activity of CAR-expressing cells. In one embodiment, dasatinib is administered orally at a dose of at least 10 mg/day, 20 mg/day, 40mg/day, 60mg/day, 70mg/day, 90 mg/day, 100mg/day, 140mg/day, 180mg/day, 210mg/day, 250mg/day or 280mg/day.

[00560] In one embodiment, Ponatinib is administered to the patient after the administration of CAR-expressing cells to control or terminate the activity of CAR-expressing cells. In one embodiment, ponatinib is administered orally at a dose of at least 15 mg/day, 30 mg/day, 45mg/day, 60mg/day.

[00561] T lymphocytes have a limited replicative life span until they reach the terminally differentiated state and then enter into a replicative senescence phase due to progressive loss of telomeres with age. Human T lymphocytes display a limited life-span of about 30–50 population doublings when cultured *in vitro*.

[00562] Further contemplated herein are methods for promoting the survival and proliferation of peripheral blood mononuclear cells and T cells and preventing their replicative senescence to extend the life span of immune cells (*e.g.*, lymphocytes and NK cells) for the purpose of adoptive cell therapy. The method entails ectopic expression of viral and/or cellular proteins that promote survival and proliferation and block activation induced cell death. An exemplary protein suitable for this purpose includes viral FLICE Inhibitory Protein (vFLIP) K13 encoded by the Kaposi's sarcoma associated herpesvirus (also known as human herpesvirus 8). In some embodiments, the above viral and cellular proteins are expressed in the immune cells (*e.g.*, T cells and NK cells) in their native state or carrying small epitope tags and are functionally active in a constitutive manner. In other embodiments, the above viral and cellular proteins are expressed in the immune cells (*e.g.*, T cells and NK cells) in fusion with one or more copies of a switch domain (or a dimerization domain), such as FKBP and FKBPx2. In other embodiment, the FKBP or the FKBP-x2 domain may additionally carry an N-terminal myristoylation (Myr) sequence to anchor the fusion proteins to the cell membrane. The fusion proteins carrying the switch domains are functionally inactive in their basal state but are activated upon addition of a dimerizer agent, such as

AP20187 as described in PCT/US2017/024843, which is incorporated by reference in its entirety herein.

[00563] Further contemplated herein are methods for promoting lentiviral mediated transduction and/or expression of a foreign gene and/or cDNA. The method involves expression of HIV1-Vif protein. In some embodiment, the Vif protein is encoded on the same vector as the foreign gene and/or cDNA. In some embodiment, the Vif protein is encoded on a different vector as the foreign gene/cDNA. Exemplary foreign gene/cDNA whose transfer and/or expression can be enhanced by coexpression of HIV1 Vif protein include CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR), recombinant TCR, globin, and adenosine deaminase *etc.* In some embodiment, co-expression of HIV1 Vif protein can be used to promote the lentiviral mediated transduction and/or expression of any encoded gene/cDNA or nucleic acid fragment. In some embodiment, the HIV1 Vif protein is encoded on the same vector as the gene/cDNA of interest. An exemplary vector encoding a CAR and coexpressing HIV1 Vif protein is presented in SEQ ID NO: 11268. Exemplary nucleic acid cassettes encoding CARs and HIV Vif are presented in SEQ ID NOs: 11244-11267. In some embodiment, the HIV1 Vif protein is encoded on a different vector as the gene/cDNA of interest. The HIV1 Vif protein can be used to enhance gene transfer/expression in any mammalian cell. In some embodiments, the HIV1 Vif protein is used to enhance gene transfer/expression into peripheral blood mononuclear cells, T cells, NK cells, NKT cells, B cells, hematopoietic stem cells, induced pluripotent stem cells, liver cells, brain cells or skin cells. The HIV1 Vif protein can be used to enhance gene transfer/expression into

[00564] In an embodiment, an immune effector cell, *e.g.*, a T cell, ectopically expresses one or more of a viral or cellular signaling protein selected from the group of K13-vFLIP (SEQ ID NO: 4107), MC159 (SEQ ID NO:4108), cFLIP-L/MRIT-alpha (SEQ ID NO: 4109), cFLIP-p22 (SEQ ID NO: 4110), HIV1-Vif (SEQ ID NO: 4117), HTLV1-TAX (SEQ ID NO: 4113), HTLV2-TAX (SEQ ID NO: 4114), HTLV2-TAX-RS (SEQ ID NO: 4115) or a protein with 70-99% identity to amino acid sequences of the above proteins.

[00565] In an embodiment, an immune effector cell, *e.g.*, a T cell, ectopically expresses a fusion protein containing one or more switch domains, *e.g.*, FKBP, FKBPx2 or Myr-FKBP, and one or more viral or cellular signaling protein selected from the group K13-vFLIP (SEQ ID NO: 4107), MC159 (SEQ ID NO:4108), cFLIP-L/MRIT-alpha (SEQ ID NO: 4109), cFLIP-p22 (SEQ ID NO: 4110), HIV1-Vif (SEQ ID NO: 4117), HTLV1-TAX (SEQ ID NO: 4113), HTLV2-TAX (SEQ ID NO: 4114), HTLV2-TAX-RS (SEQ ID NO: 4115).

[00566] In some aspects, this disclosure provides a method of producing an immune effector cell suitable for adoptive cellular therapy, comprising contacting the cell with a nucleic acid encoding partially or completely one or more of a viral or cellular signaling protein selected from the group of K13-vFLIP (SEQ ID NO: 4107), MC159 (SEQ ID NO:4108), cFLIP-L/MRIT-alpha (SEQ ID NO: 4109), cFLIP-p22 (SEQ ID NO: 4110), HIV1-Vif (SEQ ID NO: 4117), HTLV1-TAX (SEQ ID NO: 4113), HTLV2-TAX (SEQ ID NO: 4114), HTLV2-TAX-RS (SEQ ID NO: 4115) or proteins with 70-99% identity to amino acid sequences of the above proteins.

[00567] In some aspects, this disclosure provides a method of producing an immune effector cell suitable for adoptive cellular therapy, comprising contacting the cell with a nucleic acid encoding a fusion protein containing one or more switch domains, *e.g.*, FKBP, FKBPx2 or Myr-FKBP, and one or more viral or cellular signaling protein is selected from the group of K13-vFLIP (SEQ ID NO: 4107), MC159 (SEQ ID NO:4108), cFLIP-L/MRIT-alpha (SEQ ID NO: 4109), cFLIP-p22 (SEQ ID NO: 4110), HIV1-Vif (SEQ ID NO: 4117), HTLV1-TAX (SEQ ID NO: 4113), HTLV2-TAX (SEQ ID NO: 4114), HTLV2-TAX-RS (SEQ ID NO: 4115) or proteins with 70-99% identity to amino acid sequences of the above proteins.

[00568] In an embodiment, the cell suitable for adoptive cell therapy expresses a natural or synthetic immune receptor. Exemplary such immune receptors include a chimeric antigen receptor (CAR), a T cell receptor (TCR), a chimeric T cell receptor (cTCR), a synthetic T cell receptor, a TCR fusion protein (TFP), a Ab-TCR and a synthetic notch receptor. In an embodiment, the cell may be contacted with the nucleic acid encoding the viral and cellular signaling proteins before, simultaneous with, or after being contacted with a construct encoding a natural or synthetic immune receptor. In an embodiment, the cell may be contacted with the nucleic acid encoding the viral and cellular signaling proteins containing a switch or dimerization domain before, simultaneous with, or after being contacted with a construct encoding a natural or synthetic immune receptor.

[00569] In one aspect, the disclosure features a method of making a population of immune effector cells (*e.g.*, T cells, NK cells). In an embodiment, the method comprises: providing a population of immune effector cells (*e.g.*, T cells or NK cells), contacting the population of immune effector cells with a nucleic acid encoding an immune receptor (*e.g.*, CAR, TCR, synthetic TCR) and contacting the population of immune effector cells with a nucleic acid encoding a viral or cellular signaling protein, under conditions that allow for immune receptor and viral or cellular signaling protein co-expression.

[00570] In an embodiment, the nucleic acid encoding the viral or cellular signaling protein is DNA. In an embodiment, the nucleic acid encoding the viral or cellular signaling protein contains promoter capable of driving expression of the viral and cellular signaling proteins. In an embodiment, the nucleic acid encoding the viral or cellular signaling protein and the nucleic acid encoding the immune receptor is expressed from the same vector. In an embodiment, the nucleic acid encoding the viral or cellular signaling protein and the nucleic acid encoding the immune receptor is expressed from separate vectors. In an embodiment, the nucleic acid encoding the viral or cellular signaling protein (subunit 1) and the nucleic acid encoding the immune receptor (subunit 2) is expressed from the same polynucleotide fragment containing an internal ribosomal entry site (IRES) that allows the translation of the second subunit. In an embodiment, the nucleic acid encoding the viral or cellular signaling protein (subunit 1) and the nucleic acid encoding the immune receptor (subunit 2) is expressed from a single polynucleotide fragment that encodes and the different subunits are separated by a cleavable linker.

[00571] In an embodiment, the nucleic acid encoding the viral or cellular signaling protein is an *in vitro* transcribed RNA. In an embodiment, the viral or cellular signaling protein (subunit 1) and the immune receptor (subunit 2) is expressed from the same RNA containing an internal ribosomal entry site (IRES) that allows the translation of the second subunit. In an embodiment, the viral or cellular signaling protein (subunit 1) and the immune receptor (subunit 2) are expressed from a single RNA and the different subunits are separated by cleavable linkers.

[00572] In an embodiment, the nucleic acid encoding the cellular signaling protein is the genomic copy of the said protein that is been activated by activation of its promoter by genetic or chemical means.

[00573] Therapeutic methods described herein comprise using compositions comprising genetically modified cells comprising nucleic acids encoding CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) described herein. In various embodiments, the therapeutic methods described herein may be combined with existing therapies and agents. The therapeutic compositions described herein, comprising genetically modified cells comprising nucleic acids encoding the CARs (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) described herein, are administered to the subject with at least one additional known therapy or therapeutic agent. In some embodiments, the compositions described herein and the additional therapy or therapeutic agents are administered sequentially. In some embodiments, the compositions described herein and the additional therapy or

therapeutic agents are administered simultaneously. The optimum order of administering the compositions described herein and the existing therapies will be apparent to a person of skill in the art, such as a physician.

[00574] A CAR or next generation CAR-expressing cell described herein and the at least one additional therapeutic agent can be administered simultaneously, in the same or in separate compositions, or sequentially. For sequential administration, the CAR-expressing cell described herein can be administered first, and the additional agent can be administered second, or the order of administration can be reversed.

[00575] Combinations therapies may be administered to the subject over the duration of the disease. Duration of the disease includes from diagnosis until conclusion of treatment, wherein the treatment results in reduction of symptoms and/or elimination of symptoms. In various embodiments, the effect of the two treatments can be partially additive, wholly additive, or greater than additive. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered.

[00576] The CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) therapy and/or other therapeutic agents, procedures or modalities can be administered during periods of active disorder, or during a period of remission or less active disease. The CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) therapy can be administered before the other treatment, concurrently with the treatment, post-treatment, or during remission of the disorder.

[00577] When administered in combination, the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) therapy and the additional agent (*e.g.*, second or third agent), or all, can be administered in an amount or dose that is higher, lower or the same than the amount or dosage of each agent used individually, *e.g.*, as a monotherapy. In certain embodiments, the administered amount or dosage of the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) therapy, the additional agent (*e.g.*, second or third agent), or all, is lower (*e.g.*, at least 20%, at least 30%, at least 40%, or at least 50%) than the amount or dosage of each agent used individually, *e.g.*, as a monotherapy. In other embodiments, the amount or dosage of the CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) therapy, the additional agent (*e.g.*, second or third agent), or all, that results in a desired effect (*e.g.*, treatment of cancer) is lower (*e.g.*, at least 20%, at least 30%, at least 40%, or at least 50% lower) than the amount or dosage of each agent used individually, *e.g.*, as a monotherapy, required to achieve the same therapeutic effect.

[00578] Further method aspects relate administering to the subject an effective amount of a cell, *e.g.*, an immune effector cell, or a population thereof, each cell comprising a CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) molecule, optionally in combination with an agent that increases the efficacy and/or safety of the immune cell. In further aspects, the agent that increases the efficacy and/or safety of the immune cell is one or more of: (i) a protein phosphatase inhibitor; (ii) a kinase inhibitor; (iii) a cytokine; (iv) an inhibitor of an immune inhibitory molecule; or (v) an agent that decreases the level or activity of a TREG cell; (vi) an agent that increase the proliferation and/or persistence of CAR-modified cells (vii) a chemokine (viii) an agent that increases the expression of CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) (ix) an agent that allows regulation of the expression or activity of CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like), (x) an agent that allows control over the survival and/or persistence of CAR-modified cells, (xi) an agent that controls the side effects of CAR-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 CAR (*e.g.*, CAR I, CAR II, SIR, zSIR, Ab-TCR, TFP and the like) is directed; (xv) an adenosine A2a receptor antagonist; (xvi) an agent that depletes monocytes and/or macrophages; (xvii) Etoposide

[00579] In some embodiments, a the genetically modified cells described herein may be used in a treatment regimen in combination with surgery, chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies or other antibody therapies, cytoxin, fludarabine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, cytokines, and irradiation, peptide vaccine, such as that described in Izumoto *et al.* 2008 J Neurosurg 108:963-971. In one embodiment, a CAR-expressing cell described herein can be used in combination with a chemotherapeutic agent. Exemplary chemotherapeutic agents include an anthracycline (*e.g.*, doxorubicin (*e.g.*, liposomal doxorubicin)), a vinca alkaloid (*e.g.*, vinblastine, vincristine, vindesine, vinorelbine), an alkylating agent (*e.g.*, cyclophosphamide, decarbazine, melphalan, ifosfamide, temozolomide), an immune cell antibody (*e.g.*, alemtuzumab, gemtuzumab, rituximab, ofatumumab, tositumomab, brentuximab), an antimetabolite (including, *e.g.*, folic acid antagonists, pyrimidine analogs, purine analogs and adenosine deaminase inhibitors (*e.g.*, fludarabine)), an mTOR inhibitor, a TNFR glucocorticoid induced TNFR related protein (GITR) agonist, a proteasome inhibitor (*e.g.*, aclacinomycin A, gliotoxin or

bortezomib), an immunomodulator such as thalidomide or a thalidomide derivative (*e.g.*, lenalidomide).

[00580] In some embodiments, a CAR- expressing cell described herein is administered to a subject in combination with cyclophosphamide and fludarabine.

[00581] In some embodiments, a CAR- expressing cell described herein is administered to a subject who has been previously administered both myeloablative and lymphodepleting chemotherapy. Exemplary myeloablative and lymphodepleting conditioning regimens include FCE (Fludarabine 25 mg/m²/day, days -7 to -3; cyclophosphamide 200 mg/m²/day, days -7 to -3; and etoposide 250 mg/m²/day, days -4 to -3), FCIE (Fludarabine 25 mg/m²/day, days -7 to -3; cyclophosphamide 200 mg/m²/day, days -7 to -3; idarubicin 12 mg/m²/day, days -7 to -5 and etoposide 250 mg/m²/day, days -4 to -3), FluCyE (fludarabine 30 mg/m²/day, cytarabine 1.5 g/m² /day administered following fludarabine and etoposide 100 mg/m²/day with each of the drugs given on days -6 to -1), or FE (fludarabine 30 mg/m²/day and Etoposide 100 mg/m²/day on days -5 to day -1). In some embodiments, CAR- expressing cell are administered to the subject between 1 day to 5 days after the last dose of chemotherapy.

[00582] In some embodiments, a CAR- expressing cell described herein is administered to a subject who has been previously administered etoposide. In some embodiments, Etoposide is administered intravenously at a dose of 50 mg/m²/day to 250 mg/m²/day for 1-5 days. In some embodiments, Etoposide is dosed at 5 mg/kg per dose for between 1 to 5 doses. In some embodiments, CAR- expressing cell are administered to the subject between 1 day to 5 days after the last dose of Etoposide.

[00583] In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with bendamustine and rituximab.

[00584] In some embodiments, a CAR-expressing cell described herein is administered to a subject in combination with rituximab, cyclophosphamide, doxorubicin, vincristine, and/or a corticosteroid (*e.g.*, prednisone). In embodiments, a CAR or next generation CAR-expressing cell described herein is administered to a subject in combination with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP). In embodiments, the subject has diffuse large B-cell lymphoma (DLBCL).

[00585] In some embodiments, a CAR-expressing cell described herein is administered to a subject in combination with etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and/or rituximab. In embodiments, a CAR or next generation CAR-expressing cell described herein is administered to a subject in combination with etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab (EPOCH-R). In embodiments, a

CAR-expressing cell described herein is administered to a subject in combination with dose adjusted EPOCH-R (DA-EPOCH-R). In embodiments, the subject has a B cell lymphoma, *e.g.*, a Myc-rearranged aggressive B cell lymphoma.

[00586] In some embodiments, a CAR-expressing cell described herein is administered to a subject in combination with brentuximab. Brentuximab is an antibody-drug conjugate of anti-CD30 antibody and monomethyl auristatin E. In some embodiments, the subject has Hodgkin's lymphoma (HL), *e.g.*, relapsed or refractory HL. In some embodiments, the subject comprises CD30+ HL. In embodiments, the subject has undergone an autologous stem cell transplant (ASCT).

[00587] In some embodiments, a CAR-expressing cell described herein is administered to a subject in combination with a CD20 inhibitor, *e.g.*, an anti-CD20 antibody (*e.g.*, an anti-CD20 mono- or bispecific antibody) or a fragment thereof.

[00588] In one embodiment, a CAR-expressing cell described herein is administered to a subject in combination with an mTOR inhibitor, *e.g.*, an mTOR inhibitor described herein, *e.g.*, a rapalog such as everolimus. In one embodiment, the mTOR inhibitor is administered prior to the administration of CAR -expressing cell. For example, in one embodiment, the mTOR inhibitor can be administered prior to apheresis of the cells. In one embodiment, the subject has CLL.

[00589] In one embodiment, a CAR-expressing cell described herein can be used in combination with a kinase inhibitor. In one embodiment, the kinase inhibitor is a CDK4 inhibitor, *e.g.*, a CDK4 inhibitor described herein, *e.g.*, a CD4/6 inhibitor, such as, *e.g.*, palbociclib or PD0332991. In one embodiment, the kinase inhibitor is a BTK inhibitor, *e.g.*, a BTK inhibitor described herein, such as, *e.g.*, ibrutinib. In some embodiments, ibrutinib is administered at a dosage of about 300-600 mg/day (*e.g.*, about 300-350, 350-400, 400-450, 450-500, 500-550, or 550-600 mg/day, *e.g.*, about 420 mg/day or about 560 mg/day), *e.g.*, orally. In embodiments, the ibrutinib is administered at a dose of about 250 mg, 300 mg, 350 mg, 400 mg, 420 mg, 440 mg, 460 mg, 480 mg, 500 mg, 520 mg, 540 mg, 560 mg, 580 mg, 600 mg (*e.g.*, 250 mg, 420 mg or 560 mg) daily for a period of time, *e.g.*, daily for 21 day cycle, or daily for a 28 day cycle. In one embodiment, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more cycles of ibrutinib are administered.

[00590] In one embodiment, the kinase inhibitor is an mTOR inhibitor, *e.g.*, an mTOR inhibitor described herein, such as, *e.g.*, rapamycin, a rapamycin analog, OSI- 027. In one embodiment, the kinase inhibitor is a dual PI3K/mTOR inhibitor described herein, such as, *e.g.*, PF-04695102.

[00591] In one embodiment, the kinase inhibitor is a Src kinase inhibitor. In one embodiment, the kinase inhibitor is Dasatinib. In one embodiment, the Src kinase inhibitor is administered to the patient after the administration of CAR expressing cells to control or terminate the activity of CAR-expressing cells. In one embodiment, Dasatinib is administered to the patient after the administration of CAR-expressing cells to control or terminate the activity of CAR-expressing cells. In one embodiment, dasatinib is administered orally at a dose of at least 10 mg/day, 20 mg/day, 40mg/day, 60mg/day, 70 mg/day, 90 mg/day, 100mg/day, 140 mg/day, 180 mg/day or 210 mg/day.

[00592] In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with an anaplastic lymphoma kinase (ALK) inhibitor.

[00593] Drugs that inhibit either the calcium dependent phosphatase calcineurin (cyclosporine and FK506) or inhibit the p70S6 kinase that is important for growth factor induced signaling (rapamycin). (Liu *et al.*, Cell 66:807-815, 1991; Henderson *et al.*, Immun. 73:316-321, 1991; Bierer *et al.*, Curr. Opin. Immun. 5:763-773, 1993) can also be used. In a further aspect, the cell compositions of the disclosure may be administered to a patient in conjunction with (*e.g.*, before, simultaneously or following) bone marrow transplantation, T cell ablative therapy using chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, and/or antibodies such as OKT3 or CAMPATH. In one aspect, the cell compositions of the disclosure are administered following B-cell ablative therapy such as agents that react with CD20, *e.g.*, Rituxan. For example, in one embodiment, subjects may undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. In certain embodiments, following the transplant, subjects receive an infusion of the expanded immune cells of the disclosure. In an additional embodiment, expanded cells are administered before or following surgery.

[00594] In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with an autologous stem cell transplant, an allogeneic stem cell transplant, an autologous bone marrow transplant or an allogeneic bone marrow transplant.

[00595] In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with microtransplant or HLA mismatched allogeneic cellular therapy (Guo M *et al.*, J Clin Oncol. 2012 Nov 20;30(33):4084-90).

[00596] In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with an indoleamine 2,3-dioxygenase (IDO) inhibitor.

[00597] In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with a modulator of myeloid-derived suppressor cells (MDSCs).

[00598] In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with a Brd4 or BET (bromodomain and extra-terminal motif) inhibitor. Exemplary Brd4 inhibitors that can be administered in combination with CAR-expressing cells include but are not limited to JQ1, MS417, OTX015, LY 303511 and Brd4 inhibitor as described in US 20140256706 A1 and any analogs thereof.

[00599] In some embodiments, a CAR-expressing cell described herein is administered to a subject in combination with an interleukin-15 (IL-15) polypeptide, a interleukin-15 receptor alpha (IL-15Ra) polypeptide, or a combination of both a IL-15 polypeptide and a IL-15Ra polypeptide *e.g.*, hetIL-15 (Admune Therapeutics, LLC). In some embodiments, het-IL-15 is administered subcutaneously.

[00600] In one embodiment, the subject can be administered an agent which reduces or ameliorates a side effect associated with the administration of a CAR-expressing cell. Side effects associated with the administration of a CAR-expressing cell include, but are not limited to CRS, and hemophagocytic lymphohistiocytosis (HLH), also termed Macrophage Activation Syndrome (MAS).

[00601] Accordingly, the methods described herein can comprise administering a CAR-expressing cell described herein to a subject and further administering one or more agents to manage elevated levels of a soluble factor resulting from treatment with a CAR-expressing cell. In one embodiment, the soluble factor elevated in the subject is one or more of IFN- γ , TNF α , IL-2 and IL-6. In an embodiment, the factor elevated in the subject is one or more of IL-1, GM-CSF, IL-10, IL-8, IL-5 and fractalkine. Therefore, an agent administered to treat this side effect can be an agent that neutralizes one or more of these soluble factors. In one embodiment, the agent that neutralizes one or more of these soluble forms is an antibody or antigen binding fragment thereof. Examples of such agents include, but are not limited to a steroid (*e.g.*, corticosteroid), Src inhibitors (*e.g.*, Dasatinib) an inhibitor of TNF α , and an inhibitor of IL-6. An example of a TNF α inhibitor is an anti-TNF α antibody molecule such as, infliximab, adalimumab, certolizumab pegol, and golimumab. Another example of a TNF α inhibitor is a fusion protein such as entanercept. An example of an IL-6 inhibitor is an anti-IL-6 antibody molecule or an anti-IL-6 receptor antibody molecule such as tocilizumab (toe), sarilumab, elsilimomab, CNTO 328, ALD518/BMS-945429, CNTO 136, CPSI-2364, CDP6038, VX30, ARGX-109, FE301, and FM101. In one embodiment, the anti-IL-6 receptor antibody molecule is tocilizumab. In one embodiment, the IL-6 inhibitor is a camelid bispecific antibody that binds to IL6R and human serum albumin (*e.g.*, IL6R-304-Alb8) (SEQ ID NO: 2649). An example of an IL-1R based inhibitor is anakinra. In one embodiment,

an agent administered to treat the side effects of CAR-expressing cells is a Src inhibitor (*e.g.*, Dasatinib). In one embodiment, an agent administered to treat the side effects of CAR-expressing cells is the Src inhibitor Dasatinib. In embodiments, Dasatinib is administered at a dose of about 10 mg/day to 240 mg/day (*e.g.*, 10mg/day, 20mg/day, 40mg/day, 50mg/day, 70 mg/day, 80mg/day, 100mg/day, 110mg/day, 120mg/day, 140mg/day, 180mg/day, 210 mg/day, 240mg/day or 300mg/day).

[00602] In one embodiment, the subject can be administered an agent which enhances the activity of a CAR-expressing cell. For example, in one embodiment, the agent can be an agent which inhibits an inhibitory molecule. Inhibitory molecules, *e.g.*, Programmed Death 1 (PD-1), can, in some embodiments, decrease the ability of a CAR-expressing cell to mount an immune effector response. Examples of inhibitory molecules include PD-1, PDL1, CTLA-4, TIM-3, CEACAM (*e.g.*, CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG-3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4 and TGFR beta. Inhibition of an inhibitory molecule, *e.g.*, by inhibition at the DNA, RNA or protein level, can optimize a CAR-expressing cell performance. In embodiments, an inhibitory nucleic acid, *e.g.*, an inhibitory nucleic acid, *e.g.*, a dsRNA, *e.g.*, an siRNA or shRNA, a clustered regularly interspaced short palindromic repeats (CRISPR), a transcription-activator like effector nuclease (TALEN), or a zinc finger endonuclease (ZFN), *e.g.*, as described herein, can be used to inhibit expression of an inhibitory molecule in the CAR-expressing cell. In an embodiment the inhibitor is an shRNA. In an embodiment, the inhibitory molecule is inhibited within a CAR-expressing cell. In these embodiments, a dsRNA molecule that inhibits expression of the inhibitory molecule is linked to the nucleic acid that encodes a component, *e.g.*, all of the components, of the CAR. In one embodiment, the inhibitor of an inhibitory signal can be, *e.g.*, an antibody or antibody fragment that binds to an inhibitory molecule. For example, the agent can be an antibody or antibody fragment that binds to PD-1, PD-L1, PD-L2 or CTLA4. In an embodiment, the agent is an antibody or antibody fragment that binds to TIM3. In an embodiment, the agent is an antibody or antibody fragment that binds to CEACAM (CEACAM-1, CEACAM-3, and/or CEACAM-5). In an embodiment, the agent is an antibody or antibody fragment that binds to LAG3.

[00603] Antibodies, antibody fragments, and other inhibitors of PD-1, PD-L1 and PD-L2 are available in the art and may be used combination with a CAR of the disclosure described herein. Pembrolizumab is a humanized IgG4 monoclonal antibody that binds to PD-1. In other embodiments, the agent that enhances the activity of a CAR-expressing cell is a CEACAM inhibitor (*e.g.*, CEACAM-1, CEACAM-3, and/or CEACAM-5 inhibitor).

[00604] In one embodiment, the agent which enhances activity of a CAR-described herein is another agent that increases the expression of the target antigen against which the CAR is directed. The agents that can be administered to the subject receiving a CAR-expressing cell described herein include: Arsenic trioxide, ATRA (all-trans-retinoic acid), compounds 27, 40, 49 of (Du et al, Blood; Prepublished online October 12, 2016), IDH2 inhibitors (*e.g.*, AG-221) or a combination thereof. In an embodiment, the agents are administered prior to, concurrently or after administration of CAR-expressing cells. In preferred embodiments these agents are administered prior to administration of CAR-expressing cells. In preferred embodiment, the CAR expressing cells that are administered with the above agents target a B cell antigen (*e.g.*, CD19, CD20, or CD22 *etc.*).

[00605] In one embodiment, the agent which enhances activity of a CAR or next generation CAR described herein is a soluble receptor. Soluble receptor that can be administered to the subject receiving a CAR-expressing cell described herein include: sHVEM (SEQ ID NO: 2664), sHVEM-Alb8-vHH fusion protein (SEQ ID NO: 2665), or a combination thereof. The soluble receptor can be administered once a day or more than once a day, *e.g.*, twice a day, three times a day, or four times a day. The soluble receptor can be administered for more than one day, *e.g.* the soluble receptor is administered for 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, or 4 weeks. For example, the soluble receptor is administered once a day for 7 days.

[00606] In one embodiment, the subject can be administered an agent which protects against the toxicity of a CAR-expressing cell on normal tissues. One of the limitations of CAR-T cell therapy could be toxicity on normal tissue. For example, CAR or next generation CAR targeting CD19 could lead to long-term depletion of normal B cells, which also express CD19 antigen. In one embodiment, CD19 CAR-T cell therapy can be combined with knock-out or mutation of endogenous CD19 in normal hematopoietic stem cells. In one embodiment, the knock out or mutation of the endogenous CD19 is achieved using CRIPS/Cas9, Talons or other suitable gene editing methods which are known in the art. The epitope of CD19 bound by the CD19 CAR-T cells in current clinical use has been mapped to exon2-4. In one embodiment, missense or nonsense mutations are generated in exon 2 (or other suitable exons/regions that are recognized by CD19 targeted CAR T-cells) of autologous or allogeneic hematopoietic stem cells using CRISP/Cas9, Zn finger nucleases, Talons or other methods known in the art. In one embodiment, the subject is given CD19 CAR-T cells infusion to control his/her disease and an autologous or allogeneic stem cell transplant using CD19 deleted/mutated hematopoietic stem cells. As the B cells that will

originate from the modified stem cells will not be targeted by CD19-CAR-T cells, the patient will escape B cell aplasia which is a common side effect of CD19 CAR-T cells. In another embodiment, MPL CAR-T cell therapy is combined with knock-out or mutation of endogenous MPL in normal hematopoietic stem cells. In another embodiment, CD123 CAR-T cell therapy is combined with knock-out or mutation of endogenous CD123 in normal hematopoietic stem cells. In another embodiment, CD33 CAR-T cell therapy is combined with knock-out or mutation of endogenous CD33 in normal hematopoietic stem cells. In another embodiment, CD20 CAR-T cell therapy is combined with knock-out or mutation of endogenous CD20 in normal hematopoietic stem cells. In another embodiment, CD22 CAR-T cell therapy is combined with knock-out or mutation of endogenous CD22 in normal hematopoietic stem cells. In another embodiment, CS1 CAR-T cell therapy is combined with knock-out or mutation of endogenous CS1 in normal hematopoietic stem cells. In another embodiment, BCMA CAR-T cell therapy is combined with knock-out or mutation of endogenous BCMA in normal hematopoietic stem cells. In another embodiment, CD45 CAR-T cell therapy is combined with knock-out or mutation of endogenous CD45 in normal hematopoietic stem cells or immune effector cells (*e.g.*, T cells or NK cells). Essentially, a similar approach could be used to mitigate the toxicity of CAR-T cells against normal tissue where the antigen targeted by the CAR or next generation CAR is also expressed on normal hematopoietic stem cells or one of its progenies.

[00607] In another embodiment, CAR-T cell therapy is combined with knock-out or mutation of endogenous gene or protein targeted by the CAR or next generation CAR in the immune effector cell (*e.g.*, T cells or NK cells) or stem cells that give rise to immune effector cells. For example, since CD45 is expressed on all hematopoietic cells, CAR-T cells targeting CD45 would be difficult to generate as they would be killed off by neighboring CD45-CAR-T cells. However, such cells can be generated if expression of CD45 CAR in T cells is combined with knock-down or deletion of endogenous CD45 in the T cells in which CD45 CAR or next generation CAR is being expressed. Essentially a similar approach can be used to generate CAR or next generation CAR targeting other antigens that are expressed on immune effector cells. Exemplary such antigens include, but are not limited to, CD5, TCR α , TCR β 1, TCR β 2, TCR γ , TCR δ , preTCR α and various receptors expressed on NK cells.

[00608] Cytokines that can be administered to the subject receiving a CAR-expressing cell described herein include: IL-2, IL-4, IL-7, IL-9, IL-15, IL-18, LIGHT, and IL-21, or a combination thereof. In preferred embodiments, the cytokine administered is IL-7, IL-15, or IL-21, IL12F, or a combination thereof. The cytokine can be administered once a day or more

than once a day, *e.g.*, twice a day, three times a day, or four times a day. The cytokine can be administered for more than one day, *e.g.* the cytokine is administered for 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, or 4 weeks. For example, the cytokine is administered once a day for 7 days. In a preferred embodiment, the cytokine administered after administration of CAR-expressing cells is IL-7.

[00609] In one embodiment, the agent which enhances activity of a CAR-expressing cell described herein is a Brd4 inhibitor or an siRNA or an shRNA targeting BRD4 as described in (Tolani, B *et al.*, *Oncogene*, 29; 33(22):2928-37. PMID: 23792448) (Tolani, Gopalakrishnan, Punj, Matta, & Chaudhary, 2014).

[00610] Also provided herein are pharmaceutical compositions comprising any one or more of the chimeric antigen receptors, the polynucleotides, the polypeptides, the vectors, the viruses, and/or the genetically engineered cells and/or chemical compounds described herein and a pharmaceutically acceptable carrier. 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 are in one aspect formulated for intravenous administration.

[00611] Pharmaceutical compositions of the disclosure may be administered in a manner appropriate to the disease to be treated. 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, although appropriate dosages may be determined by clinical trials.

[00612] When a "therapeutically effective amount" is indicated, the precise 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). It can generally be stated that a pharmaceutical composition comprising the genetically modified cells (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).

[00613] In some embodiments, it may be desired to administer activated genetically modified cells (T cells, NK cells) to a subject and then subsequently redraw blood (or have an

apheresis performed), activate the genetically modified cells therefrom and reinfuse the patient with these activated and expanded genetically modified 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.

[00614] “Pharmaceutically acceptable excipient” means an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic, and desirable, and includes excipients that are acceptable for veterinary use as well as for human pharmaceutical use. Such excipients may be solid, liquid, semisolid, or, in the case of an aerosol composition, gaseous.

[00615] In various embodiments, the pharmaceutical compositions according to the disclosure may be formulated for delivery via any route of administration. “Route of administration” may refer to any administration pathway known in the art, including but not limited to aerosol, nasal, oral, intravenous, intramuscular, intraperitoneal, inhalation, transmucosal, transdermal, parenteral, implantable pump, continuous infusion, topical application, capsules and/or injections.

[00616] The pharmaceutical compositions according to the disclosure can also contain any pharmaceutically acceptable carrier. “Pharmaceutically acceptable carrier” as used herein refers to a pharmaceutically acceptable material, composition, or vehicle that is involved in carrying or transporting a compound of interest from one tissue, organ, or portion of the body to another tissue, organ, or portion of the body. For example, the carrier may be a liquid or solid filler, diluent, excipient, solvent, or encapsulating material, or a combination thereof. Each component of the carrier must be “pharmaceutically acceptable” in that it must be compatible with the other ingredients of the formulation. It must also be suitable for use in contact with any tissues or organs with which it may come in contact, meaning that it must not carry a risk of toxicity, irritation, allergic response, immunogenicity, or any other complication that excessively outweighs its therapeutic benefits.

[00617] The pharmaceutical compositions according to the disclosure can also be encapsulated, tableted or prepared in an emulsion or syrup for oral administration. Pharmaceutically acceptable solid or liquid carriers may be added to enhance or stabilize the composition, or to facilitate preparation of the composition. Liquid carriers include syrup, peanut oil, olive oil, glycerin, saline, alcohols and water. Solid carriers include starch, lactose, calcium sulfate, dihydrate, terra alba, magnesium stearate or stearic acid, talc, pectin,

acacia, agar or gelatin. The carrier may also include a sustained release material such as glyceryl monostearate or glyceryl distearate, alone or with a wax.

[00618] The pharmaceutical preparations are made following the conventional techniques of pharmacy involving milling, mixing, granulation, and compressing, when necessary, for tablet forms; or milling, mixing and filling for hard gelatin capsule forms. When a liquid carrier is used, the preparation will be in the form of syrup, elixir, emulsion or an aqueous or non-aqueous suspension. Such a liquid formulation may be administered directly p.o. or filled into a soft gelatin capsule.

[00619] The pharmaceutical compositions according to the disclosure may be delivered in a therapeutically effective amount. The precise therapeutically effective amount is that amount of the composition that will yield the most effective results in terms of efficacy of treatment in a given subject. This amount will vary depending upon a variety of factors, including but not limited to the characteristics of the therapeutic compound (including activity, pharmacokinetics, pharmacodynamics, and bioavailability), the physiological condition of the subject (including age, sex, disease type and stage, general physical condition, responsiveness to a given dosage, and type of medication), the nature of the pharmaceutically acceptable carrier or carriers in the formulation, and the route of administration. One skilled in the clinical and pharmacological arts will be able to determine a therapeutically effective amount through routine experimentation, for instance, by monitoring a subject's response to administration of a compound and adjusting the dosage accordingly. For additional guidance, see *Remington: The Science and Practice of Pharmacy* (Gennaro ed. 20th edition, Williams & Wilkins PA, USA) (2000).

[00620] The administration of the subject compositions may be carried out in any convenient manner, including by aerosol inhalation, injection, transfusion, implantation or transplantation. The compositions described herein may be administered to a patient trans-arterially, subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous (i.v.) injection, or intraperitoneally. In one aspect, the T cell compositions of the disclosure are administered to a patient by intradermal or subcutaneous injection. In one aspect, the T cell compositions of the disclosure are administered by i. v. injection. The compositions of immune effector cells (*e.g.*, T cells, NK cells) may be injected directly into a tumor, lymph node, or site of infection.

[00621] In a particular exemplary aspect, 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 such that one or more CAR or next generation CAR constructs of the disclosure may be introduced, thereby creating a CAR-T cell of the disclosure. 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 of the disclosure. In an additional aspect, expanded cells are administered before or following surgery.

[00622] In one embodiment, the CAR is introduced into immune effector cells (*e.g.*, T cells, NKT cells), *e.g.*, using *in vitro* transcription, and the subject (*e.g.*, human) receives an initial administration of CAR or next generation CAR immune effector cells (*e.g.*, T cells, NKT cells) of the disclosure, and one or more subsequent administrations of the CAR or next generation CAR immune effector cells (*e.g.*, T cells, NK cells) of the disclosure, wherein the one or more subsequent administrations are administered less than 15 days, *e.g.*, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, or 2 days after the previous administration. In one embodiment, more than one administration of the CAR or next generation CAR immune effector cells (*e.g.*, T cells, NK cells) of the disclosure are administered to the subject (*e.g.*, human) per week, *e.g.*, 2, 3, or 4 administrations of the CAR or next generation CAR immune effector cells (*e.g.*, T cells, NK cells) of the disclosure are administered per week. In one embodiment, the subject (*e.g.*, human subject) receives more than one administration of the CAR immune effector cells (*e.g.*, T cells, NK cells) per week (*e.g.*, 2, 3 or 4 administrations per week) (also referred to herein as a cycle), followed by a week of no CAR or next generation CAR immune effector cells (*e.g.*, T cells, NK cells) administrations, and then one or more additional administration of the CAR or next generation CAR immune effector cells (*e.g.*, T cells, NK cells) (*e.g.*, more than one administration of the CAR or next generation CAR immune effector cells (*e.g.*, T cells, NK cells) per week) is administered to the subject. In another embodiment, the subject (*e.g.*, human subject) receives more than one cycle of CAR or next generation CAR immune effector cells (*e.g.*, T cells, NK cells), and the time between each cycle is less than 10, 9, 8, 7, 6, 5, 4, or 3 days. In one embodiment, the CAR or next generation CAR immune effector cells (*e.g.*, T cells, NK cells) are administered every other day for 3 administrations per week. In one embodiment, the CAR immune effector cells (*e.g.*, T cells, NK cells) of the disclosure are administered for at least two, three, four, five, six, seven, eight or more weeks.

[00623] A potential issue that can arise in patients being treated using transiently expressing CAR or next generation CAR immune effector cells (*e.g.*, T cells, NK cells) (particularly with murine scFv bearing CAR-Ts) is anaphylaxis after multiple treatments.

[00624] Without being bound by this theory, it is believed that such an anaphylactic response might be caused by a patient developing humoral anti-CAR response, *i.e.*, anti-CAR antibodies having an anti-IgE isotype. It is thought that a patient's antibody producing cells undergo a class switch from IgG isotype (that does not cause anaphylaxis) to IgE isotype when there is a ten to fourteen days break in exposure to antigen.

[00625] If a patient is at high risk of developing an anaphylactic response to CAR or next generation CAR therapy or generating an IgE type CAR antibody response during the course of CAR or next generation CAR therapy, then omalizumab (Xolair) can be administered before or during the CAR or next generation CAR therapy.

[00626] If a patient is at high risk of generating an anti-CAR antibody response during the course of transient CAR or next generation CAR therapy (such as those generated by RNA transductions), CAR-T infusion breaks should not last more than ten to fourteen days.

[00627] Kits to practice the disclosure are also provided. For example, kits for treating a cancer in a subject, or making a CAR-T cell that expresses one or more of the CARs or next generation CARs disclosed herein. The kits may include a nucleic acid molecule or a polypeptide molecule encoding a CAR or next generation CAR or a vector encoding a CAR or next generation CAR 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 CAR or next generation CAR and chemicals, such as polybrene, to enhance the virus transduction. The kit may contain components for isolation of T cells for expressing a CAR or next generation CAR. Alternatively, the kit may contain immune effector cells (*e.g.*, T cells or NK cells) or stem cells expressing a CAR or next generation CAR. More than one of the disclosed CAR can be included in the kit. The kit can include a container and a label or package insert on or associated with the container.

[00628] 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 expressing a CAR or next generation CAR. 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 nucleic acid molecules, CARs or next generation CARs or T cells expressing a CAR or next generation CAR, 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 CAR or next generation CAR on T cells or of determining the number or percentage of T cells that express the CAR or of determining the functionality of CAR-T 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.

[00629] Animal models can also be used to measure CAR activity. For example, xenograft model using human cancer associated antigen described herein-specific CAR⁺ T cells to treat a primary human pre-B-ALL in immunodeficient mice can be used. See, *e.g.*, Milone *et al.*, Molecular Therapy 17(S): 1453-1464 (2009).

[00630] Dose dependent CAR treatment response can be evaluated. See, *e.g.*, Milone *et al.*, Molecular Therapy 17(S): 1453-1464 (2009).

[00631] Assessment of cell proliferation and cytokine production has been previously described, *e.g.*, at Milone *et al.*, Molecular Therapy 17(S): 1453-1464 (2009).

[00632] Cytotoxicity can be assessed by Matador assay or using a standard ⁵¹Cr-release assay. See, *e.g.*, Milone *et al.*, Molecular Therapy 17(8): 1453-1464 (2009).

[00633] Imaging technologies can be used to evaluate specific trafficking and proliferation of zSIRs in tumor-bearing animal models. Such assays have been described, for example, in Barrett *et al.*, Human Gene Therapy 22:1575-1586 (2011).

[00634] Other assays, including those described in the Example section herein as well as those that are known in the art can also be used to evaluate the CAR described herein.

EXAMPLES

[00635] The activity of a CAR can be tested by several *in vitro* and *in vivo* assays described herein and below. A general scheme for generating, selecting and using suitable CARs is provided below:

[00636] **Identification of target for CAR generation.** A suitable target against which the CAR is designed is selected based on search of the literature or gene expression databases. In

general, a suitable target for a CAR shows higher expression on the disease causing or disease associated cells as compared to normal healthy cells.

[00637] **Generation of CAR.** Once a candidate target antigen for CAR is identified, the antigen binding domain of CAR is designed based on information available in the literature. In general, the antigen binding domain of CAR is typically based on an antibody, an antibody fragments, scFV, or camelid vHH domains. The sequences of the variable chains of heavy (vH) and light (vL) chains of antibodies, the camelid vHH domains and various receptors and ligands can be obtained by sequencing or by publically available databases and can be used for synthesis of a CAR using the methods described herein as shown in different examples. The sequences comprising the antigen binding domains of CAR are codon optimized and synthesized artificially using publically available software (*e.g.* ThermoFisher or IDT) and commercial vendors (*e.g.* IDT). The resulting fragments are PCR amplified and cloned in different vectors containing the different CAR backbones using standard Molecular Biology techniques. The different CAR backbones are described in WO 2016/187349 A1, PCT/US2016/058305, US 62/429,597, PCT/US17/64379 and PCT/US2017/024843, which are incorporated in their entirety herein by reference. In general, CAR constructs are typically cloned in a lentiviral vector. The sequences of the constructs are confirmed using automated sequencing.

[00638] Another exemplary construct encoding a zSIR is pLenti-EF1 α -CD8SP-BCMA-Am06-HL-vL-IgCL-Bam-CD3zECDTMCP-opt-F-P2A-Spe-SP-Bst-BCMA-Am06-HL-vH-IgG1-CH1-KPN-CD3zECDTMCP-opt2-F-F2A-Xba-PAC-DWPRE (SEQ ID NO: 154). This construct has many convenient restriction sites so that the antigen binding domain fragments (*e.g.*, vL and vH domains) can be cut out and replaced with the antigen binding domain fragments targeting other antigens. The vector carries an Nhe I site upstream of the CD8 Signal peptide (CD8SP), which can be also used along with the Xho I site to clone in a new vL fragment carrying a 5' signal peptide. The BstB I and Mlu I sites can be used to replace the vH fragment. The Xho I and Spe I sites can be used to replace the module encoding IgCL-[IgCL-Bam-CD3zECDTMCP-opt-F-P2A with a different module. Similarly, the MluI and Xba sites can be used to replace the module containing IgG1-CH1-KPN-CD3zECDTMCP-opt2-F-F2A. The accessory module encoding PAC can be replaced using the Xba I (or Nde I) and SalI restriction sites. Thus, a person with ordinary skills in the art can use this vector and the sequence of the antigen binding domain (*e.g.*, vL and vH domains of an antibody) to generate zSIRs targeting any other new antigen.

[00639] Generation of secretory antigen-NLuc fusion proteins and antigen binding domain (ABD)-NLuc fusion proteins. (Optional step).

[00640] Secretory antigen-NLuc fusion proteins and antigen binding domain (ABD)-NLuc fusion proteins were generated and used as described in PCT/US2017/025602, which is incorporated herein in its entirety by reference. A panel of cell lines are tested for binding to the ABD-NLuc fusion protein to identify cell lines that express high level of CAR target and therefore can be used to test the activity of CAR. **Table A** provides an exemplary list of cell lines expressing different antigen targets that can be used to assay for the activity of a CAR of this disclosure. The cell lines expressing the target of CAR can be also identified using alternate methods such as literature search, immunostaining with commercially available antibodies or by searching publically available gene expression databases.

[00641] The immune effector cells expressing CAR are tested in the following assays to identify the functional CAR.

[00642] **(A) Topanga Assay (NLuc binding assay):** The control vector- and CAR-expressing Jurkat-NFAT-GFP or T cells are stained with the target Antigen-Nluc fusion protein (as described above) and their ability to bind to the target antigen is assayed by measuring Nluc activity. For example, Jurkat-NFAT-GFP cells expressing FMC63 based CAR targeting CD19 show increased binding to CD19-NLuc fusion protein as compared to control vector expressing Jurkat-NFAT-GFP cells or parental Jurkat-NFAT-GFP cells.

[00643] **(B) Induction of NFAT promoter driven GFP expression.** The control vector- and CAR-expressing Jurkat-NFAT-GFP cocultured for 4-24 hours with the target antigen-expressing cell line (described above) and their ability to bind to the target antigen is assayed by measuring induction of GFP expression using Flow Cytometry. Cellular supernatant is collected and assayed for the induction of cytokines (*e.g.*, IL2).

[00644] **(C) Assay for cytokine production:** The control vector- and CAR-expressing Jurkat-NFAT-GFP or T cells are cocultured with the target cell lines for 4-96 hours and supernatant examined for induction of cytokines (*e.g.*, IL2, IFN γ , TNF α *etc.*) expression using ELISA.

[00645] **(D) Assay for Cytotoxic Activity *in vitro* and *in vivo*:** The uninfected T cells or those expressing a control vector or CAR are cocultured with the target cell lines expressing a non-secretory form of a luciferase (such as GLuc, NLuc, Turboluc 16 *etc.*) for 4-96 hours and induction of cell lysis examined by measuring the luciferase activity as described in PCT/US17/52344. Alternate methods for measurement of cytotoxic activity (*e.g.*, ^{51}Cr release

assay or LDH release assay) can be used as well. The activity of T cells expressing a CAR can be also assayed *in vivo* using appropriate xenograft models in immunodeficient mice.

[00646] Based on the above methods, a person with ordinary skilled in the art can easily design, construct, test and select the appropriate functioning CAR or pool of CARs against any antigen. The CAR or a pool of CARs can be used for human clinical trials and clinical use for the prevention and treatment of various disease conditions. **Table 9** provides an exemplary list of human disease conditions that can be treated using the CARs of the disclosure.

[00647] 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.

[00648] 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.

[00649] Generating a diverse pool of CARs 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.*, v_L and v_H 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. The diversity of the CAR 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 and therapeutic controls described in the disclosure.

[00650] 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 (SEQ ID NO: 123-128) 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 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.

[00651] Gene fragments encoding the different signal peptides, antibody binding domains, linkers, TCR constant chains, cleavable linkers and selection markers (*e.g.*, PAC, EGFP, CNB30 *etc.*) were artificially synthesized in single or multiple fragments using a commercial supplier (IDT) and used as templates in PCR reactions with primers containing appropriate restriction enzymes. The amplified fragments were digested with appropriate restriction enzymes and then cloned in the pLENTI-EF1 α (SEQ ID NO: 129), pLENTI-EF1 α -DWPRE (SEQ ID NO: 130), pCCLc-MNDU3-WPRE (SEQ ID NO: 12639) or MSCV-Bgl2-AvrII-Bam-EcoRI-Xho-BstBI-Mlu-Sal-ClaI.I03 (SEQ ID NO: 131) vectors using standard molecular biology techniques. The CAR fragments were cloned between the Nhe I and Sal I sites in the pLENTI-EF1 α (SEQ ID NO: 129), pLENTI-EF1 α -DWPRE (SEQ ID NO: 130), pCCLc-MNDU3-WPRE (SEQ ID NO: 12639) vectors. The resulting fragment can then be used as a template in PCR reaction with primers containing appropriate restriction enzymes. The amplified fragment can be digested with appropriate restriction enzymes and then cloned in the appropriate vector using standard molecular biology techniques.

[00652] 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.

[00653] 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
RS4:11	RPMI, 20% FCS	CD19, Folate Receptor beta (FRbeta), TGFbeta, CD179b, NKG2D, FLT3, CD179a, CD133
MV4;11	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, Cripto
Jurkat	RPMI, 10% FCS	TnAg, TSLRP, TSHR, CD4, CD38
Daudi	RPMI, 10% FCS	BCMA, FRbeta
REC-1	RPMI, 10% FCS	NKG2D, ROR1, CD19, FCRH5, BAFF-R
KG-1	RPMI, 20% FCS	CD33, CD34, CD123, TSLRP, EMR2, VISTA, BST1
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, EMR2, BST1
A431	DMEM, 10% FCS	EGFR, Folate Receptor Alpha (FOLR1), Her3
P19	DMEM, 10% FCS	SSEA
THP-1	RPMI, 10% FCS	CD32, CD33, CXCR4, CD123, CD44, IL6R, Folate Receptor beta, CD70, LAMP1, FLT3, CSF2RA, IL1RAP
U87MG	DMEM, 10% FCS	CD276, gpNMB, IL13RA2, MMP16
LoVo	DMEM, 10% FCS	Tissue Factor, CDH17, EGFR, CEA, RNF43
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,

Cell line	Culture Conditions	Exemplary CAR Target Antigens Expressed
Jeko-1	RPMI, 20% FCS	BCMA, ROR1, BAFF-R
PC-3	DMEM, 10% FCS	CGH, TROP2, PSCA, PSMA, EPCAM, FSHR, CLD18A2 (CLDN18.2), STEAP1
HeLa	DMEM, 10% FCS	EGFR, FR1, MSLN, TSHR
LNCaP	DMEM, 10% FCS	EGFR, FSHR, PSCA, PSMA, CD22, Her3, LHR, CLD18A2 (CLDN18.2), STEAP1, BMPR1B
OVCAR-3	DMEM, 10% FCS	B7H4, CDH6, DLL3, FR1, FSH, LHR, MSLN, PTK7, TnAg, TSHR, L1CAM, LYPD1, CLDN6, UPK1B, CD133, SLC34A2
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
MDA-MB-453	DMEM, 10% FCS	Nectin-4,; WISP1
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, NKG2D
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, Prolactin Receptor (PRLR), Liv-1
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, CLDN6

[00654] Jurkat cell line (clone E6-1) engineered with a NFAT-dependent GFP reporter gene was a gift from Dr. Arthur Weiss at UCSF. Jurkat cells were maintained in RPMI-1640 medium supplemented with 10% FBS, penicillin and streptomycin.

[00655] **Generation of Lentiviruses and retroviruses.** Lentiviruses were generated by calcium phosphate based transfection in 293FT cells essentially as described previously (Matta, Hozayev, Tomar, Chugh, & Chaudhary, 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 µg of lentiviral expression plasmid encoding different genes, 7.5 µg of PSPAX2 plasmid and 2 µg of PLP/VSVG plasmid. In some experiment, the transfection mixture also contained between 2.5 to 5 µg of an HIV1 Vif encoding plasmid (SEQ ID NO: 11269). 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.

[00656] **Infection of T cells and PBMC.** 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, 10 ng/ml CD28 antibody and 100 IU recombinant human-IL2. Alternatively, CD3/CD28 beads and 100 IU recombinant human-IL2 were used. 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 10 ng/ml CD3 antibody, 10 ng/ml CD28 antibody and 100 IU recombinant human-IL2 and supplemented with respective antibiotics (if indicated) and placed in the cell culture flask for selection,

unless indicated otherwise. Alternatively, CD3/CD28 beads and 100 IU recombinant human-IL2 were used. 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.

[00657] 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.

[00658] **Antibodies and drug.** 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 30 µg/ml unless indicated otherwise.

[00659] **Clinical grade CAR-T manufacturing and administration**

For clinical grade CAR-T manufacturing, cGMP grade lentiviruses encoding the CARs are generated using commercial sources (*e.g.*, Lentigen, Lonza *etc.*). The T cell are collected from donors (autologous or allogeneic) using leukapheresis. CAR-T cells are manufactured using CLINIMAC Prodigy (Miltenyi Biotech) automated closed system as described (Zhu F, Shah N *et al*, Cytotherapy, 2017) and following the instructions of the manufacturer. The multiplicity of infection (MOI) of between 5 to 10 is used. Alternate methods for clinical grade CAR-T manufacturing, such as Cocoon (Lonza) and manual open systems, are known in the art and can be used in alternate embodiment of the invention. CAR-T cells are administered to the patient after lympho-depleting chemotherapy at escalating doses starting at approximately of 1×10^6 CD3 CAR-T cells/kg.

[00660] **IL2 ELISA.** Human IL2, IFN γ , IL6 and TNF α was 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 ELISA kits from R&D systems (Minneapolis, MN) and following the recommendations of the manufacturer.

[00661] **FACS analysis.** 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.

[00662] 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 μ l of APC-conjugated Myc antibody and incubated in dark for 1 hour followed by two washings with ice cold wash buffer.

[00663] 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 μ g 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 10 μ l 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.

[00664] **Cell death assay.** To measure cell death, a novel assay based on ectopic cytosolic expression of Gluc or NLuc was utilized as described in PCT/US17/52344 “*A Non-Radioactive Cytotoxicity Assay*”.

[00665] **Development of an Assay to detect the expression of CAR and their antigens.** To detect the expression of CAR and their target antigens, a luciferase based reporter assay, designated Matador Assay was utilized as described in PCT/US2017/025602 “*A Highly Sensitive And Specific Luciferase Based Reporter Assay For Antigen Detection*”.

[00666] **Jurkat NFAT-GFP Assay for CARs.** The Jurkat-NFAT-GFP cells are engineered in such a way that the IL-2 promoter, which carries NFAT binding sites, is cloned upstream of the GFP gene. These cells have been used to study signaling via TCR and CAR. The different CARs were stably expressed in Jurkat NFAT-GFP cells by lentiviral mediated gene transfer, followed by selection with puromycin. The CAR-expressing Jurkat-NFAT-GFP cells were cocultured with the target cells at E:T ratio of approximately 1:2 for approximately 4 hours to 18 hours. When the interaction between CARs and their target antigen results in activation of the NFAT pathway, GFP expression is induced. Hence Jurkat-NFAT-GFP cells expressing CAR show increased levels of GFP expression when they interact with target cell lines expressing the receptor for the CAR.

[00667] Induction of GFP expression by coculturing of Jurkat-NFAT-GFP cells expressing different CAR constructs and the different target cells was studied essentially as described previously (Wu, Roybal, Puchner, Onuffer, & Lim, 2015). GFP expression was monitored by FACS analysis. The Jurkat-NFAT-GFP (parental) cells were used as control. The results with different CARs are summarized in the following summary **Table 14**. The names of different CARs, their SEQ ID NOs, component antigen binding domains and TCR chains can be determined by reference to **Tables 14**. A CAR is considered positive in the assay in case the CAR expressing Jurkat-NFAT-GFP cells show greater % GFP positive cells when cultured with the target cell line as compared to parental Jurkat-NFAT-GFP cells. Thus, the Jurkat-NFAT-GFP cells expressing the BCMA CAR represented by SEQ ID NO: 495 showed greater induction of GFP expression when cocultured with the L363 and U266 cells as compared to parental Jurkat-NFAT-GFP cells. The signs +/-, +, 2+ *etc.* after the name of the cell lines indicate the relative degree of positivity on the Jurkat-NFAT-GFP assay as measured by the % GFP positive cells after culture of the CAR expressing Jurkat-NFAT-GFP cells with that cell line. The results also demonstrate that different CARs containing the binding domain derived from the same antibody show great diversity in their ability to activate NFAT signaling using this assay when exposed to the identical cell line depending upon the CAR type. In addition, great diversity of response against the same target cell line is observed with Jurkat cells expressing CARs containing different antigen binding domains targeting the same antigen (*e.g.*, CARs having antigen binding domains derived from different BCMA antibodies) even when the CARs share the same CAR architecture (*e.g.*, BBz CAR or SIR). Finally, Jurkat cells expressing CARs targeting different antigens (*e.g.* CD19 vs CD20) show a diversity of response when exposed to the same target cell line. Thus, a diverse immune response can be generated against a single target cell by combining CARs with different TCR chains, linkers, antigen binding domains and target specificity. **Table 14** also summarizes the results of GLuc based T cell cytotoxicity assay (Matador Assay) observed with different CARs when exposed to their target cell lines. The signs +/-, + and 2+ *etc.* indicate the degree of cytotoxicity observed using the Gluc cytotoxicity assay following 4-96 hours co-culture of the target cell line with CAR-expressing T cells as compared to control T cells, *i.e.*, T cells expressing no CAR or an irrelevant CAR (*e.g.*, a CAR targeting an antigen not expressed on the particular target cell line), when the assay is performed under similar conditions. Again, similar to the results obtained with Jurkat-NFAT-GFP cells, T cells expressing different CARs show great diversity in their ability to exert cytotoxicity when exposed to their target antigen expressing cells depending upon their

TCR chains, linkers, antigen binding domains, target specificity and the target cell line. A similar diversity in the ability to induce cytokine production (*e.g.* IL2, TNF α and IFN γ) is observed among T cells expressing different CARs depending upon their TCR chains, linkers, antigen binding domains, target specificity and the target cell line when they were exposed to the target cell line under comparable conditions. Based on the results of this analysis, different antigen binding domains (*e.g.*, scFv), linkers, CAR type and class, and TCR chains and their configurations that were positive in the NFAT-GFP and T cell cytotoxicity assays were selected for further studies.

[00668] **TABLE 14**

			NFAT-GFP ASSAY	T-CELL Cytotoxicity Assay
Target	SEQ ID NO	Constructs Name	Positive Cell lines	Positive cell line
BCMA	446	CD8SP-BCMA-Am08-HL-vH-Gly-Ser-Linker-vL-Myc-CD8TM-BBz	L363(2+), U266 (2+)	
BCMA	543	CD8SP-BCMA-BB-CAR02-vL-Gly-Ser-Linker-BCMA-BB-CAR02-vH-Myc-CD8TM-BBz		L363 (+), U266 (+)
BCMA	545	CD8SP-BCMA-BB-CAR02-vL-Gly-Ser-Linker-BCMA-BB-CAR02-vH-Myc-CD8TM-z-P2A-K13-FLAG-T2A-PAC		L363 (+), U266 (+)
BCMA	495	CD8SP-BCMA-Am14-HL-vH-Gly-Ser-Linker-vL-Myc-CD8TM-BBz	L363(2+), U266 (2+)	L363(+), U266 (+)
BST1	8506	CD8SP-hu-BST1-A1-vL-Gly-Ser-Linker-hu-BST1-A1-vH-Myc-CD8TM-z-P2A-K13-FLAG-T2A-PAC	Kasumi (+/-)	HL60 (+)
BST1	8604	CD8SP-hu-BST1-A3-vL-Gly-Ser-Linker-hu-BST1-A3-vH-Myc-CD8TM-z-P2A-K13-FLAG-T2A-PAC	Kasumi (+/-), MOLM13 (+/-)	HL60 (+)
BST1	8553	CD8SP-hu-BST1-A2-vL-Gly-Ser-Linker-hu-BST1-A2-vH-Myc-CD8TM-BBz	HL60 (+/-)	
BST1	8602	CD8SP-hu-BST1-A3-vL-Gly-Ser-Linker-hu-BST1-A3-vH-Myc-CD8TM-BBz	Kasumi (2+), MOLM13 (+/-), HL60 (+/-)	
CD19	791	CD8SP-CD19-AM1-vL-[hTCRa-CSDVP]-F-F2A-SP-CD19-AM1-vH-[hTCRb-KACIAH]-F-P2A-Xba-PAC	RAJI (2+), Nalm6 (+), REH (+/-)	
CD19	984	CD8SP-hu-CD19-USC1-LH4-vL-Gly-Ser-Linker-hu-CD19-USC1-LH4-vH-Myc-CD8TM-BBz	RAJI (2+), Nalm6 (+), REH (+)	
CD19	742	CD8SP-CD19-9B7-vL-[hTCRa-CSDVP]-F-F2A-SP-CD19-9B7-vH-[hTCRb-KACIAH]-F-P2A-Xba-PAC	RAJI (+), Nalm6 (+/-), REH (+/-)	

CD19	937	CD8SP-CD19-DART1-vL-Gly-Ser-Linker-CD19-DART1-vH-Myc-CD8TM-z-P2A-K13-FLAG-T2A-PAC	RAJI (2+), Nalm6 (2+)	RAJI (+), Nalm6 (+)
CD19	935	CD8SP-CD19-DART1-vL-Gly-Ser-Linker-CD19-DART1-vH-Myc-CD8TM-BBz	RAJI (2+), Nalm6 (2+)	
CD19	788	CD8SP-CD19-AM1-vL-Gly-Ser-Linker-CD19-AM1-vH-Myc-CD8TM-BBz	RAJI (2+), Nalm6 (2+)	
CD19	739	CD8SP-CD19-9B7-vL-Gly-Ser-Linker-CD19-9B7-vH-Myc-CD8TM-BBz	RAJI (2+), Nalm6 (2+)	
CD19	887	CD8SP-hu-CAT18-1-HL-vH-Gly-Ser-Linker-vL-Myc-CD8TM-BBz	RAJI (3+), Nalm6 (3+)	
CD19	838	CD8SP-hCD19-CAT17-HL-vH-Gly-Ser-Linker-vL-Myc-CD8TM-BBz	RAJI (4+), Nalm6 (3+)	RAJI (+)
CD19	3956	CD8-hCD19-EUK5-13-vL-IgCL-Xho-CD3zECDTMCP-opt-F-P2A-Spe-SP-Bst-hCD19-EUK5-13-vH-IgG1-CH1-Mlu-CD3zECDTMCP-opt2-F-F2A-PAC	RAJI(2+), NALM6 (2+)	
CD19	8663	CD8SP-hu-Bu13-vL-[hTCRb-KAC-ECD-Bam-CD3zECDTMCP-opt]-F-P2A-SP-hu-Bu13-vH-[hTCRa-CSDVP-ECD-Kpn-CD3zECDTMCP-opt2]	RAJI (+), Nalm6 (+)	
CD19	8651	CD8SP-hu-Bu13-vL-Gly-Ser-Linker-hu-Bu13-vH-Myc-CD8TM-BBz	RAJI (2+), Nalm6 (+)	RAJI (+/-), Nalm6 (+)
CD19	8655	CD8SP-hu-Bu13-vL-PG4SP-v2-[hTCRb-KACIAH]-F-P2A-SP-hu-Bu13-vH-PG4SP-[hTCRa-CSDVP]-F-F2A-PAC	RAJI (2+), Nalm6 (2+)	
CD19	8654	CD8SP-hu-Bu13-vL-[hTCRa-CSDVP]-F-F2A-SP-hu-Bu13-vH-[hTCRb-KACIAH]-F-P2A-Xba-PAC	RAJI (3+), Nalm6 (3+)	RAJI (+), Nalm6 (+), Bv173 (+)
CD19	8634	CD8SP-V5-[hTCRb-KACIAH]-F-P2A-CD8SP-hu-Bu13-vL-Gly-Ser-Linker-hu-Bu13-vH-Myc-[hTCRa-CSDVP]-F-F2A-PAC	RAJI (+) NALM6 (+/-)	RAJI (+), Nalm6 (+), Bv173 (+)
CD19	3955	CD8-hCD19-EUK5-13-vL-IgCL-Bam-CD3zECDTMCP-opt-F-P2A-Spe-SP-Bst-hCD19-EUK5-13-vH-IgG1-CH1-KPN-CD3zECDTMCP-opt2-F-F2A-Xba-PAC	RAJI (3+) NALM6 (2+)	
CD19	3961	CD8SP-CD19Bu12-scFv-Xho-CD3zECDTMCP-opt-F-P2A-SP-CD19MM-scFv-Mlu-CD3zECDTMCP-opt2-F-F2A-PAC	RAJI (2+), NALM6 (2+)	
CD19-AFP	3964	CD8SP-CD19Bu12-scFv-Xho-CD3zECDTMCP-opt-F-P2A-SP-AFP-61-scFv-Mlu-CD3zECDTMCP-opt2-F-F2A-PAC	RAJI(2+), NALM6(+)	
CD19-CD20	3963	CD8SP-CD19Bu12-scFv-Xho-CD3zECDTMCP-opt-F-P2A-SP-CD20-2F2-scFv-Mlu-CD3zECDTMCP-opt2-F-F2A-PAC	RAJI(+), NALM6 (+)	

CD19- CD22	3966	CD8SP-CD19Bu12-scFv-Xho-CD3zECDTMCP-opt-F-P2A-SP-hSC22-10-HL-scFv-Mlu-CD3zECDTMCP-opt2-F-F2A-PAC	RAJI(+), NALM6 (+)	
CD19- CD22	3965	CD8SP-CD19Bu12-scFv-Xho-CD3zECDTMCP-opt-F-P2A-SP-CD22-h10F4v2-scFv-Mlu-CD3zECDTMCP-opt2-F-F2A-PAC	RAJI(+), NALM6 (+)	
CD19- CD123	3967	CD8SP-CD19Bu12-scFv-Xho-CD3zECDTMCP-opt-F-P2A-SP-CD123-DART1-scFv-Mlu-CD3zECDTMCP-opt2-F-F2A-PAC	RAJI (+), NALM6 (+), L428(+)	
CD19- CD123	3962	CD8SP-CD19Bu12-scFv-Xho-CD3zECDTMCP-opt-F-P2A-SP-CD123-DART2-scFv-Mlu-CD3zECDTMCP-opt2-F-F2A-PAC	RAJI (+), NALM6 (+), L428 (2+)	
CD19- WT1	3968	CD8SP-CD19Bu12-scFv-Xho-CD3zECDTMCP-opt-F-P2A-SP-WT1-Ab5-scFv-Mlu-CD3zECDTMCP-opt2-F-F2A-PAC	RAJI(+), Nalm6 (+)	
CD20	1084	CD8SP-hu-Ubli-1-v4-vL-Gly-Ser-Linker-hu-Ubli-1-v4-vH-Myc-CD8TM-z-P2A-K13-FLAG-T2A-PAC	RAJI (2+)	RAJI (+)
CD20	1082	CD8SP-hu-Ubli-1-v4-vL-Gly-Ser-Linker-hu-Ubli-1-v4-vH-Myc-CD8TM-BBz	RAJI (2+)	RAJI (+)
CD20	1034	CD8SP-CD20-HL-vH-Gly-Ser-Linker-vL-Myc-CD8TM-BBz	RAJI (2+)	
CD22	1131	CD8SP-hCD19-hu-HA22-1-vL-Gly-Ser-Linker-hCD19-hu-HA22-1-vH-Myc-CD8TM-BBz	RAJI (2+), Nalm6 (+/-)	
CD44v6	1181	CD8SP-CD44v6-USC1-HL4-vH-Gly-Ser-Linker-vL-Myc-CD8TM-BBz	L363 (+/-) U266 (+/-)	
CLDN6	1526	CD8SP-CLDN6-USC2-LH4-vL-[hTCRa-CSDVP]-F-F2A-SP-CLDN6-USC2-LH4-vH-[hTCRb-KACIAH]-F-P2A-PAC		HepG2 (+/-), OVCAR3 (+/-)
CLDN6	1523	CD8SP-CLDN6-USC2-LH4-vL-Gly-Ser-Linker-CLDN6-USC2-LH4-vH-Myc-CD8TM-BBz	HepG2(+), OVCAR (+)	
CLDN6	1476	CD8SP-CLDN6-USC1-LH4-vL-Gly-Ser-Linker-CLDN6-USC1-LH4-vH-Myc-CD8TM-z-P2A-K13-FLAG-T2A-PAC	HepG2(+/-), OVCAR3 (+/-)	
CRIPTO	8879	CD8SP-V5-[hTCRb-KACIAH]-F-P2A-CD8SP-hu-Cripto-L1H2-vL-Gly-Ser-Linker-hu-Cripto-L1H2-vH-Myc-[hTCRa-CSDVP]-F-F2A-PAC		HEL (+)
DLL3	1575	CD8SP-DLL3-AM6-HL-vL-[hTCRa-CSDVP]-F-F2A-SP-DLL3-AM6-HL-vH-[hTCRb-KACIAH]-F-P2A-PAC	SKMEL 37 (+/-)	
DLL3	1622	CD8SP-DLL3-AM14-HL-vH-Gly-Ser-Linker-vL-Myc-CD8TM-BBz	LAN5 (+), SKMEL 37 (+/-)	SKMEL 37 (+)
DLL3	1573	CD8SP-DLL3-AM6-HL-vH-Gly-Ser-Linker-vL-Myc-CD8TM-BBz	LAN5 (+/-), SKMEL 37 (+/-)	

EGFRvIII	1771	CD8SP-EGFRviii-H2M1863N2-HL-vL-[hTCRa-CSDVP]-F-F2A-SP-EGFRviii-H2M1863N2-HL-vH-[hTCRb-KACIAH]-F-P2A-Xba-PAC		HeLa (+), HeLa EGFRvIII (+)
EGFRvIII	1769	CD8SP-EGFRviii-H2M1863N2-HL-vH-Gly-Ser-Linker-vL-Myc-CD8TM-BBz	Hela (+/-), HeLa EGFR (+/-), LoVo (+/-), A431(4+)	
EMR2	1915	CD8SP-EMR2-USC2-V4-vL-Gly-Ser-Linker-EMR2-USC2-V4-vH-Myc-CD8TM-BBz	MOLM13 (+), HL60(+/-)	
FOLR1	2064	CD8SP-FOLR1-USC1-HL4-vL-Gly-Ser-Linker-FOLR1-USC1-HL4-vH-Myc-CD8TM-z-P2A-K13-FLAG-T2A-PAC	Hela (2+), A431 (+/-)	
gpA33	9026	CD8SP-V5-[hTCRb-KACIAH]-F-P2A-CD8SP-hu-gpA33-vL-Gly-Ser-Linker-hu-gpA33-vH-Myc-[hTCRa-CSDVP]-F-F2A-PAC		MOLM13 (+)
GPC3	2210	CD8SP-GPC3-USC2-HL-V4-vH-Gly-Ser-Linker-vL-Myc-CD8TM-BBz	HepG (3+) MOLM13 (+/-)	
GPC3	2161	CD8SP-GPC3-USC1-HL-V4-vH-Gly-Ser-Linker-vL-Myc-CD8TM-BBz	HepG (3+) MOLM13 (+/-)	
Her2	2309	CD8SP-Her2-169-vL-Gly-Ser-Linker-Her2-169-vH-Myc-CD8TM-z-P2A-K13-FLAG-T2A-PAC		MCF7 (+/-)
Her2	2457	CD8SP-Her2-XMT-1520-vL-[hTCRa-CSDVP]-F-F2A-SP-Her2-XMT-1520-vH-[hTCRb-KACIAH]-F-P2A-Xba-PAC	MCF7(2+)	
Her2	2408	CD8SP-Her2-XMT-1518-vL-[hTCRa-CSDVP]-F-F2A-SP-Her2-XMT-1518-vH-[hTCRb-KACIAH]-F-P2A-Xba-PAC	MCF7(+)	
Her2	2405	CD8SP-Her2-XMT-1518-vL-Gly-Ser-Linker-Her2-XMT-1518-vH-Myc-CD8TM-BBz	MCF7(+)	
Her2	2454	CD8SP-Her2-XMT-1520-vL-Gly-Ser-Linker-Her2-XMT-1520-vH-Myc-CD8TM-BBz	MCF7 (2+)	
Her2	2456	CD8SP-Her2-XMT-1520-vL-Gly-Ser-Linker-Her2-XMT-1520-vH-Myc-CD8TM-z-P2A-K13-FLAG-T2A-PAC	MCF7 (+/-)	
Her2	2405	CD8SP-Her2-XMT-1518-vL-Gly-Ser-Linker-Her2-XMT-1518-vH-Myc-CD8TM-BBz	MCF7 (2+)	
Her2	2356	CD8SP-Her2-huMab4D5-D98W-vL-Gly-Ser-Linker-Her2-huMab4D5-D98W-vH-Myc-CD8TM-BBz	MCF7 (+/-)	
IL1RAP	9242	CD8SP-IL1RAP-IAPB63-vL-[hTCRa-CSDVP]-F-F2A-SP-IL1RAP-IAPB63-vH-[hTCRb-KACIAH]-F-P2A-Xba-PAC	MOLM13 (+/-)	MOLM13 (+)
IL1RAP	9222	CD8SP-V5-[hTCRb-KACIAH]-F-P2A-CD8SP-IL1RAP-IAPB63-vL-Gly-Ser-Linker-IL1RAP-IAPB63-vH-Myc-[hTCRa-CSDVP]-F-F2A-PAC		MOLM13 (+)

Integrin B7	2552	CD8SP-Hu-IntB7-MMG49-vL-Gly-Ser-Linker-Hu-IntB7-MMG49-vH-Myc-CD8TM-BBz		L363 (+), U266 (+)
LYPD1	2650	CD8SP-LYPD1-HL-V4-vL-Gly-Ser-Linker-LYPD1-HL-V4-vH-Myc-CD8TM-BBz	OVCAR3 (2+)	
MSLN	2849	CD8SP-MSLN-237-HL-vL-[hTCRa-CSDVP]-F-F2A-SP-MSLN-237-HL-vH-[hTCRb-KACIAH]-F-P2A-Xba-PAC	SKOV3 (+)	SKOV3 (7+)
MSLN	2800	CD8SP-MSLN-5-HL-vL-[hTCRa-CSDVP]-F-F2A-SP-MSLN-5-HL-vH-[hTCRb-KACIAH]-F-P2A-Xba-PAC	SKOV3 (+)	SKOV3 (5+)
MSLN	2944	CD8SP-MSLN-HuAM15-vL-Gly-Ser-Linker-MSLN-HuAM15-vH-Myc-CD8TM-BBz	SKOV3 (+)	
MSLN	2749	CD8SP-MSLN-3-HL-AM-vH-Gly-Ser-Linker-vL-Myc-CD8TM-BBz	SKOV3 (+)	
MSLN	2798	CD8SP-MSLN-5-HL-vH-Gly-Ser-Linker-vL-Myc-CD8TM-BBz	SKOV3 (+/-)	
MSLN	9435	CD8SP-MSLN-hu22A10-vL-Gly-Ser-Linker-MSLN-hu22A10-vH-Myc-CD8TM-BBz	SKOV3 (2+)	
MSLN	9387	CD8SP-MSLN-7D9-v3-vH-Gly-Ser-Linker-vL-Myc-CD8TM-BBz	SKOV3 (3+)	
MSLN	9389	CD8SP-MSLN-7D9-v3-vL-[hTCRa-CSDVP]-F-F2A-SP-MSLN-7D9-v3-vH-[hTCRb-KACIAH]-F-P2A-Xba-PAC	SKOV3 (2+)	
MSLN	9398	CD8SP-MSLN-7D9-v3-vL-[hTCRb-KAC-ECD-Bam-CD3zECDTMCP-opt]-F-P2A-SP-MSLN-7D9-v3-vH-[hTCRa-CSDVP-ECD-Kpn-CD3zECDTMCP-opt2]	SKOV3 (+)	
MSLN	9439	CD8SP-MSLN-hu22A10-vL-PG4SP-v2-[hTCRb-KACIAH]-F-P2A-SP-MSLN-hu22A10-vH-PG4SP-[hTCRa-CSDVP]-F-F2A-PAC	SKOV3 (2+)	SKOV3 (+)
MSLN	9447	CD8SP-MSLN-hu22A10-vL-[hTCRb-KAC-ECD-Bam-CD3zECDTMCP-opt]-F-P2A-SP-MSLN-hu22A10-vH-[hTCRa-CSDVP-ECD-Kpn-CD3zECDTMCP-opt2]	SKOV3 (+)	
MSLN	9419	CD8SP-V5-[hTCRb-KACIAH]-F-P2A-CD8SP-MSLN-hu22A10-vL-Gly-Ser-Linker-MSLN-hu22A10-vH-Myc4-[preTCRa-Del48]-F-F2A-PAC	SKOV3 (+/-)	SKOV3 (+)
MSLN	2898	CD8SP-MSLN76923-HL-vL-[hTCRa-CSDVP]-F-F2A-SP-MSLN76923-HL-vH-[hTCRb-KACIAH]-F-P2A-PAC		SKOV3 (+/-)
MSLN	9369	CD8SP-V5-[hTCRb-KACIAH]-F-P2A-CD8SP-MSLN-7D9-v3-vL-Gly-Ser-Linker-MSLN-7D9-v3-vH-Myc-[hTCRa-CSDVP]-F-F2A-PAC	SKOV3 (+)	SKOV3 (+)
Muc17	3045	CD8SP-Muc17-11-CN-vL-[hTCRa-CSDVP]-F-F2A-SP-Muc17-11-CN-vH-[hTCRb-KACIAH]-F-P2A-Xba-PAC	SKOV3 (+)	

SLC34A2	3581	CD8SP-huMX35-LH4-vL-Gly-Ser-Linker-huMX35-LH4-vH-Myc-CD8TM-BBz	OVCAR3 (3+)	
Nectin-4	3092	CD8SP-Nectin4-66-HL-vH-Gly-Ser-Linker-vL-Myc-CD8TM-BBz	MDAMB231 (+/-), MCF7 (2+)	MDAMB231 (+), MCF7 (+)
PRLR	3143	CD8SP-PRLR-CN-vL-[hTCRa-CSDVP]-F-F2A-SP-PRLR-CN-vH-[hTCRb-KACIAH]-F-P2A-Xba-PAC	MCF7 (+)	
PRLR	3191	CD8SP-PRLR-USC2-HL-V4-vL-Gly-Ser-Linker-PRLR-USC2-HL-V4-vH-Myc-CD8TM-z-P2A-K13-FLAG-T2A-PAC	MCF7 (2+)	MCF7 (+/-)
PRLR	3140	CD8SP-PRLR-CN-vL-Gly-Ser-Linker-PRLR-CN-vH-Myc-CD8TM-BBz	MCF7 (2+)	
PSMA	3241	CD8SP-hu106mPSMA-4-HL-vL-[hTCRa-CSDVP]-F-F2A-SP-hu106mPSMA-4-HL-vH-[hTCRb-KACIAH]-F-P2A-Xba-PAC		PC3 (+), LNCAP (+)
PSMA	3290	CD8SP-PSMA-76-HL-AM-vL-[hTCRa-CSDVP]-F-F2A-SP-PSMA-76-HL-AM-vH-[hTCRb-KACIAH]-F-P2A-Xba-PAC	PC3 (+/-)	
PSMA	3239	CD8SP-hu106mPSMA-4-HL-vH-Gly-Ser-Linker-vL-Myc-CD8TM-BBz	PC3 (+/-)	
PSMA	3337	CD8SP-PSMA-83A12-HL-AM-vH-Gly-Ser-Linker-vL-Myc-CD8TM-BBz	PC3 (+/-)	
RNF43	3386	CD8SP-RNF43-USC1-HL4-vH-Gly-Ser-Linker-vL-Myc-CD8TM-BBz	LoVo (+/-)	LoVo (+/-)
ROR1	9516	CD8SP-V5-[hTCRb-KACIAH]-F-P2A-CD8SP-ROR1-DART4-vL-Gly-Ser-Linker-ROR1-DART4-vH-Myc-[hTCRa-CSDVP]-F-F2A-PAC		Jeko1 (+)
STEAP	3534	CD8SP-STEAP1-USC1-HL4-vL-Gly-Ser-Linker-STEAP1-USC1-HL4-vH-Myc-CD8TM-z-P2A-K13-FLAG-T2A-PAC	PC3 (+) LNCAP (+/-)	
STEAP	9565	CD8SP-V5-[hTCRb-KACIAH]-F-P2A-CD8SP-STEAP1-hu120-vL-Gly-Ser-Linker-STEAP1-hu120-vH-Myc-[hTCRa-CSDVP]-F-F2A-PAC		PC3 (+) LNCAP (+)
TSHR	3630	CD8SP-TSHR-hu-3BD10-vL-Gly-Ser-Linker-TSHR-hu-3BD10-vH-Myc-CD8TM-BBz		MOLM13 (1.5+)
UISP1	3924	CD8SP-hu-UISP1-USC2-LH4-vL-Gly-Ser-Linker-hu-UISP1-USC2-LH4-vH-Myc-CD8TM-BBz	MDAMB231 (+/-)	
UPK1B	3730	CD8SP-hUPK1B-USC2-LH4-vL-Gly-Ser-Linker-hUPK1B-USC2-LH4-vH-Myc-CD8TM-z-P2A-K13-FLAG-T2A-PAC	OVCAR3 (2+) Sudhl1 (+)	
UPK1B	3681	CD8SP-hUPK1B-USC1-LH4-vL-Gly-Ser-Linker-hUPK1B-USC1-LH4-vH-Myc-CD8TM-z-P2A-K13-FLAG-T2A-PAC	Sudhl-1(+)	

UPK1B	3682	CD8SP-hUPK1B-USC1-LH4-vL-[hTCRa-CSDVP]-F-F2A-SP-hUPK1B-USC1-LH4-vH-[hTCRb-KACIAH]-F-P2A-Xba-PAC	OVCAR3 (2+) Sudhl1 (+)	OVCAR (2+), Sudhl1 (1.5+)
VISTA	3780	CD8SP-huVISTA-JJ-USC2-v4-vL-[hTCRa-CSDVP]-F-F2A-SP-huVISTA-JJ-USC2-v4-vH-[hTCRb-KACIAH]-F-P2A-Xba-PAC	KG1 (+/-) MOLM13 (+/-) HL60 (+/-)	
VISTA	3826	CD8SP-huVISTA-USC1-v4-vL-Gly-Ser-Linker-huVISTA-USC1-v4-vH-Myc-CD8TM-BBz		MOLM13(+)
BCMA	12755	CD8SP-BCMA-Am06-HL-Mlu-MYC-CD8TM-BBZ-XS-T2A-Pac	L363(+), U266 (+)	
BCMA	12894	CD8SP-BCMA-huC13-F12-vL-V5-[hTCRb-KACIAH]-F-P2A-SP-BCMA-huC13-F12-vH-Myc-[hTCRa-CSDVP]-F-F2A-PAC	L363(2+), U266 (2+)	
BCMA	546	CD8SP-BCMA-BB-CAR02-vL-[hTCRa-CSDVP]-F-F2A-SP-BCMA-BB-CAR02-vH-[hTCRb-KACIAH]-F-P2A-PAC	L363(2+), U266 (+)	
BCMA	497	CD8SP-BCMA-Am14-HL-vL-[hTCRa-CSDVP]-F-F2A-SP-BCMA-Am14-HL-vH-[hTCRb-KACIAH]-F-P2A-PAC	L363(2+), U266 (+)	
BCMA	12921	BCMA-huC13-F12vL-Myc2-[hTCRb-KACIAH]-F-P2A-BCMA-huC13-F12vH-MYC4-[hTCRa-CSDVP]-F-F2A-PAC	L363(+), U266 (+)	L363(+), U266 (+)
BCMA	449	CD8SP-BCMA-Am08-HL-vL-PG4SP-v2-[hTCRb-KACIAH]-F-P2A-SP-BCMA-Am08-HL-vH-PG4SP-[hTCRa-CSDVP]-F-F2A-PAC	L363(+), U266 (+)	
BCMA	16316	CD8SP-BCMA-huC13-F12-BBz	L363(2+), U266 (+)	L363 (+), U266 (+). Raji(+), Nalm6 (+)
BCMA	13028	CD8SP-BCMA-J6M0-vL-[hTCR β -KACIAH]-F-P2A-SP-BCMA-J6M0-vH-Mlu-huTCR α -CSDVP	L363(+/-)	
BCMA	12922	CD8SP-BCMA-huC13-F12-vL-[hTCRb-KACIAH]-F-P2A-SP-BCMA-huC13-F12-vH-[hTCRa-CSDVP]-F-F2A-PAC	L363(+), U266 (+)	L363(+), U266 (+)
BCMA	12944	CD8SP-BCMA-huC13-F12-vL-[hTCRb-S57C]-F-P2A-SP-BCMA-huC13-F12-vH-[hTCRa-T48C]-F-F2A-K13-opt	L363(3+), U266 (3+)	
BCMA		BCMA-HuC12A3-L3H3-BBZ	L363(1.5+), U266 (+)	L363 (+), U266 (+). Raji(+), Nalm6 (+)
BCMA	12890	CD8SP-BCMA-huC12A3-L3H3-vL-[hTCRb-S57C]-F-P2A-SP-BCMA-huC12A3-L3H3-vH-[hTCRa-T48C]		L363 (+), U266 (+). Raji(+), Nalm6 (+)

BCMA	12943	CD8SP-BCMA-huC13-F12-vL-[hTCRb-S57C]-F-P2A-SP-BCMA-huC13-F12-vH-[hTCRa-T48C]		L363 (+), U266 (+). Raji(+), Nalm6 (+)
BCMA	13102	CD8SP-BCMA-mJ22-9-vL-[hTCRb-S57C]-F-P2A-SP-BCMA-mJ22-9-vH-[hTCRa-T48C]	L363 (+), U266 (+). Raji(+), Nalm6 (+/-), Daudi (+)	L363 (+), U266 (+)
BCMA	12996	CD8SP-BCMA-huJ22-10-vL-[hTCRb-S57C]-F-P2A-SP-BCMA-huJ22-10-vH-[hTCRa-T48C]	L363 (+), U266 (+). Raji(+), Nalm6 (+/-), Daudi (+)	L363 (+), U266 (+)
BCMA	12807	CD8SP-BCMA-hu72-vL-Gly-Ser-Linker-BCMA-hu72-vH-Myc-CD8TM-BBz	RAJI (2.5+), L363 (2.5+), U266 (3+)	
BCMA	12837	CD8SP-BCMA-hu72-vL-[hTCRb-S57C]-F-P2A-SP-BCMA-hu72-vH-[hTCRa-T48C]	RAJI (3+), L363 (4+), U266 (4+)	
BCMA	12839	CD8SP-BCMA-hu72-vL-[hTCRa-T48C]-F-P2A-SP-BCMA-hu72-vH-[hTCRa-S57C]	RAJI (3+), L363 (4+), U266 (2.5+)	
CD19	895	CD8SP-hu-CAT18-1-HL-vL-[hTCRb-KACIAH]-F-P2A-SP-hu-CAT18-1-HL-vH-[hTCRa-CSDVP]-F-F2A-PAC	RAJI (2+), Nalm6 (2+)	RAJI (+), Nalm6 (+)
CD19	846	CD8SP-hCD19-CAT17-HL-vL-[hTCRb-KACIAH]-F-P2A-SP-hCD19-CAT17-HL-vH-[hTCRa-CSDVP]-F-F2A-PAC	RAJI (2+), Nalm6 (2+)	RAJI (+), Nalm6 (+)
CD19	16328	CD8SP-huCD19-mROO5-1-(vL-vH)-CD3c-ECDTMCP-opt2-T2A-PAC	RAJI (2+), NALM6 (2+)	
CD19	16239	CD8SP-huCD19-mROO5-1-(vL-vH)-CD3d-ECDTMCP-opt2-T2A-PAC	RAJI (+), NALM6 (+)	
CD19	16317	CD8SP-CD19-hu-mROO5-1-Mlu-CD8TM-BBZ	RAJI (1.5+), NALM6 (1.5+)	RAJI (+), Nalm 6(+)
CD19	14057	CD8SP-huCD19-mROO5-1-vL-[hTCRb-S57C]-F-P2A-SP-huCD19-mROO5-1-vH-[hTCRa-T48C]-F-F2A-K13-opt	RAJI(2+), NALM6(4+)	RAJI (+), Nalm6 (+)
CD19	14056	CD8SP-huCD19-mROO5-1-vL-[hTCRb-S57C]-F-P2A-SP-huCD19-mROO5-1-vH-[hTCRa-T48C]	RAJI(2+), NALM6(3+)	RAJI (+), Nalm6 (+)
CD19	16318	CD8SP-huCD19-mROO5-1-vL-[hTCRb-KACIAH]-F-P2A-SP-huCD19-mROO5-1-vH-[hTCRa-CSDVP]-F-F2A-K13-opt	RAJI(+), NALM6(2+)	RAJI (+), Nalm 6(+)
CD19	16311	CD8SP-FMC63-BBz		RAJI (+), Nalm 6(+)
CD19	14035	CD8SP-huCD19-mROO5-1-vL-[hTCRb-KACIAH]-F-P2A-SP-huCD19-mROO5-1-vH-[hTCRa-CSDVP]-F-F2A-PAC		RAJI(+), NALM6(+)

CD19	16317	CD8SP-CD19-hu-mROO5-1-Mlu-CD8TM-BBZ		RAJI(+), NALM6(+), Bv173(+), Rec1(+)
CD19	16311	CD8SP-FMC63-BBz		RAJI(+), NALM6(+), Bv173(+), Rec1(+)
CD19	8651	CD8SP-hu-Bu13-vL-Gly-Ser-Linker-hu-Bu13-vH-Myc-CD8TM-BBz		RAJI(+), NALM6(+), Bv173(+), Rec1(+)
CD19-Nemo		CD8SP-CD19-hu-mROO5-1-Mlu-CD8TM-Asc-hCD3z-xba-P2A-Bam-hNEMO-D23V-K277A		RAJI(+), NALM6(+), Bv173(+), Rec1(+)
CD19-K13	16318	CD8SP-huCD19-mROO5-1-vL-[hTCRb-KACIAH]-F-P2A-SP-huCD19-mROO5-1-vH-[hTCRa-CSDVP]-F-F2A-K13-opt		RAJI(+), NALM6(+)
CD19-MC159L		CD8SP-huCD19-mROO5-1-vL-[hTCRb-KACIAH]-F-P2A-SP-huCD19-mROO5-1-vH-[hTCRa-CSDVP]-F-F2A-MC159		RAJI (+), Nalm6 (+)
CD19	14035	CD8SP-huCD19-mROO5-1-vL-[hTCRb-KACIAH]-F-P2A-SP-huCD19-mROO5-1-vH-[hTCRa-CSDVP]-F-F2A-PAC		RAJI (+), Nalm6 (+)
CD19	16318	CD8SP-CD19-hu-mROO5-1-vL-Xho-[hTCRb-KACIAH]-F-P2A-SP-Bst-CD19-hu-mROO5-1-vH-Mlu-[hTCRa-CSDVP]-F-F2A-Xba-Bam-SynthK13		RAJI (+), Nalm 6(+)
CD19	14109	CD8SP-huCD19-USC3-vL-[hTCRb-S57C]-F-P2A-SP-huCD19-USC3-vH-[hTCRa-T48C]	Raji (2.5+), Nalm6 (2.5+), Daudi (2.5+)	
CD22	13284	CD8SP-CD22-h10F4v2-vL-Gly-Ser-Linker-CD22-h10F4v2-vH-MYC-CD8TM-BBZ-T2A-PA	RAJI (5+), Nalm6 (3+)	
CD22	13293	CD8SP-CD22-h10F4v2-vL-[hTCRb-KACIAH]-F-P2A-SP-CD22-h10F4v2-vH-[hTCRa-CSDVP]-F-F2A-PAC	RAJI (6+), Nalm6 (5+)	
CD22	13349	CD8SP-CD22-HA22-vL-[hTCRb-KAC-ECD-Bam-CD3zECDTMCP-opt]-F-P2A-SP-CD22-HA22-vH-[hTCRa-CSDVP-ECD-Kpn-CD3zECDTMCP-opt2]	RAJI (2+), Nalm6 (+/-)	
CD22	13314	CD8SP-CD22-h10F4v2-vL-[hTCRb-S57C]-F-P2A-SP-CD22-h10F4v2-vH-[hTCRa-T48C]	RAJI (4.5+), Nalm6 (+/-)	
CD22	13320	CD8SP-V5-[hTCRb-KACIAH]-F-P2A-CD8SP-CD22-HA22-vL-Gly-Ser-Linker-CD22-HA22-vH-Myc-[hTCRa-CSDVP]-F-F2A-PAC	RAJI (+)	

CD22	13321	CD8SP-V5-[hTCRb-KACIAH]-F-P2A-CD8SP-CD22-HA22-vL-Gly-Ser-Linker-CD22-HA22-vH-Myc4-[preTCRa-Del48]-F-F2A-PAC	RAJI (+/-)	
CD22		CD8SP-SC22-HA22-Mlu-CD8TM-BBZ		RAJI(+), Nalm6 (+)
CD22	13346	CD8SP-CD22-HA22-vL-[hTCRb-KACIAH]-F-P2A-SP-CD22-HA22-vH-[hTCRa-CSDVP]-F-F2A-PAC		RAJI(+), Nalm6 (+)
CD22	13369	CD8SP-CD22-HA22-vL-[hTCRa-T48C]-F-P2A-SP-CD22-HA22-vH-[hTCRa-S57C]		RAJI(+), Daudi (+)
CD22		CD22-h10F4v2-Mlu-CD8TM-BBZ		RAJI(+), Daudi (+)
CD22	13316	CD8SP-CD22-h10F4v2-vL-[hTCRa-T48C]-F-P2A-SP-CD22-h10F4v2-vH-[hTCRa-S57C]		RAJI(+), Daudi (+)
CD22	14215	CD8SP-hu-RFB4-vL-[hTCRb-S57C]-F-P2A-SP-hu-RFB4-vH-[hTCRa-T48C]	Raji (1.5+), Nalm6 (+), Daudi (2+)	Raji (+)
CD22	13343	CD8SP-CD22-INO-vL-Gly-Ser-Linker-CD22-INO-vH-Myc-CD8TM-BBz		Raji (+)
CD22	16319	CD8SP-CD22-INO-vL-[hTCRb-S57C]-F-P2A-SP-CD22-INO-vH-[hTCRa-T48C]-F-F2A-PAC	Raji (+), Nalm6 (+), Daudi (+)	
CD22	16320	CD8SP-CD22-hu-HA22-2-vL-[hTCRa-T48C]-F-P2A-SP-CD22-hu-HA22-2-vH-[hTCRa-S57C]-F-F2A-Pac	Raji (2+), Nalm6 (+), Daudi (2.5+)	
CD22	16321	CD8SP-CD22-Med-12C5-HL-vH-[hTCRb-S57C]-F-P2A-SP-CD22-Med-12C5-HL-vL-[hTCRa-T48C]-F-F2A-PAC	Raji (+), Nalm6 (+/-), Daudi (1.5+)	
CD22	16322	CD8SP-huRFB4-vL-Xho-[hTCRβ-S57C]-F-P2A-SP-Bst-huRFB4-vH-Mlu-[hTCRα-T48C-opt]-F-F2A-Xba-PAC	RAJI (2+), NALM6 (+), Daudi (3+)	
CD22	16323	CD8SP-CD22-CELL7-vL-[hTCRb-S57C]-F-P2A-SP-CD22-CELL7-vH-[hTCRa-T48C]-F-F2A-PAC	RAJI (+/-)	
CD22	16324	CD8SP-CD22-HA22-vL-[hTCRb-S57C]-F-P2A-SP-CD22-HA22-vH-[hTCRa-T48C]-F-F2A-PAC	RAJI (+), NALM6 (+/-), Daudi (1.5+)	
CLDN6	1526	CD8SP-CLDN6-USC2-LH4-vL-[hTCRa-CSDVP]-F-F2A-SP-CLDN6-USC2-LH4-vH-[hTCRb-KACIAH]-F-P2A-Xba-PAC	HepG2(3+), OVCAR3 (+)	
DLL3	1622	CD8SP-DLL3-AM14-HL-vH-Gly-Ser-Linker-vL-Myc-CD8TM-BBz	LAN5 (+), SKMEL 37 (+/-)	
Her3	2506	CD8SP-Her3-USC1-HL4-vL-[hTCRa-CSDVP]-F-F2A-SP-Her3-USC1-HL4-vH-[hTCRb-KACIAH]-F-P2A-Xba-PAC	MCF7(2+)	

MPL		CD8SP-MPL-161-(vL-vH)-Mlu-CD8TM-BBZ-ter-Sal-WPRE-G02		HEL (+)
MPL	16315	CD8SP-MPL-hu-161-2-BBz	HEL (+)	HEL (+)
MPL	13764	CD8SP-hu-161-2-vL-[hTCRa-CSDVP]-F-F2A-SP-hu-161-2-vH-[hTCRb-KACIAH]-F-P2A-Xba-PAC		HEL (+)
MPL	13791	CD8SP-hu-161-2-vL-[hTCRb-S57C]-F-P2A-SP-hu-161-2-vH-[hTCRa-T48C]	HEL (3+)	HEL (+)
MPL		CD8SP-161-vL-[hTCRb-S57C]-F-P2A-IgHSP-Bst-161-vH-[hTCRa-T48C-opt]-		HEL (+)
MSLN	2749	CD8SP-MSLN-3-HL-AM-vH-Gly-Ser-Linker-vL-Myc-CD8TM-BBz	SKOV3 (+)	
MSLN	2847	CD8SP-MSLN-237-HL-vH-Gly-Ser-Linker-vL-Myc-CD8TM-BBz	SKOV3 (+)	
MSLN	9444	CD8SP-MSLN-hu22A10-vL-[hTCRb-KACIAH]-F-P2A-SP-MSLN-hu22A10-vH-[hTCRa-CSDVP]-F-F2A-PAC	SKOV3 (+)	SKOV3 (+)
MSLN	9395	CD8SP-MSLN-7D9-v3-vL-[hTCRb-KACIAH]-F-P2A-SP-MSLN-7D9-v3-vH-[hTCRa-CSDVP]-F-F2A-PAC	SKOV3 (+)	SKOV3 (+)
MSLN	14321	CD8SP-MSLN-7D9-HL-vH-[hTCRb-S57C]-F-P2A-SP-MSLN-7D9-HL-vL-[hTCRa-T48C]	SKOV3 (+/-)	SKOV3 (+)
MSLN		CD8SP-MSLN-hu22A10-vL-Xho-[hTCRβ-KACIAH]-F-P2A-SP-Bst-MSLN-hu22A10-vH-Mlu-[hTCRα-CSDVP]-F-F2A-Xba-Bam-SynthK13	SKOV3 (+)	SKOV3 (+)
MSLN		CD8SP-MSLN-7D9-vH-Xho-[hTCRβ-KACIAH]-F-P2A-SP-Bst-MSLN-7D9-vL-Mlu-[hTCRα-CSDVP]-F-F2A-Xba-Bam-SynthK13	SKOV3 (+)	SKOV3 (+)
MSLN	14294	CD8SP-MSLN-7D9-HL-vH-[hTCRa-CSDVP]-F-F2A-SP-MSLN-7D9-HL-vL-[hTCRb-KACIAH]-F-P2A-Xba-PAC	SKOV3 (+/-)	SKOV3 (+)
MSLN	14241	CD8SP-MSLN-5-HL-vH-[hTCRa-CSDVP]-F-F2A-SP-MSLN-5-HL-vL-[hTCRb-KACIAH]-F-P2A-Xba-PAC	SKOV3 (+)	SKOV3 (+)
MSLN		CD8SP-MSLN-hu22A10-vL-Xho-[hTCRa-CSDVP]-F-F2A-IgHSP-Bst-MSLN-hu22A10-vH-Mlu-[hTCRb-KACIAH]-		SKOV3 (+)
MSLN	2847	MSLN-237-BBZ		SKOV3 (+)
MSLN	16312	CD8SP-MSLN-hu22A10-BBz	SKOV-3 +/-	SKOV3 (+)
MSLN	16313	CD8SP-MSLN-7D9-HL-BBz	SKOV-3 +/-	SKOV3 (+)
MSLN	16314	CD8SP-MSLN-5-HL-BBz	SKOV-3 +	SKOV3 (+)

MSLN	2849	CD8SP-MSLN-237-HL-vL-[hTCRa-CSDVP]-F-F2A-SP-MSLN-237-HL-vH-[hTCRb-KACIAH]-F-P2A-Xba-PAC		SKOV3 (+)
MSLN	14348	CD8SP-MSLN-hu22A10-vL-PG4SP-v2-[hTCRb-KACIAH]-F-P2A-SP-MSLN-hu22A10-vH-PG4SP-[hTCRa-CSDVP]-F-F2A-PAC	SKOV-3 +/-	
MSLN	14295	CD8SP-MSLN-7D9-HL-vH-PG4SP-v2-[hTCRb-KACIAH]-F-P2A-SP-MSLN-7D9-HL-vL-PG4SP-[hTCRa-CSDVP]-F-F2A-PAC	SKOV-3 +	
MSLN	14268	CD8SP-MSLN-5-HL-vH-[hTCRb-S57C]-F-P2A-SP-MSLN-5-HL-vL-[hTCRa-T48C]	SKOV-3 +	SKOV3 (+)
MSLN	14274	CD8SP-V5-[hTCRb-KACIAH]-F-P2A-CD8SP-MSLN-7D9-HL-vH-Gly-Ser-Linker-MSLN-7D9-HL-vL-Myc-[hTCRa-CSDVP]-F-F2A-PAC	SKOV-3 +	
SLC34A2	3584	CD8SP-huMX35-LH4-vL-[hTCRa-CSDVP]-F-F2A-SP-huMX35-LH4-vH-[hTCRb-KACIAH]-F-P2A-Xba-PAC	OVCAR3 (3+)	

[00669] Comparative analysis of CD19 CARs with different antigen binding domains

T cells are infected with CARs containing different antigen binding domains but having similar backbone. T cells expressing the CARs on the 2nd generation CAR backbone (*e.g.*, SEQ ID NO: 16317) and double chain SIR backbone (*e.g.*, SEQ ID NO: 14056 and 14109) and containing the antigen binding domains (*e.g.*, vL and vH fragments, vL-CDR1-3 and vH-CDR-1-3) derived from huCD19-mROO5-1 (SEQ ID NO: 14406 and 14437) and huCD19-USC3 (SEQ ID NO: 14400 and 14431), respectively, show superior *in vitro* cytotoxicity and cytokine secretion as compared to T cells expressing the corresponding CAR containing the antigen binding domains (*e.g.*, vL and vH fragments) derived from hu-CD19-USC1-LH4 (SEQ ID NO: 4190 and 4264), CD19-9B7 (SEQ ID NO: 4151 and 4225) and hu-Bu-13 (SEQ ID NO: 9655 and 9686). Therefore, antigen binding domains derived from hu-CD19-USC1-LH4, CD19-9B7 and hu-Bu-13 were not selected for construction of CARs (*e.g.*, 2nd generation CARs, SIR, Ab-TCR, TFP *etc.*)

[00670] *In vitro* and *in vivo* studies with CD19 CAR-T cells

Human peripheral blood T cells isolated using CD3 magnetic beads were infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 16311, 16317 and 16318) targeting CD19. The amino acid sequences of these CARs are represented by SEQ ID NO: 16335, 16341 and 16342, respectively. The CAR represented by SEQ ID NO: 16311 is a second generation CAR with antigen binding domain derived from FMC63 antibody and contains a 41BB co-stimulatory domain and a CD3z activation domain. The CAR represented by SEQ ID NO: 16317 is a second generation CAR with antigen binding domain derived from a low

affinity humanized anti-CD19 antibody and contains a 41BB co-stimulatory domain and a CD3z activation domain. In contrast, the CAR represented by SEQ ID NO: 16318 is a double chain SIR containing the antigen binding domain derived from a humanized low affinity anti-CD19 antibody. This SIR construct also expressed an accessory module encoding a codon optimized version of vFLIP-K13 (SEQ ID NO: 12734). All the CAR constructs were cloned in the pCCLc-MNDU3-WPRE vector (SEQ ID NO: 12639). CAR-T cells were expanded *in vitro* for 21 days in XVIVO medium supplemented with recombinant IL2 and CD3/CD28 beads. Staining with APC-conjugated Protein L followed by flow cytometry on day 5 after infection revealed strong expression of CAR constructs SEQ ID NO: 16311 and SEQ ID NO: 16317 on cell surface with approximately 45-50% of cells showing Protein L staining. In contrast, very little cell surface expression of CAR represented by SEQ ID NO: 16318 was seen with less than 2% of cell showing surface staining with Protein L. After 3 weeks in culture, RAJI and NALM cells stably expressing GLuc were cocultured with T cells expressing the different CARs at an Effector:Target (E:T) ratio of 1:1 for 48 hours. Supernatant was collected and used for measurement of IFN γ by ELISA. **Figure 2A** shows significant increase in IFN γ production when all CAR-T cells are co-cultured with RAJI cells that express high level of CD19. **Figure 2B** shows that CAR-T expressing the construct SEQ ID NO:16318 shows higher IFN γ induction as compared to CAR-T expressing the construct SEQ ID NO: 16311 when co-cultured with Nalm6 cells that express modest levels of CD19. Essentially similar results were obtained when the experiment is repeated to measure TNF α production. To test the *in vivo* efficacy of the different CAR-T cells, NSG mice were injected via tail vein with 0.5×10^6 RAJI cells stably expressing firefly luciferase (RAJI-Luc) and three days later injected with 4×10^6 T cells expressing the CAR constructs (SEQ ID NO: 16311, 16317 and 16318). Animals were imaged weekly by bioluminescence imaging following injection of D-luciferin. **Figure 3** shows that there was significant tumor growth in animals given no T cells or control T cells and they all died by day 23. The animals given T cells expressing SEQ ID NO: 16311 and 16317 initially cleared the disease but showed disease relapse after day 28. In contrast, animals given T cells expressing CAR with SEQ ID NO: 16318 remained disease-free until day 51. Mice given T cells expressing CAR with SEQ ID NO: 16318 had improved survival as compared to mice given no T cells, control T cells or T cells expressing SEQ ID NO: 16311. Mice given T cells expressing SEQ ID NO: 16317 had intermediate survival.

[00671] *In vitro* and *in vivo* studies with CD19 CAR-T cells

Human peripheral blood T cells isolated using CD3 magnetic beads were infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 16311, and 14056) targeting CD19. The CAR represented by SEQ ID NO: 16311 has been described. In contrast, the CAR represented by SEQ ID NO: 14056 is a double chain SIR containing the antigen binding domain derived from a humanized low affinity antibody. The amino acid sequence of this construct is represented by SEQ ID NO: 15800. Both the CAR constructs were cloned in the pCCLc-MNDU3-WPRE vector (SEQ ID NO: 12639). The nucleic acid sequence of the pCCLc-MNDU3-WPRE vector encoding the CAR with SEQ ID NO: 14056 is represented by SEQ ID NO: 12641. CAR-T cells were expanded *in vitro*. Staining with APC-conjugated Protein L followed by flow cytometry on day 6 after infection revealed strong expression of CAR constructs SEQ ID NO: 16311 on cell surface with approximately 71.07% of cells showing Protein L staining. In contrast, less cell surface expression of CAR represented by SEQ ID NO: 14056 was seen with 22.04% of cell showing surface staining with Protein L. After approximately 1 week expansion, RAJI and NALM cells stably expressing GLuc were cocultured with T cells expressing the different CARs at an E:T ratio of 1:1 for 48 hours. Supernatant was collected and used for measurement of IFN γ , TNF α and IL2 by ELISA. T cells expressing the next generation CAR (*i.e.*, double chain SIR) represented by SEQ ID NO: 14056 showed robust production of IFN γ , TNF α and IL2 when co-cultured with RAJI cells while the T cells expressing the FMC63-BBz CAR (SEQ ID NO: 16311) showed weak induction of these cytokines. The experiment was repeated after 4 weeks expansion of CAR-T cells in culture in XVIVO medium in the presence IL2 and CD3 and CD28 antibodies. It was observed that T cells expressing the CAR represented by SEQ ID NO: 14056 continue to show robust production of IFN γ , TNF α and IL2 when co-cultured with RAJI cells while the T cells expressing the FMC63-BBz CAR (SEQ ID NO: 16311) showed very low to negligible induction of these cytokines, suggesting evidence of functional exhaustion. To test the *in vivo* efficacy of the different CAR-T cells, NSG mice were injected via tail vein with 0.5×10^6 NALM6 cells (B cell Acute Lymphocytic Leukemia) stably expressing firefly luciferase (NALM6-Luc) and three days later injected with 3×10^6 T cells expressing the CAR constructs (SEQ ID NO: 16311 and 14056) that had been expanded *in vitro* for 2-3 weeks. Animals were imaged weekly by bioluminescence imaging following injection of D-luciferin. **Figure 4** shows that there was significant tumor growth in animals given no T cells or control T cells and they all died by day 29. The animals given T cells expressing SEQ ID NO: 16311 initially cleared the disease but showed disease relapse after day 29. In contrast, animals given T cells expressing CAR with SEQ ID NO: 14056 remained disease-free until

day 36. Mice given T cells expressing CAR with SEQ ID NO: 14056 had improved survival as compared to mice given no T cells, control T cells or T cells expressing CAR represented by SEQ ID NO: 16311. Essentially similar results are obtained when T cells expressing the SIR (SEQ ID NO:16330) are administered to NSG mice xenografted with Nalm6 cells.

[00672] Comparision of antigen masking by CD19 targted CAR, SIR and TFP

Accidental insertion of a conventional 2nd generation CD19 CAR into a single B cell Acute lymphocytic leukemia cell was recently shown to result to disease relapse due to masking of CD19 expressed on leukemia cells by the CAR polypeptide expressed in the leukemia cells (Ruella M *et al*, Nat Med. 2018 Oct;24(10):1499-1503). This prevented the CAR-T cells from recognizing and killing the leukemia cells expressing the CAR, leading to clonal proliferation, disease relapse and death of the patient. To test, whether the next generation CAR are also susceptible to this problem, lentiviruses encoding second generation CARs (SEQ ID NO: 16311, 16317), SIRs (SEQ ID NO: 14035, 14056, 14065, 14109 and 16330) and TFP (SEQ ID NO: 16328 and 14098) are stably expressed in CD19-expressing RAJI and Nalm6 cells. The CAR-expressing cells are subsequently stained with PE-conjugated CD19 antibody (*e.g.*, FMC63-PE). It is observed that expression of second generation CARs (SEQ ID NO: 16311, 16317), and TFP (SEQ ID NO: 16328 and 14098) results in maksing of CD19 on RAJI and Nalm6 cells as revealed by decrease in cell surface staining for CD19 as determined by binding with CD19-PE antibody and flow cytometry. In contrast, expression of SIRs (SEQ ID NO: 14035, 14056, 14065, 14109 and 16330) has no significant effect on expression of CD19 in RAJI and Nalm6 cells. Furthermore, RAJI cells expressing the second generation CARs (SEQ ID NO: 16311, 16317), and TFP (SEQ ID NO: 16328 and 14098) show reduced killing by T cells expressing the corresponding CAR and TFP, while RAJI cells expressing the SIRs (SEQ ID NO: 14035, 14056, 14065, 14109 and 16330) retain their susceptibility to killing by T cells expressing the correspondning SIR.

[00673] Comparison of CARs using Cytokine release syndrome (CRS) model

The ability of different CD19 CAR constructs to induce CRS was tested using a recently described mouse model (Giavridis T *et al*, Nature Medicine, 2018). Briefly, SCID-Biege mice were injected i.p (intraperitoneal) with 3 million Raji-pLenti-Luc cells on day1. 30 million CAR-Ts expressing the CAR (SEQ ID NO: 16315 and 16330) were injected on day 21 (intraperitoneal). Similar to the published study (Giavridis T et al, Nature Medicine) one third of animals given the 2nd generation CAR (SEQ ID NO: 16315) died after the injection of CAR-T cells. None of the animals receiving SIR (SEQ ID NO: 16330) died. Essentially,

similar results are obtained when the experiment is repeated using T cells expressing the SIR (SEQ ID NO: 14056).

[00674] Comparative analysis of MPL CARs with different antigen binding domains

T cells are infected with CARs containing different antigen binding domains but having similar backbone. T cells expressing the MPL CARs on different CAR backbones (*e.g.*, SEQ ID NO: 16315, 13761-13770, 13780-13794) and containing the antigen binding domains (*e.g.*, vL and vH fragments, vL-CDR1-3 and vH-CDR1-3) derived from hu-161-2 (SEQ ID NO: 14409 and 14440) and hu-161-3 (SEQ ID NO: 14402 and 14433), respectively, show superior *in vitro* cytotoxicity and cytokine secretion as compared to T cells expressing the corresponding CAR containing the antigen binding domains (*e.g.*, vL and vH fragments) derived from MPL-178, MPL-12E10 and MPL-AB317 which are described in WO2019067805. Therefore, antigen binding domains derived from MPL-178, MPL-12E10 and MPL-AB317 were not selected for construction of MPL targeted CARs (*e.g.*, 2nd generation CARs, SIR, Ab-TCR, TFP *etc.*). Instead, the antigen binding domains derived from hu-161-2 (SEQ ID NO: 14409 and 14440) and hu-161-3 (SEQ ID NO: 14402 and 14433) and vL and vH containing their corresponding CDR regions were selected for MPL targeted CARs (*e.g.*, 2nd generation CARs, SIR, Ab-TCR, TFP *etc.*). The CARs with SEQ ID NO: 13791 and 13793 are compared and CAR with SEQ ID NO: 13791 is shown to exhibit superior *in vitro* cytotoxicity and cytokine production as compared to CAR with SEQ ID NO: 13793. The CARs with SEQ ID NO: 13791 is also found to be superior to the corresponding CAR on the same backbone but containing antigen binding domain derived from murine MPL-161 as described in WO2019067805.

[00675] *In vitro* and *in vivo* studies with MPL CAR-T cells

Human peripheral blood T cells isolated using CD3 magnetic beads were infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 16315 and 13791) targeting human MPL (Thrombopoietin Receptor). The CAR (CD8SP-MPL-hu-161-2-BBz) represented by SEQ ID NO: 16315 is a second generation CAR with antigen binding domain derived from a humanized MPL antibody and contains a 41BB co-stimulatory domain and a CD3z activation domain. In contrast, the CAR represented by SEQ ID NO: 13791 is a double chain SIR containing the antigen binding domain derived from a humanized MPL antibody. Both the CAR constructs were cloned in the pCCLc-MNDU3-WPRE vector (SEQ ID NO: 12639). CAR-T cells were expanded *in vitro* for up to 2-4 weeks. HEL.92.1.7 cells stably expressing GLuc were cocultured with T cells expressing the different CARs at an E:T ratio of 1:1 for 48 hours. Cell death was measured using the Matador Assay. T cells expressing the next

generation CAR (*i.e.*, double chain SIR) represented by SEQ ID NO: 13791 showed robust induction of target cell death and cytokine production while the T cells expressing the MPL-hu-161-2-BBz CAR (SEQ ID NO: 16315) showed weak induction of target cell death and cytokine production. To test the *in vivo* efficacy of the different CAR-T cells, NSG mice were injected via tail vein with 0.5×10^6 HEL.92.1.7 cells (Acute Myeloid Leukemia) stably expressing firefly luciferase (HEL-Luc) and three days later injected with 3×10^6 T cells expressing the CAR constructs (SEQ ID NO: 16315 and 13791) that had been expanded *in vitro* for 2-3 weeks. Mice given T cells expressing CAR with SEQ ID NO: 13791 had improved survival as compared to mice given no T cells, control T cells or T cells expressing SEQ ID NO: 16315.

[00676] Comparison of antigen masking by MPL targeted CAR, SIR and TFP

HEL.92.1.7 cells are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 16315, 13780 and 13791) targeting human MPL. The effect of CAR expression on masking of MPL is determined by immunofluorescence staining with MPL antibody (1.6.1) and FACS analysis. Alternatively, the expression of unbound MPL is determined by binding with a 161-scFv-Nluc fusion protein (SEQ ID NO: 2245 as described in WO2017173403, which is incorporated herein in its entirety by reference). The 161-scFv-Nluc fusion protein contains the antigen binding domain derived from an MPL targeted antibody (1.6.1) fused to Nluc. The expression of CAR with SEQ ID NO: 16315 and 13780 in HEL.92.1.7 cells is found to result in antigen masking while expression of CAR with SEQ ID NO: 13791 does not result in significant masking of MPL.

[00677] Comparative analysis of BCMA CARs with different antigen binding domains

T cells are infected with CARs containing different antigen binding domains but having similar backbone. T cells expressing the BCMA CARs on different CAR backbones (*e.g.*, SEQ ID NO: 12913, 12916-12946) and containing the antigen binding domains (*e.g.*, vL and vH fragments, vL-CDR1-3 and vH-CDR1-3) derived from BCMA-huC13-F12 (SEQ ID NO: 14413 and 14444), BCMA-huC12A3-L3H3 (SEQ ID NO: 14414 and 14445), BCMA-J6M0 (SEQ ID NO: 14415 and 14446), BCMA-huJ22-10 (SEQ ID NO: 14398 and 14229) and BCMA-hu72 (SEQ ID NO: 14401 14432) respectively, show superior *in vitro* cytotoxicity and cytokine secretion. Therefore, antigen binding domains derived from the above antigen binding domains and vL and vH containing their corresponding CDR regions were selected for BCMA targeted CARs (*e.g.*, 2nd generation CARs, SIR, Ab-TCR, TFP *etc.*).

[00678] *In vitro* and *in vivo* studies with BCMA CAR-T cells

Human peripheral blood T cells isolated using CD3 magnetic beads were infected with lentiviruses expressing the CAR constructs (SEQ ID NO (DNA): 16316 and 12890, 12943) targeting human BCMA. The corresponding amino acid sequences of these constructs are represented by SEQ ID NO: 16340, 14634 and 14687). The CAR represented by SEQ ID NO: 16316 is a second generation CAR with antigen binding domain derived from a humanized MPL antibody and contains a 41BB co-stimulatory domain and a CD3 ζ activation domain. In contrast, the CAR represented by SEQ ID NO: 12890 and 12943 are double chain SIRs. Both the CAR constructs were cloned in the pCCLc-MNDU3-WPRE vector (SEQ ID NO: 12639). The complete nucleic acid sequence of the lentiviral vector encoding the CARs SEQ ID NO: 12890 and 12943 is represented by SEQ ID NO: 14378-14385, respectively. CAR-T cells were expanded *in vitro* for upto 2-4 weeks. L363 cells stably expressing GLuc were cocultured with T cells expressing the different CARs at an E:T ratio of 1:1 for 48 hours. Cell death was measured using the Matador Assay. All CAR-T cells showed modest induction of target cell death and cytokine (IFN γ and TNF α) production. To test the *in vivo* efficacy of the different CAR-T cells, NSG mice were injected via tail vein with 0.5×10^6 L363 (plasma cell leukemia) stably expressing firefly luciferase (L363-Luc) and two days later injected with 2×10^6 T cells expressing the CAR constructs (SEQ ID NO: 16316 and 12890, 12943) that had been expanded *in vitro* for 2-3 weeks. Mice given T cells expressing CARs with SEQ ID NO: 16316 and 12890, 12943 had improved survival as compared to mice given control T cells. Essentially similar results are obtained using T cells expressing the CARs represented by SEQ ID NO: 13049, 12996, and 12837.

[00679] The ability of different CAR constructs to mask the BCMA antigen is tested by stably expressing the CAR constructs with SEQ ID NO (DNA): 16316 and 12890, 12943 in L363 and U266 cell lines. It is observed that stable expression of CAR 16316 results in antigen masking of BCMA while no significant antigen masking is observed upon stable expression of constructs with SEQ ID NO: 12890, 12943. Similarly, CARs with SEQ ID NO: 13049, 12996, and 12837 do not result in antigen masking when expressed in BCMA expressing L363 or U266 cells.

[00680] Comparative analysis of Mesothelin (MSLN) CARs with different antigen binding domains

T cells are infected with CARs containing different antigen binding domains but having similar backbone. T cells expressing the MSLN CARs on different CAR backbones (*e.g.*, SEQ ID NO: 14291-14323) and containing the antigen binding domains (*e.g.*, vL and vH

fragments, vL-CDR1-3 and vH-CDR1-3) derived from MSLN-3-HL-AM (SEQ ID NO: 4136 and 4210), MSLN-5 (SEQ ID NO: 14412 and 14443), MSLN-7D9-HL (SEQ ID NO: 14411 and 14442), and MSLN-hu22A10 (SEQ ID NO: 14410 and 14441) respectively, show superior *in vitro* cytotoxicity and cytokine secretion as compared to antigen binding domains derived from MSLN-HuAM15 and MSLN76923-HL. Therefore, antigen binding domains derived from MSLN-3-HL-AM (SEQ ID NO: 4136 and 4210), MSLN-5 (SEQ ID NO: 14412 and 14443), MSLN-7D9-HL (SEQ ID NO: 14411 and 14442), and MSLN-hu22A10 (SEQ ID NO: 14410 and 14441) and vL and vH containing their corresponding CDR regions were selected for MSLN targeted CARs (*e.g.*, 2nd generation CARs, SIR, Ab-TCR, TFP *etc.*).

[00681] Comparative analysis of Mesothelin (MSLN) CARs with different antigen binding domains and backbones

[00682] T cells expressing the MSLN CARs containing different antigen binding domains and on different CAR backbones (SEQ ID NO: 16312-16314; 16361-16363) were generated using gene transfer involving pCCLc-MNDU3-WPRE vector (SEQ ID NO: 12639). The CAR-expressing T cells were tested against SKOV3 cells for cytotoxicity using Matador assay and for production of cytokines. All CAR-T cells showed mild to modest cytotoxicity and varying levels of cytokine production upon co-culture with SKOV3 cells. Next, the CAR-T cells were tested in a SKOV3 xenograft model in NSG mice. For this purpose, 1×10^6 SKOV3-Luc cells were injected subcutaneously followed a week later by injection of intravenous injection of 3×10^6 CAR-expressing T cells. Tumor growth was monitored by bioluminescence imaging and tumor volume measurement. A mild to modest inhibition of tumor growth was seen in mice given T cells expressing CARs with SEQ ID NO: 16312-16314 and 16361-16362.

[00683] Next, T cells expressing the MSLN CARs containing different antigen binding domains and on different CAR backbones (SEQ ID NO: 16313-16314; 16331-16334; 14268-14269; 14321, and 14374) were generated using gene transfer involving pCCLc-MNDU3-WPRE vector (SEQ ID NO: 12639). The amino acid sequence of these CAR constructs are represented by SEQ ID NO: 16337-16338; 16354-16357; 16012-16013, 16065 and 16118, respectively. The complete nucleic acid sequence of the lentiviral vector encoding the CARs SEQ ID NO: 14374, 14321 and 14268 is represented by SEQ ID NO: 14381-14383, respectively. The CAR-expressing T cells were tested against SKOV3 cells for cytotoxicity using Matador assay and for production of cytokines. All CAR-T cells showed modest cytotoxicity and varying levels of cytokine production upon 72 hour co-culture with SKOV3 cells at an E:T ratio of 1:1. T cells expressing the CARs showed high baseline

production of IFN γ and TNF α which was absent in T cells expressing the CARs 16331-16334; 14268-14269; 14321, and 14374. Next, the CAR-T cells were tested in a SKOV3 xenograft model in NSG mice. For this purpose, 1×10^6 SKOV3-Luc cells were injected subcutaneously followed a week later by injection of intravenous injection of 3×10^6 CAR-expressing T cells. Tumor growth was monitored by bioluminescence imaging and tumor volume measurement. While mice given T cells expressing CARs with SEQ ID NO: 16313-16314 failed to control tumor, mice given CARs with SEQ ID NO: 16331-16334; 14268-14269; 14321, and 14374 completely eradicated the tumor after day 18 and no measureable tumor was observed in these animal until day 67. The results were confirmed using bioluminescence imaging. This resulted in significant improvement in survival in animals given CARs with SEQ ID NO: 16331-16334; 14268-14269; 14321, and 14374.

[00684] Comparative analysis of CD22 CARs with different antigen binding domains

T cells are infected with CARs containing different antigen binding domains but having similar backbone. T cells expressing the CD22 CARs on the 2nd generation CAR backbone (*e.g.*, SEQ ID NO: 13443, 13390, 13284 and 14185) and double chain SIR backbone (*e.g.*, SEQ ID NO: 13473, 13420, 13314 and 14215) and containing the antigen binding domains (*e.g.*, vL and vH fragments, vL-CDR1-3 and vH-CDR-1-3) derived from CD22-INO (SEQ ID NO: 14387 and 14418), CD22-hu-HA22-2 (SEQ ID NO: 14399 and 14430) CD22-h10F4v2 (SEQ ID NO: 14407 and 14438) and CD22-hu-RFB4 (SEQ ID NO: 14396 and 14427), respectively, show superior *in vitro* cytotoxicity and cytokine secretion as compared to T cells expressing the corresponding CAR containing the antigen binding domains (*e.g.*, vL and vH fragments) derived from hu-HA22-1 (SEQ ID NO: 4123 and 4197) and CD22-CELL7 (SEQ ID NO: 14390 and 14421). Therefore, antigen binding domains derived from hu-HA22-1 and CD22-CELL7 were not selected for construction of CD22 targeted CARs (*e.g.*, 2nd generation CARs, SIR, Ab-TCR, TFP *etc.*). Instead, the antigen binding domains derived from CD22-INO (SEQ ID NO: 14387 and 14418), CD22-hu-HA22-2 (SEQ ID NO: 14399 and 14430) CD22-h10F4v2 (SEQ ID NO: 14407 and 14438) and CD22-hu-RFB4 (SEQ ID NO: 14396 and 14427) and vL and vH containing their corresponding CDR regions were selected for CD22 targeted CARs.

[00685] T cells expressing Folate Receptor 1 (FR1 or FOLR1) CARs induce cytotoxicity in FR1-expressing SKOV3 cells. Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 2062-2102; 2111-2140) targeting FR1. CAR-T cells are expanded *in vitro* for 10-14 days. SKOV3 cells stably expressing GLuc are cocultured with T cells expressing the

CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00686] T cells expressing BAFF-R CARs induce cytotoxicity in BAFF-R-expressing Jeko-1 and REC-1 cells. Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the different CAR constructs (*e.g.*, SEQ ID NO: 13922-13953; 13848-13858, 13869- 13900, 13954-13964; 13975-14006) targeting BAFF-R. CAR-T cells are expanded *in vitro* for 10-14 days. Jeko-1 and REC-1 cells stably expressing hGLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The production of IFN γ and TNF α is determined by ELISA. The *in vivo* activity of the CARs is demonstrated using a Jeko-1 xenograft model in NSG mice. T cells expressing the CARs (*e.g.*, SEQ ID NO: 13897, 13950, 14003 *etc*) are shown to activate T cell signaling when encountered by BAFF-R positive cells.

[00687] T cells expressing Mesothelin (MSLN) CARs induce cytotoxicity in MSLN-expressing SKOV3 cells. Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the different CAR constructs (SEQ ID NO: 2748-2777; 2797-2826; 2846-2875; 2895-2924; 2944-2973; 9386-9415; 9435-9464) targeting MSLN. CAR-T cells are expanded *in vitro* for 10-14 days. SKOV3 cells stably expressing hGLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00688] T cells expressing Her2 CARs induce cytotoxicity in Her2-expressing MCF7 cells. Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 2346, 2356-2385; 2395, 2405-2434; 2444, 2454- 2483; 9092-9121; 9141-9170) targeting Her2. CAR-T cells are expanded *in vitro* for 10-14 days. MCF7 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00689] T cells expressing EGFRviii CARs induce cytotoxicity in EGFRviii-expressing U87MG cells. Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 1660, 1670-1699, 1709, 1719-1748, 1758, 1768-1797, 1807, 1817-1846) targeting EGFRviii. CAR-T cells are expanded *in vitro* for 10-14 days. **U87MG-EGFRviii** cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00690] T cells expressing EMR2 CARs induce cytotoxicity in EMR2-expressing cells. Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 1856, 1866-1895, 1905, 1915-1944, 1954, 1964-1993) targeting EMR2. CAR-T cells are expanded *in vitro* for 10-14 days. Molm13 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00691] T cells expressing DLL3 CARs induce cytotoxicity in DLL3-expressing cells. Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 1553-1650) targeting **DLL3**. CAR-T cells are expanded *in vitro* for 10-14 days. SK-MEL-37 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00692] T cells expressing CD19 CARs induce cytotoxicity in CD19-expressing cells. Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 769-817, 720-768, 867-915, 965-1013, 818- 866, 8632-8680) targeting **CD19**. CAR-T cells are expanded *in vitro* for 10-14 days. RAJI or NALM6 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 4 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00693] T cells expressing CD20 CARs induce cytotoxicity in CD20-expressing cells.

Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 1063-1111, 1014-1062) targeting **CD20**. CAR-T cells are expanded *in vitro* for 10-14 days. RAJI or NALM6 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 4 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00694] T cells expressing BCMA CARs induce cytotoxicity in BCMA-expressing cells.

Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 377-572, 8093-8484) targeting **BCMA**. CAR-T cells are expanded *in vitro* for 10-14 days. U266 and L363 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00695] T cells expressing FLT3 CARs induce cytotoxicity in FLT3-expressing cells.

Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 8926-9023) targeting **FLT3**. CAR-T cells are expanded *in vitro* for 10-14 days. RS4;11 and MV4;11 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00696] T cells expressing CLL1 CARs induce cytotoxicity in CLL1-expressing cells.

Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 8779-8876) targeting **CLL1**. CAR-T cells are expanded *in vitro* for 10-14 days. RAJI and U937 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00697] T cells expressing BST1 CARs induce cytotoxicity in BST1-expressing cells.

Human peripheral blood T cells isolated using CD3 magnetic beads are infected with

lentiviruses expressing the CAR constructs (SEQ ID NO: 8485-8631) targeting **BST1**. CAR-T cells are expanded *in vitro* for 10-14 days. KG1 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00698] T cells expressing IL1RAP CARs induce cytotoxicity in IL1RAP-expressing cells. Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 9171-9317) targeting **IL1RAP**. CAR-T cells are expanded *in vitro* for 10-14 days. THP-1 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00699] T cells expressing gpA33 CARs induce cytotoxicity in gpA33-expressing cells. Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 9024-9072) targeting **gpA33**. CAR-T cells are expanded *in vitro* for 10-14 days. Molm-13 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00700] T cells expressing GPC3 CARs induce cytotoxicity in GPC3-expressing cells. Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 9024-9072) targeting **GPC3**. CAR-T cells are expanded *in vitro* for 10-14 days. HepG2 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00701] T cells expressing CLDN6 CARs induce cytotoxicity in CLDN6-expressing cells. Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 1455-1552) targeting **CLDN6**. CAR-T cells are expanded *in vitro* for 10-14 days. HepG2 cells stably expressing GLuc are

cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00702] T cells expressing UPK1B CARs induce cytotoxicity in UPK1B-expressing cells. Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 1455-1552) targeting **UPK1B**. CAR-T cells are expanded *in vitro* for 10-14 days. OVCAR-3 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00703] T cells expressing BMPR1B CARs induce cytotoxicity in BMPR1B-expressing cells. Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 573-670) targeting **BMPR1B**. CAR-T cells are expanded *in vitro* for 10-14 days. LNCaP and COV434 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00704] T cells expressing WISP1 CARs induce cytotoxicity in WISP1-expressing cells. Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 3856-3953) targeting **WISP1**. CAR-T cells are expanded *in vitro* for 10-14 days. MDA-MB-453 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00705] T cells expressing CD133 CARs induce cytotoxicity in CD133-expressing cells. Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 11312-11458) targeting **CD133**. CAR-T cells are expanded *in vitro* for 10-14 days. Reh and RS4;11 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador

assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00706] **T cells expressing Prolactin Receptor (PRLR) CARs induce cytotoxicity in PRLR -expressing cells.** Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 3121-3218) targeting **PRLR**. CAR-T cells are expanded *in vitro* for 10-14 days. MCF7 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00707] **T cells expressing IL13Ra2 CARs induce cytotoxicity in IL13Ra2-expressing cells.** Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 14132-14165) targeting **IL13Ra2**. CAR-T cells are expanded *in vitro* for 10-14 days. U87MG cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00708] **T cells expressing Nectin-4 CARs induce cytotoxicity in Nectin-4 -expressing cells.** Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 3072-3120, 9465-9513) targeting **Nectin-4**. CAR-T cells are expanded *in vitro* for 10-14 days. MCF7 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00709] **T cells expressing PSMA CARs induce cytotoxicity in PSMA -expressing cells.** Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 3219-3365) targeting **PSMA**. CAR-T cells are expanded *in vitro* for 10-14 days. PC3 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00710] T cells expressing TSHR (Thyroid Stimulating Hormone Receptor) CARs induce cytotoxicity in TSHR-expressing cells. Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 3611-3659) targeting **TSHR**. CAR-T cells are expanded *in vitro* for 10-14 days. TT cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00711] T cells expressing CDH19 CARs induce cytotoxicity in CDH19-expressing cells. Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 1308-1405) targeting **CDH19**. CAR-T cells are expanded *in vitro* for 10-14 days. MEL-624 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00712] T cells expressing VISTA CARs induce cytotoxicity in VISTA-expressing cells. Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 3758-3855) targeting **VISTA**. CAR-T cells are expanded *in vitro* for 10-14 days. MOLM-13 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 4 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00713] T cells expressing ROR1 CARs induce cytotoxicity in ROR1-expressing cells. Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 9514-9562) targeting **ROR1**. CAR-T cells are expanded *in vitro* for 10-14 days. JEKO-1 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 4 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00714] T cells expressing Liv1 CARs induce cytotoxicity in Liv1-expressing cells. Human peripheral blood T cells isolated using CD3 magnetic beads are infected with

lentiviruses expressing the CAR constructs (SEQ ID NO: 9514-9562) targeting **Liv1**. CAR-T cells are expanded *in vitro* for 10-14 days. MCF7 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 4 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00715] **T cells expressing Integrin B7 CARs induce cytotoxicity in Integrin B7-expressing cells.** Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 2533-2581) targeting **Integrin B7**. CAR-T cells are expanded *in vitro* for 10-14 days. U266 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 4 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00716] **T cells expressing SLC34A2 CARs induce cytotoxicity in SLC34A2-expressing cells.** Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 3562-3610) targeting **SLC34A2**. CAR-T cells are expanded *in vitro* for 10-14 days. OVCAR-3 and OVCAR-4 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00717] **T cells expressing LY6E CARs induce cytotoxicity in LY6E-expressing cells.** Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 2582-2630) targeting **LY6E**. CAR-T cells are expanded *in vitro* for 10-14 days. Molm13 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 4 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00718] **T cells expressing LYPD1 CARs induce cytotoxicity in LYPD1-expressing cells.** Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 2631-2679) targeting **LYPD1**. CAR-T cells are expanded *in vitro* for 10-14 days. OVCAR-3 cells stably expressing GLuc

are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00719] T cells expressing STEAP1 CARs induce cytotoxicity in STEAP1-expressing cells. Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 3513-3561, 9563-9611) targeting **STEAP1**. CAR-T cells are expanded *in vitro* for 10-14 days. PC3 and LNCaP cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00720] T cells expressing Muc5Ac CARs induce cytotoxicity in Muc5Ac-expressing cells. Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 2974-3022) targeting **Muc5Ac**. CAR-T cells are expanded *in vitro* for 10-14 days. Capan-1 (Pancreatic cancer) and NCI-H1437 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00721] T cells expressing Muc17 CARs induce cytotoxicity in Muc17-expressing cells. Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 3023-3071) targeting **Muc17**. CAR-T cells are expanded *in vitro* for 10-14 days. SW1463 and SW403 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00722] T cells expressing RNF43 CARs induce cytotoxicity in RNF43-expressing cells. Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 3366-3463) targeting **RNF43**. CAR-T cells are expanded *in vitro* for 10-14 days. Lovo cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by

measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00723] T cells expressing Robo4 CARs induce cytotoxicity in Robo4-expressing cells.

Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 3464-3512) targeting **Robo4**. CAR-T cells are expanded *in vitro* for 10-14 days. ME-1 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00724] T cells expressing gPNMB CARs induce cytotoxicity in gPNMB-expressing cells.

Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 2239-2287) targeting **gPNMB**. CAR-T cells are expanded *in vitro* for 10-14 days. U87MG cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00725] T cells expressing FCRH5 CARs induce cytotoxicity in FCRH5-expressing cells.

Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CAR constructs (SEQ ID NO: 1994-2042) targeting **FCRH5**. CAR-T cells are expanded *in vitro* for 10-14 days. REC-1 cells stably expressing GLuc are cocultured with T cells expressing the different CARs at an E:T ratio of 10:1 for 48 hours. CAR-T cells mediated induction of lysis of target cells is assayed using the Matador assay by measurement of GLuc activity. The *in vivo* activity of the CARs is demonstrated using a xenograft model in NSG mice.

[00726] *In vivo* efficacy of CARs targeting CD19.

Human peripheral blood T cells isolated using CD3 magnetic beads are infected with lentiviruses expressing the CD19 CAR constructs (*e.g.*, SEQ ID NO: 8633-8680). NSG mice (Jackson Lab) are sub-lethally irradiated at a dose of 175 cGy. Approximately 24 hours post irradiation (day 2), mice are injected with 2.5×10^4 RAJI cells via tail-vein. On day 3, the mice (n=5 for each group) are treated with 5 million CD19 CAR-T cells. Control mice (n=5) receive no T cells or uninfected T cells. Mice are given human IL2 (400 IU intraperitoneally) on alternate days till the death of all mice in control group. Mice receiving the CD19 CAR-T cells survive longer

than the control mice. Essentially a similar approach is used to test the *in vivo* efficacy of other CAR T cells of the disclosure using xenografts of cell lines expressing their target antigens as shown in **Table A** or using information available in the literature.

[00727] **Use of CAR-T cells for adoptive cell therapy.** CAR-T cells of the disclosure can be used for adoptive cell therapy. As an example, patients with relapsed Acute Lymphocytic Leukemia (ALL), Chronic Lymphocytic Leukemia (CLL), or high-risk intermediate grade B-cell lymphomas may receive immunotherapy with adoptively transferred CAR-T cells targeting CD19. A leukapheresis product collected from each patient undergoes selection of CD3-positive T lymphocytes using the CliniMACS Prodigy® System from Miltenyi Biotec and following the manufacturer's recommendations. Cells are transduced with clinical grade CD19-CAR virus (*e.g.*, SEQ ID NO: 14056, SEQ ID NO: 14109, SEQ ID NO: 16330; SEQ ID NO:903, SEQ ID NO: 791] and then selection and expansion of the CAR-T cells occur in a closed system. After the resulting cell products have undergone quality control testing (including sterility and tumor specific cytotoxicity tests), they are cryopreserved. Meanwhile, following leukapheresis, study participants commence with lymphodepletive chemotherapy (30 mg/m²/day fludarabine plus 500 mg/m²/day cyclophosphamide x 3 days). One day after completion of their lymphodepleting regimen, the previously stored CAR-T cell product is transported, thawed and infused at the patient's bedside. The study participant receives CAR-transduced lymphocytes infused intravenously followed by high-dose (720,000 IU/kg) IL-2 (Aldesleukin; Prometheus, San Diego, CA) every 8 hours to tolerance. The dose of CAR-T product varies from 1×10^4 CAR+ve CD3 cells/kg to 5×10^9 CAR+ve CD3 cells/kg as per the study protocol. The CAR-T product may be administered in a single infusion or split infusions. Research participants can be pre-medicated at least 30 minutes prior to T cell infusion with 15 mg/kg of acetaminophen P.O. (max. 650 mg.) and diphenhydramine 0.5-1 mg/kg I.V. (max dose 50 mg). The study participant may optionally receive daily injections of human IL-2. Clinical and laboratory correlative follow-up studies can then be performed at the physician's discretion, and may include quantitative RT-PCR studies for the presence of CD19-expressing ALL/lymphoma cells and/or the adoptively transferred T cells; FDG-PET and/or CT scans; bone marrow examination for disease specific pathologic evaluation; lymph node biopsy; and/or long-term follow up per the guidelines set forth by the FDA's Biologic Response Modifiers Advisory Committee that apply to gene transfer studies. Essentially a similar approach can be used to treat other diseases using immune cells (*e.g.*, T cells) that have been engineered to express the

CAR of the disclosure where the CAR targets an antigen or antigens expressed on the disease causing or disease-associated cells.

[00728] Use of CAR-T cells targeting multiple antigens for adoptive cell therapy.

Patients many cancers are enrolled in an IRB approved phase I clinical trial of to immunotherapy with adoptively transferred CAR-T cells targeting different disease causing or disease associated antigens. The CAR for different diseases are 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 is confirmed by binding with ABD-GGS-NLuc fusion protein in which the antigen binding domain (ABD) of CAR is fused to non-secretory form of NLuc protein via a flexible linker. Alternatively, immunohistochemistry or flow cytometry using commercially available antibodies is used to confirm the expression of the CAR target on disease causing or disease associated cells. T cells are collected from the subject using leukopheresis, transduced with the appropriate CAR encoding lentivirus vector 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 are cryopreserved. Meanwhile, study participants commence with lymphodepletive chemotherapy (30 mg/m²/day fludarabine plus 500 mg/m²/day cyclophosphamide x 3 days). One day after completion of their lymphodepleting regimen, the study participant receives CAR transduced lymphocytes infused intravenously followed by high -dose (720,000 IU/kg) IL-2 (Aldesleukin; Prometheus, San Diego, CA) every 8 hours to tolerance. The previously stored CAR-T cell product is transported, thawed and infused at the patient's bedside. The dose of CAR-T product varies from 1 x 10⁴ CAR+ve CD3 cells/kg to 5 x 10⁹ CAR+ve CD3 cells/kg as per the study protocol. The CAR-T product may be administered in a single infusion or split infusions. Research participants can be pre-medicated at least 30 minutes prior to T cell infusion with 15 mg/kg of acetaminophen P.O. (max. 650 mg.) and diphenhydramine 0.5-1 mg/kg I.V. (max dose 50 mg). The study participant may optionally receive daily injections of human IL-2. Clinical and laboratory correlative follow-up studies can then be performed at the physician's discretion.

[00729] Use of both myeloablative and lymphodepleting chemotherapy prior to adoptive cellular therapy

[00730] Essentially a similar protocol as described in the preceding example is used with the exception that the study participant receives both myeloablative and lymphodepleting chemotherapy regimen. Exemplary myeloablative conditioning regimens

include FCE (Fludarabine 25 mg/m²/day, days -7 to -3; cyclophosphamide 200 mg/m²/day, days -7 to -3; and etoposide 250 mg/m²/day, days -4 to -3), FCIE (Fludarabine 25 mg/m²/day, days -7 to -3; cyclophosphamide 200 mg/m²/day, days -7 to -3; idarubicin 12 mg/m²/day, days -7 to -5 and etoposide 250 mg/m²/day, days -4 to -3), FluCyE (fludarabine 30 mg/m²/day, cytarabine 1.5 g/m²/day administered following fludarabine and etoposide 100 mg/m²/day with each of the drugs given on days -6 to -1), or FE (fludarabine 30 mg/m²/day and Etoposide 100 mg/m²/day on days -5 to day -1) or Etoposide (50-100 mg/m²/day on days -5 to day -1). The subject receives CAR-T cell products 24-72 hours after the completion of chemotherapy. The incidence and severity of cytokine release syndrome and neurotoxicity is reduced in patients receiving both myeloablative and lymphodepleting chemotherapy prior to the administration of CAR-T cells.

[00731] Use of an mTOR inhibitor RAD001 in combination with CAR-T cells. The study is conducted as described in the preceding examples with the exception that starting 1 day after the infusion of CAR -T cells, study participants are administered an mTOR inhibitor, *e.g.*, an allosteric inhibitor, *e.g.*, RAD001, at a dosage that provides a target trough level 0.1 to 3 ng/ml, where the trough level" refers to the concentration of a drug in plasma just before the next dose, or the minimum drug concentration between two doses.

[00732] Use of Ibrutinib in combination with CAR-T cells. The study is conducted as described in the preceding examples with the exception that starting 1 day after the infusion of CAR-T cells, study participants are administered oral ibrutinib at dose of 140 mg/d to 420 mg/d. It is noted that the study participant receiving ibrutinib has less incidence of severe cytokine release syndrome as compared to participants who received CAR-T cells without ibrutinib.

[00733] Use of allogeneic CAR-T cells for adoptive cells therapy. Patients with relapsed myeloma and primary effusion lymphoma may receive immunotherapy with adoptively transferred allogeneic CAR-T cells. A leukapheresis product collected from an HLA-matched donor undergoes selection of CD3 positive T lymphocytes using the CliniMACS Prodigy® System from Miltenyi Biotec and following the manufacturer's recommendations. BCMA-specific CAR (SEQ ID NO: 12837, 12890, 12943 or 13049) is directed to the TRAC locus in T cells essentially as described in the study by Eyquem J *et al* (Nature, 543(7643):113-117). Cells are expanded for 9-12 days in a closed system. After the resulting cell products have undergone quality control testing (including sterility and tumor specific cytotoxicity tests), they are cryopreserved. Meanwhile, study participants commence with lymphodepletive chemotherapy (30 mg/m²/day fludarabine plus 500 mg/m²/day

cyclophosphamide x 3 days). One day after completion of their lymphodepleting regimen, the study participant receives transduced lymphocytes infused intravenously followed by high - dose (720,000 IU/kg) IL-2 (Aldesleukin; Prometheus, San Diego, CA) every 8 hours to tolerance. The CAR-T cell product is transported, thawed and infused at the patient's bedside. The dose of CAR-T product may vary from 1×10^4 CAR+ve CD3 cells/kg to 5×10^9 CAR+ve CD3 cells/kg as per the study protocol. The CAR product may be administered in a single infusion or split infusions. Research participants can be pre-medicated at least 30 minutes prior to CAR-T cell infusion with 15 mg/kg of acetaminophen P.O. (max. 650 mg.) and diphenhydramine 0.5-1 mg/kg I.V. (max dose 50 mg). Use of immunosuppressive drugs is also at the discretion of the physician. Essentially a similar approach can be used to treat other diseases using allogeneic immune cells (*e.g.*, T cells) expressing the CAR of the disclosure where the CAR targets an antigen or antigens expressed on the disease causing or disease-associated cells.

[00734] **CAR-T Cell Hepatic Arterial Infusion.** In addition to intravenous infusion, CAR-T cells 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 an ovarian cancer which expresses Mesothelin (MSLN). Essentially a similar approach can be used for intra-arterial infusion of CAR-T cells targeting other tumor antigens.

[00735] A mapping angiogram is 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, is embolized with microcoils. The same arterial access procedure is carried out for administration of T cells expressing the MSLN CARs either singly or in combination (SEQ ID NO: 16331-16334; 14268-14269; 14321, or 14374). The T cells is collected from the patient on day 0 and are infected with CAR encoding lentiviruses either singly or in combination and expanded as described in the previous examples. The CAR-T cells will be given in a dose escalating fashion on day 14 (10^8 CAR-T cells), day 28 (10^9 CAR-T cells) and day 44 (10^{10} CAR-T cells). The CAR- T cells are injected manually via a 60cc syringe at a rate of <2 cc/second. The total volume of infusion is approximately 100 cc. Angiography with calibrated contrast rate is performed after the first infusion of 50 cc and at completion of the CAR-T infusion to confirm preserved arterial flow. Infusions are delivered into the proper hepatic artery when possible. Certain patients 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 is split based upon

lobar volume calculations. In such cases, split doses are delivered separately into the right and left hepatic arteries to ensure proportionate CAR-T delivery to both hepatic lobes. Clinical assessments are performed at baseline, on infusion days, and 1, 2, 4, and 7 days post-infusion.

[00736] **Intraperitoneal administration of CAR-T cells.** 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 case patients with peritoneal involvement with ovarian cancer which expresses Folate Receptor alpha (FR1 or FOLR1). Essentially a similar approach can be used for intraperitoneal infusion of CAR-T cells targeting other tumor antigens.

[00737] 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 (FOLR1). In case the expression of FR1 is confirmed by immunohistochemistry, then patients will have a leukapheresis product obtained from peripheral blood. 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 a FOLR1 CAR [SEQ ID NO: 2120 or 2121] and further expanded using CD3/CD28 bead expansion protocol.

[00738] These autologous T cells will be genetically engineered to express the FOLR1 CAR [SEQ ID NO: 2120 or 2121]. 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.

[00739] Cohorts of 3–6 patients will be infused with escalating doses of modified T cells to establish the maximum tolerated dose (MTD). There are four planned dose levels: 3×10^5 , 1×10^6 , 3×10^6 , and 1×10^7 FOLR1 CAR-T cells/kg. Cohorts I and II will be treated with 3×10^5 FOLR1 [SEQ ID NO: 2120 or 2121] 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. If the first dose level exceeds the MTD, a subsequent cohort of 3–6 patients will be treated at the –1 dose level of 1×10^5 FOLR1 CAR-T cells/kg without the addition of lymphodepleting cyclophosphamide (cohort -I).

[00740] An IP catheter will be placed prior to T cell infusion. The catheter will be placed when the modified T cells are ready for administration. 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 in-patient service (subject to the clinical judgment of the treating physician).

[00741] 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.

[00742] 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.

[00743] **Use of CAR-T cells for intratumoral injection.** 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 is used in case of patients with recurrent glioblastoma (GBM) which expresses EGFRviii. Essentially a similar approach can be used for intra-tumoral injection of CAR-T cells targeting other tumor antigens.

[00744] A pilot safety and feasibility study will be conducted to test CAR (SEQ ID NO:1699 or 1700) expressing T cells in recurrent GBM. All participating patients will be required to give written informed consent. 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. 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 EGFRviii CAR (SEQ ID NO:1699 or 1700) 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 *et al.*, Clin Cancer Res. 2015 September 15; 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 weeks 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 we will utilize 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, 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.ifo.nih.gov/>) will be followed for the monitoring of toxicity and adverse event reporting.

[00745] Use of CAR-T cells for ex-vivo purging of bone marrow or peripheral blood stem cell preparation prior to transplant. 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, BCMA expressing CAR-T cells are 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. Essentially a similar approach can be used to purge bone marrow or peripheral blood stem cell preparations using CAR-T cells targeting other suitable antigens that are expressed on cancer cells and have no or negligible expression on normal hematopoietic stem cells.

[00746] 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 BCMA CAR CD8SP-BCMA-BB-CAR02-vL-[hTCRa-CSDVP]-F-F2A-SP-BCMA-BB-CAR02-vH-[hTCRb-KACIAH]-F-P2A-Xba-PAC [SEQ ID NO: 546] containing the puromycin resistance gene (PAC) following infection with the corresponding lentiviral vector as described in the previous examples. This CAR targets BCMA, an antigen

expressed on myeloma cells. CAR-T expressing the CAR CD8SP-BCMA-BB-CAR02-vL-[hTCRb-KACIAH]-F-P2A-SP-BCMA-BB-CAR02-vH-[hTCRa-CSDVP]-F-F2A-PAC [SEQ ID NO: 552] or CD8SP-BCMA-BB-CAR02-vL-IgCL-Bam-CD3zECDTMCP-opt-F-P2A-Spe-SP-Bst-BCMA-BB-CAR02-vH-IgG1-CH1-KPN-CD3zECDTMCP-opt2-F-F2A-Xba-PAC [SEQ ID NO: 553] will be used as alternatives or in combination with the above CAR-T cells targeting CS1. 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 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 was complete. Peripheral blood stem cells will be collected once the peripheral blood CD34⁺-cell count was 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 aliquote 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).

[00747] Use of *in vitro* and *vivo* selection to select CARs with desired properties.

A pool of CARs targeting CD19 listed in **Tables 7** 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. Aliquotes 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 CAR 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 TNF α production, lower expression of exhaustion markers, lower expression of markers of terminal differentiation and/or higher expression of markers of cytotoxicity.

[00748] HIV-1 Vif Protein enhance lentiviral mediated gene transduction and expression.

[00749] 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 a SEQ ID NO: 11244) or a 2nd generation CAR targeting CD19 and co-expressing HIV-1 Vif protein (SEQ ID NO: 11245)

and packaging plasmids (7.5 µg of PSPAX2 plasmid and 2 µg of PLP/VSVG). Approximately 15-16 hours post-transfection, 9 ml of media is removed and replaced with 5 ml of fresh media. Approximately, 48 hours post- transfection, 5 ml of supernatant is 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 are pooled and centrifuged at 1000 rpm for 1 minute to remove any cell debris and non-adherent cells. The cell-free supernatant are filtered through 0.45 µm syringe filter. The titer of the lentiviruses are measured using p24 ELISA. Buffy coat cells are obtained from healthy de-identified adult donors from the Blood Bank and used to isolate peripheral blood mononuclear cells (PBMC) by Ficoll-Hypaque gradient centrifugation. PBMC are used to isolate T cells using CD3 magnetic microbeads (Miltenyi Biotech) and following the manufacturer's instructions. T cells are re-suspended in XVIVO medium (Lonza) supplanted with 10 ng/ml CD3 antibody, 10ng/ml CD28 antibody and 100 IU recombinant human-IL2. Purified T cells are infected with equal amounts of lentivirus vector encoding the 2nd generation CAR targeting CD19 (SEQ ID NO: 11244) or a 2nd generation CAR targeting CD19 and co-expressing HIV-1 Vif protein (SEQ ID NO: 11245). Both the CAR constructs also carry a MYC epitope tag. The expression of CAR on the T cells is examined by immunostaining with an APC-conjugated MYC antibody and FACS analysis at 48 hours after the infection. A significantly higher percentage of T cells are found to be infected with the CAR construct co-expressing Vif as compared to the CAR construct without Vif expression.

[00750] FACS analysis is repeated at 3 days after infection. Again, a significantly higher percentage of T cells are found to be infected with the CAR construct co-expressing Vif as compared to the CAR construct without Vif expression.

[00751] The experiment is also repeated in BC-1 cell line. Again, a significantly higher percentage of BC-1 cells are found to be infected with the CAR construct co-expressing Vif as compared to the CAR construct without Vif expression.

What is claimed is:

1. At least one recombinant polynucleotide encoding at least one 1st generation or next generation chimeric antigen receptor (CAR), 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; and
 - (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) wherein the binding domain is selected from a binding domain set forth in Table 3;
 - (d) an optional third nucleic acid domain encoding a costimulatory domain; and an optional additional nucleic acid domain encoding an accessory module.
2. The at least one recombinant polynucleotide of claim 1, wherein the first nucleic acid encodes partially or entirely at least one T-cell Receptor (TCR) chain as set forth in Table 13.
3. The at least one recombinant polynucleotide of claim 2, wherein the first nucleic acid encodes at least one transmembrane domain in Table 13 operably linked to the cytoplasmic domain of the TCR-type.
4. The at least one recombinant polynucleotide of claim 1, wherein the polynucleotide encodes a CAR, wherein the CAR comprises:
 - (i) a partial or entire T-cell receptor (TCR) constant chain having an amino acid sequence that has at least 75% sequence identity to a sequence selected from SEQ ID NO:4038 to 4063, 12602-12638, and which may comprise an optional costimulatory module;
 - (ii) an optional linker; and
 - (iii) one or more non-natural TCR antigen binding domain(s) linked to (a) selected from a binding domain set forth in Table 3;
 - (iv) an optional accessor module; and
 - (v) a dimer of a polypeptide comprising (i)-(iv).

5. The at least one recombinant polynucleotide of claim 4, wherein the recombinant polynucleotide comprises a sequence encoding any one of the sequence in Table 2.
6. The at least one recombinant polynucleotide of any of any of claims 1-5, wherein the accessory module comprises an amino acid sequence selected from SEQ ID NO: 4103-4117 and 4090-4096.
7. The at least one recombinant polynucleotide of any one of claims 1-6, wherein the encoded CAR comprises (1) any of CARs 1-16 of Table 1 and/or (2) a backbone of Table 2; and (3) a binding domain of Table 3.
8. The at least one recombinant polynucleotide of claim 4, wherein (i) is a CD3z TCR constant chain.
9. The at least one recombinant polynucleotide of any one of claims 1-6 encoding two first generation or next generation chimeric antigen receptors.
10. The at least one recombinant polynucleotide of any one of claims 1-6, encoding a dimer of CD3z constant chains.
11. At least one recombinant polynucleotide encoding at least one next generation chimeric antigen receptor (CAR), 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 CD3z protein having a sequence selected from the group consisting of SEQ ID NO:4064-4066, 4070-4072, and 4075-4078, 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; and
 - (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) wherein the binding domain is selected from a binding domain set forth in Table 3; and
 - (d) an optional third nucleic acid domain encoding a costimulatory module; and

an optional additional nucleic acid encoding an accessory module.

12. The at least one recombinant polynucleotide of claim 11, wherein nucleic acid sequences encoding the endogenous CD3z protein are selected from the group consisting of SEQ ID NO: 67 and 71.

13. The at least one recombinant polynucleotide of claim 11 or 12, wherein the at least one next generation CAR comprises two CARs each CAR comprising a CD3z chain.

14. The at least one recombinant polynucleotide of claim 13, wherein a vL fragment of an antibody is operably linked to one of the two CD3z chains and a vH fragment of the antibody is operably linked to the other CD3z chain.

15. The at least one recombinant polynucleotide of claim 14, wherein the vL and vH chains are selected from pairs in Table 3 and 4 for a specific antigen target.

16. The at least one recombinant polynucleotide of claim 14 or 15, wherein a linker is provided between the vL/vH and/or the CD3z chains.

17. The at least one recombinant polynucleotide of claim 16, wherein an encoded linker is selected from the group consisting of IgCL (SEQ ID NO (DNA): 28 and SEQ ID NO (PRT): 4027) and IgCH domains (SEQ ID NO (DNA): 29 and SEQ ID NO (PRT): 4028).

18. The at least one recombinant polynucleotide of claim 11, further comprising the third nucleic acid domain encoding a costimulatory module.

19. The at least one recombinant polynucleotide of claim 17, wherein the costimulatory module comprises a 41BB or CD28 protein.

20. The at least one recombinant polynucleotide of claim 18 or 19, wherein the costimulatory module comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 4067 and 4068.

21. The at least one recombinant polynucleotide of claim 18, wherein the consitmulatory module comprises a signaling domain from any one or more of CD134 (OX40), Dap10, CD27, CD2, CD5, ICAM-1, LFA-1, Lck, TNFR-I, TNFR-II, Fas, CD30, CD40 and combinations thereof.
22. The at least one recombinant polynucleotide of any of any of claims 11-21, further comprising the accessory module, wherein the accessory module comprises an amino acid sequence selected from SEQ ID NO: 4103-4117 and 4090-4096.
23. A recombinant cell expressing a homo- or hetero-dimer of a 1st generation or next generation chimeric antigen receptor (CAR), the homo- or hetero-dimer comprising:
- (a) a first 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 peptide linker; and
 - (c) a second domain operably linked to the first domain, wherein the second domain comprises one or more non-natural TCR antigen binding domain(s) wherein the binding domain is selected from a binding domain set forth in Table 3; and
 - (d) an optional third domain encoding a costimulatory module, and
- wherein the cell optionally comprises an accessory module, wherein the homo- or hetero-dimer associate on the surface of the recombinant cell.
24. The recombinant cell of claim 23, wherein the cell is transformed with the at least one recombinant polynucleotide or any of claims 1-22.
25. The recombinant cell of clause 23 or 24, wherein the cell is a T-lymphocyte (T-cell).
26. The recombinant cell of clause 25, wherein the cell is a naïve T cells, a central memory T cells, an effector memory T cell, Treg or a combination thereof.
27. The recombinant cell of clause 23, wherein the cell is a natural killer (NK) cell, a hematopoietic stem cell (HSC), an embryonic stem cell, or a pluripotent stem cell.

28. The recombinant cell of any of any of claims 23-27, wherein the accessory module comprises an amino acid sequence selected from SEQ ID NO: 4103-4117 and 4090-4096.
29. A chimeric antigen receptor (CAR) comprising:
- (a) a first 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 peptide linker; and
 - (c) a second domain operably linked to the first domain, wherein the second domain comprises one or more non-natural TCR antigen binding domain(s) wherein the binding domain is selected from a binding domain set forth in Table 3; and
 - (d) an optional third domain encoding a costimulatory module.
30. The chimeric antigen receptor of claim 29, wherein the endogenous protein comprises a sequence selected from the group consisting of SEQ ID NO:4064-4066, 4070-4072, 4075-4078 and 12637.
31. The at least one recombinant polynucleotide of claim 29, wherein the first nucleic acid encodes partially or entirely at least one T-cell Receptor (TCR) chain as set forth in Table 13.
32. The chimeric antigen receptor of claim 31, wherein the first comprises a transmembrane domain in Table 13 operably linked to the cytoplasmic domain of a corresponding TCR-type.
33. The chimeric antigen receptor of claim 29, wherein the CAR comprises:
- (i) a partial or entire T-cell receptor (TCR) constant chain having an amino acid sequence that has at least 75% sequence identity to a sequence selected from SEQ ID NO:4038 to 4063, 12602-12638, and which may comprise an optional costimulatory module.
34. A polynucleotide encoding the chimeric antigen receptor of any of claims 29-33.
35. A vector comprising the polynucleotide of clause 34.

36. A virus comprising the polynucleotide of claim 35.
37. The virus of claim 36, wherein the virus is a retrovirus, an adenovirus, an adeno-associated virus, a lentivirus, a pox virus or a herpes virus.
38. A pharmaceutical composition comprising: any one or more of the at least one polynucleotides of claims 1-22, the recombinant cell of claims 23-28, the CAR of claim 29-33, the vector of claim 35 or the virus of claim 36.
39. A method for treating cancer comprising: providing the composition of claim 38 or a recombinant cell of claim 23; and administering a therapeutically effective amount of the composition or cell to the subject so as to treat cancer.
40. The method of claim 39, wherein the cancer is blood cancer.
41. The method of claim 40, wherein the blood cancer is any one or more of acute myeloid leukemia, chronic myeloid leukemia, myelodysplastic syndrome, lymphoma, multiple myeloma and acute lymphocytic leukemia.
42. The at least one recombinant cell of claim 21, wherein the at least one recombinant polynucleotide comprises a sequence encoding HIV-1-vif.
43. The recombinant cell of claim 42, wherein the cell further comprises HIV-1-vif.

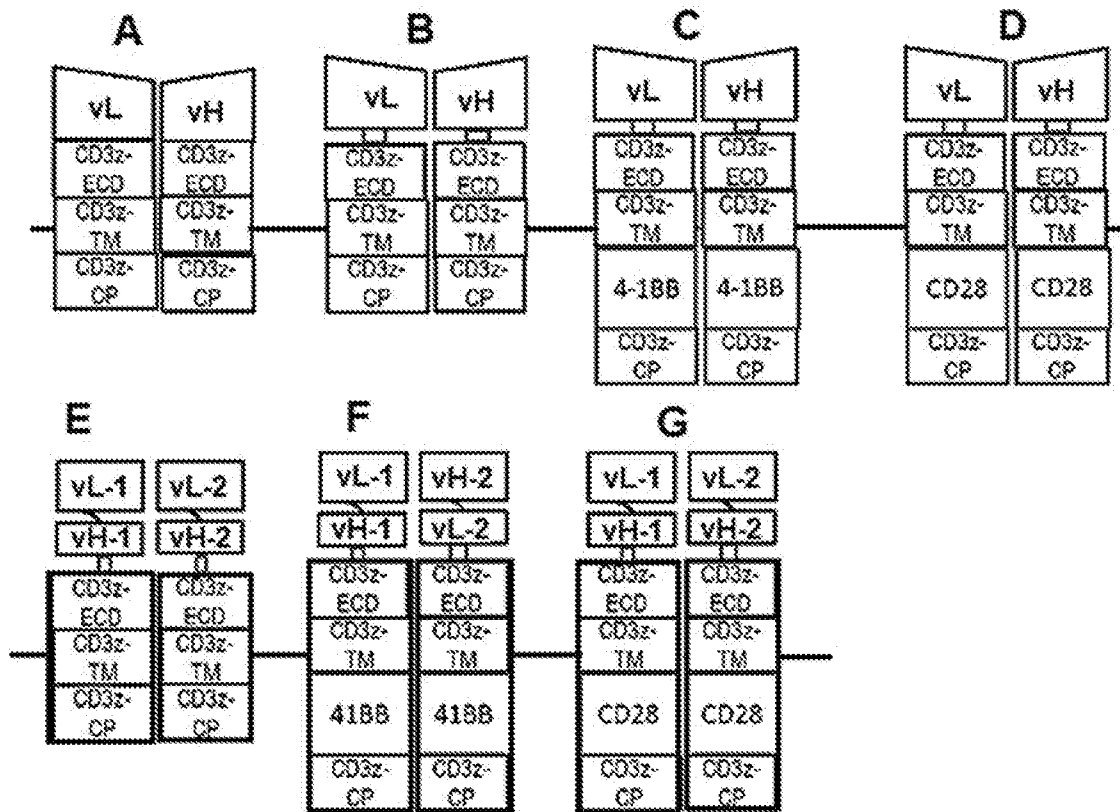


FIG. 1

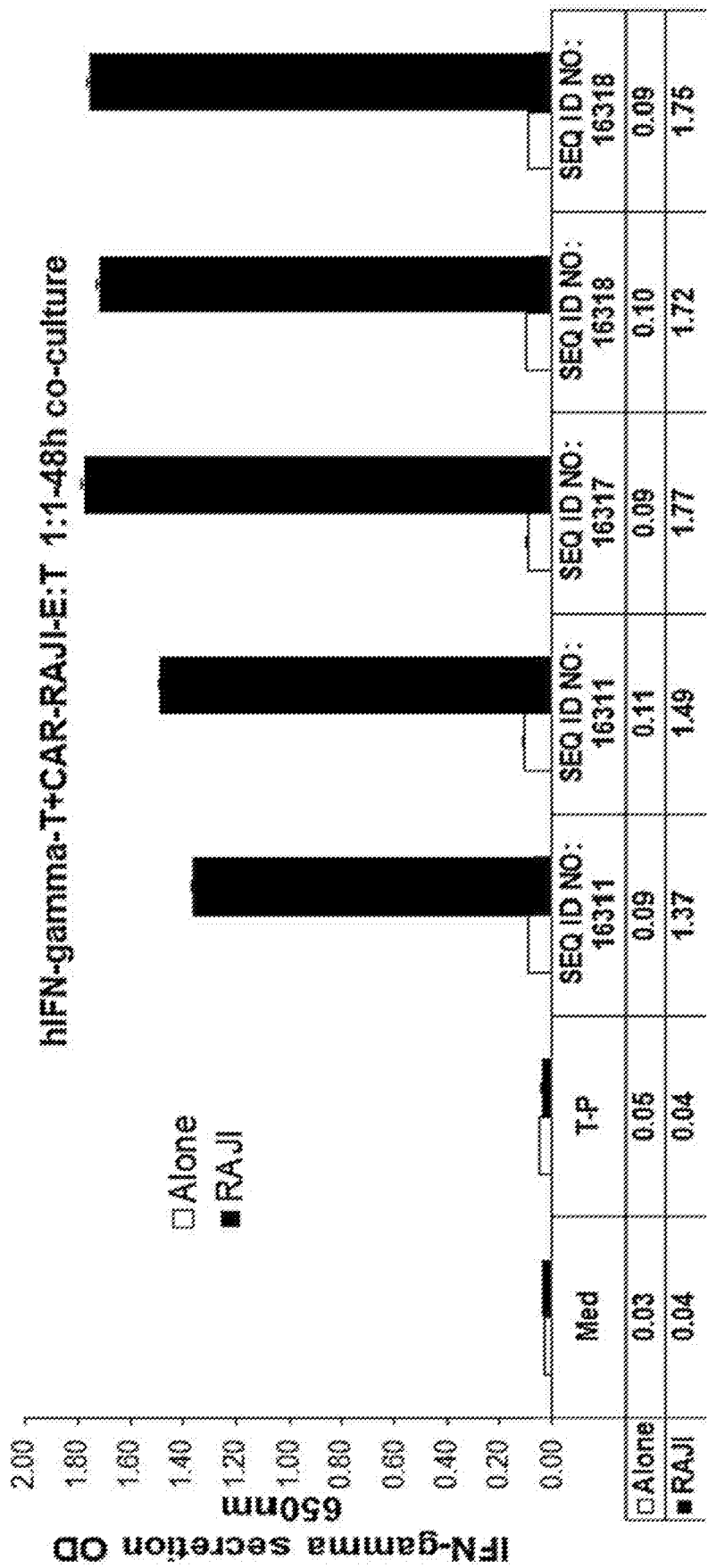


FIG. 2A

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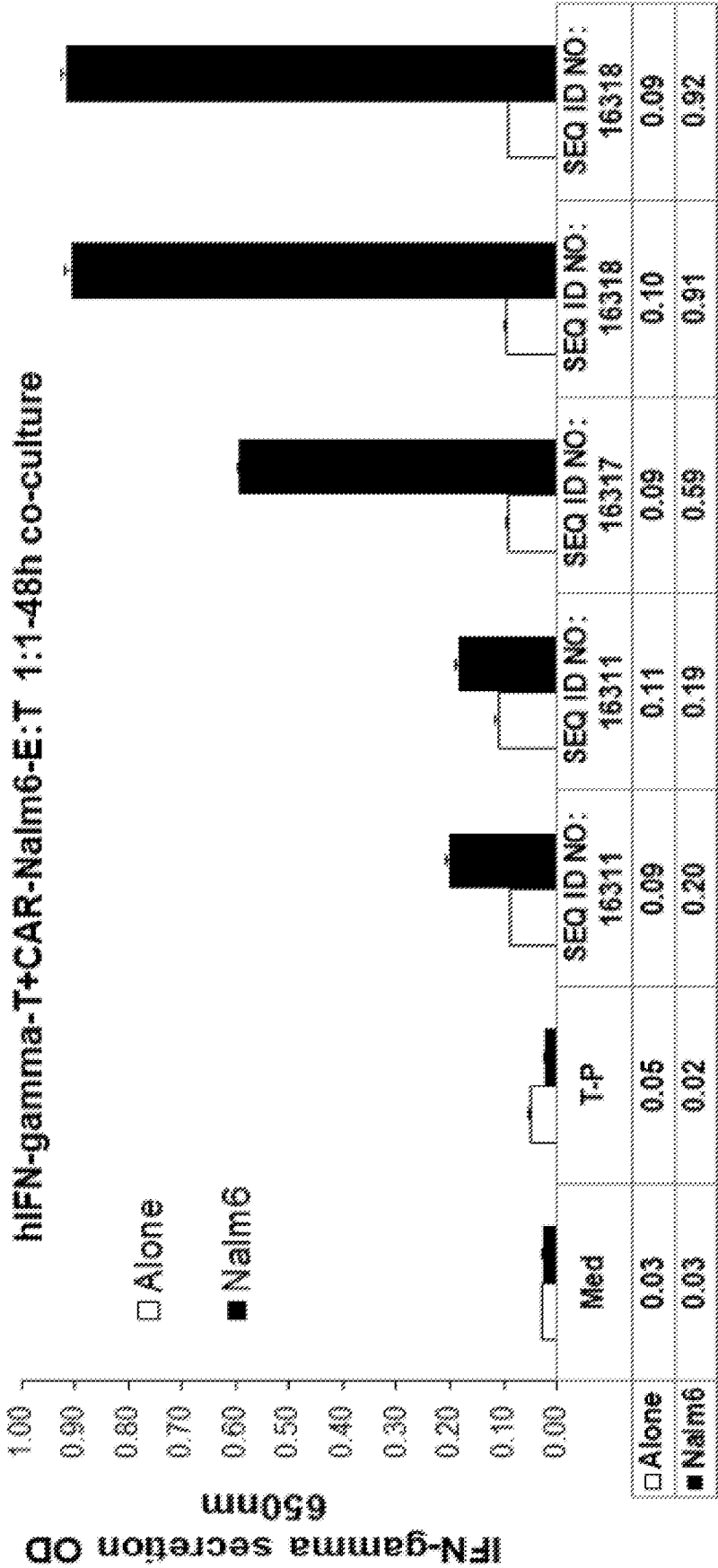


FIG. 2B

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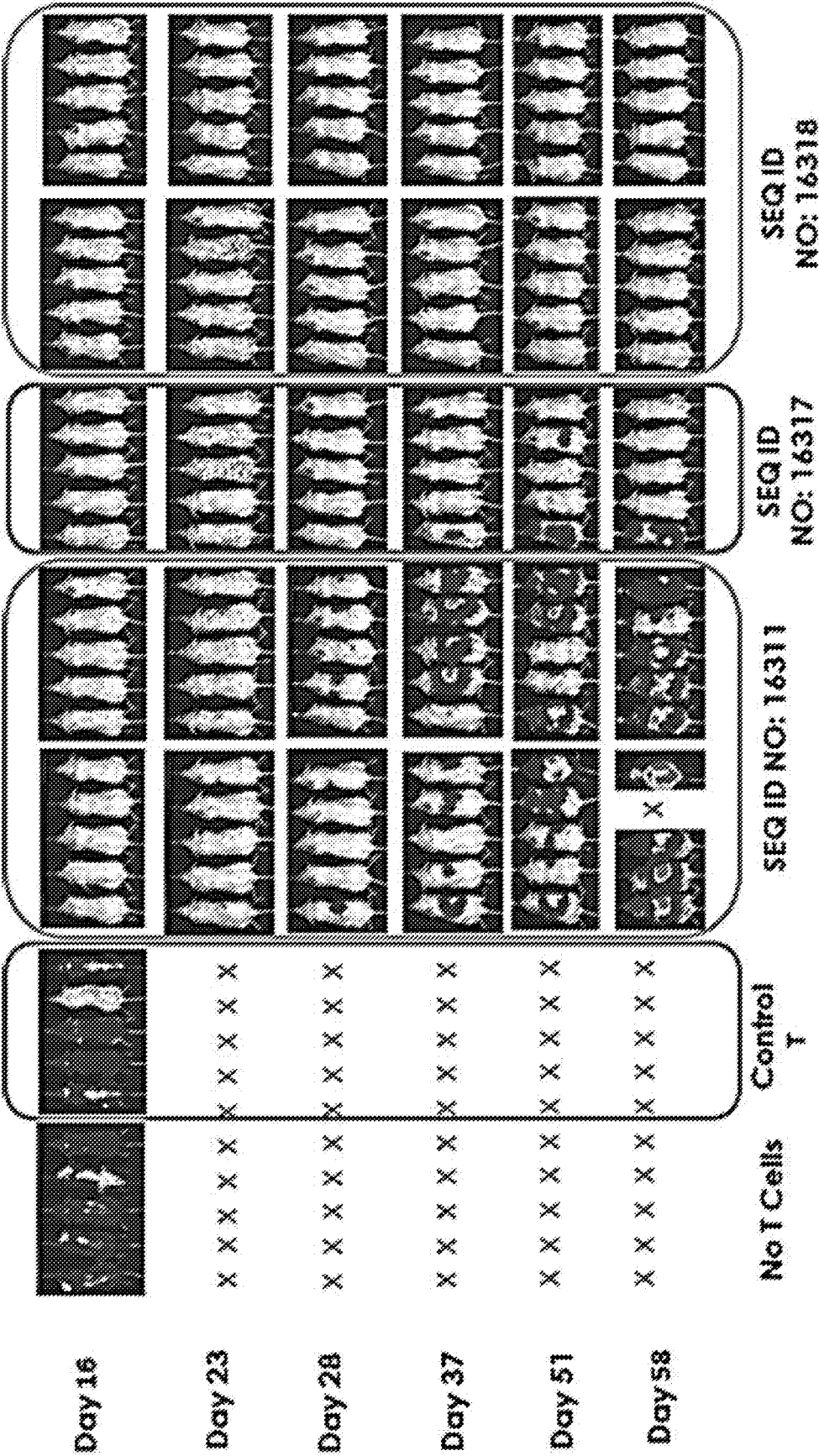


FIG. 3

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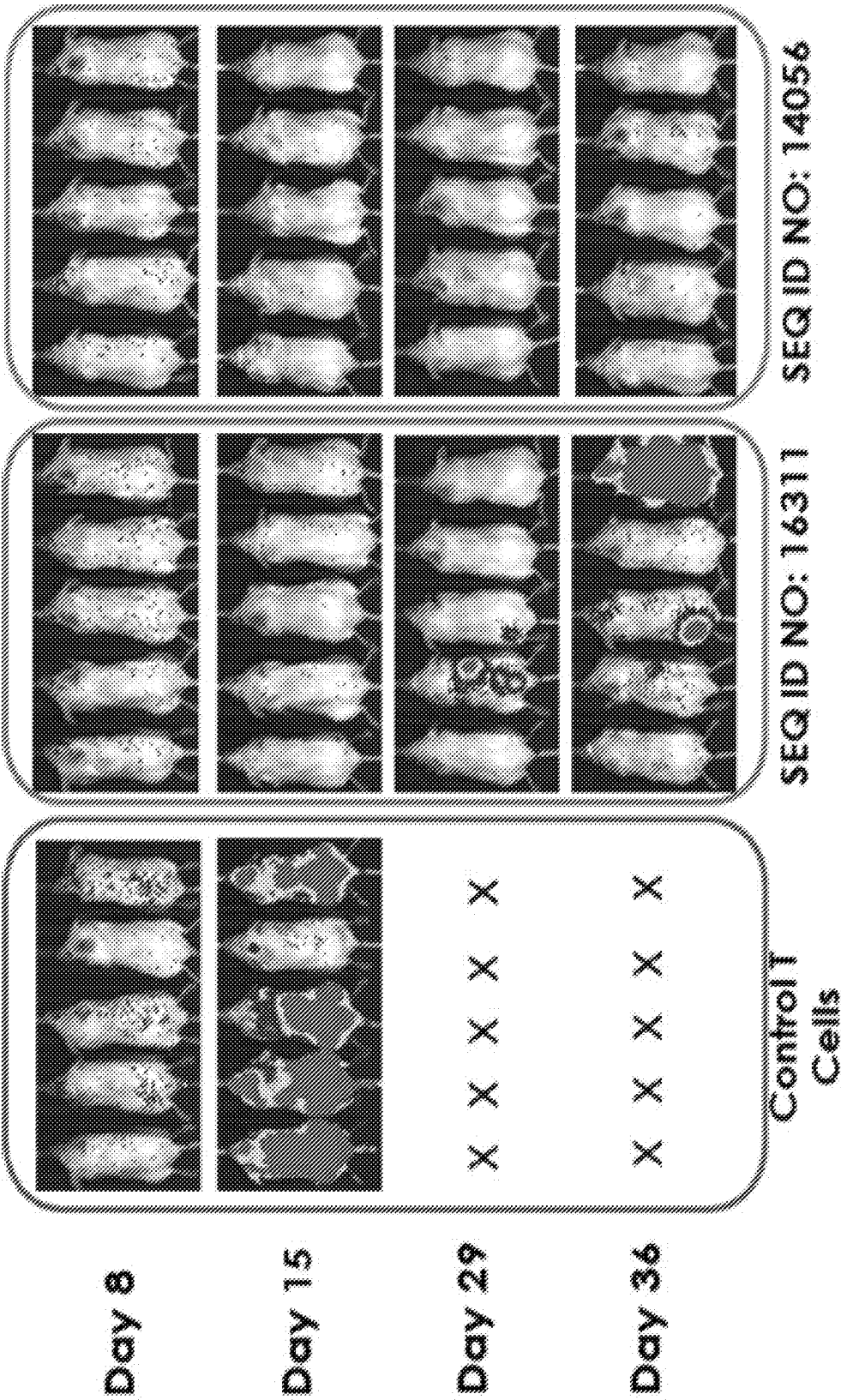


FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/35096

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 15/10, C07K 16/28, A61K 35/17 (2019.01)

CPC - C12N 15/1093, C12Q 1/6806, C07K 16/2803, A61K 35/17, C07K 14/7051

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2017/070608 A1 (EUREKA THERAPEUTICS INC.) 27 April 2017 (27.04.2017) Abstract, para [0004], [0012], [0016-0017], [0020], [0025-0026], [0045], [0101], [0103-0104], [0147], [0167], [0192-0193], [0196-0197], [0210], [0244], Figure 1A, Figure 2	1-6, 8, 11, (13-15)/11, 18, 23, 27, 29-37
Y	US 2017/0183418 A1 (CELLECTIS) 29 June 2017 (29.06.2017) Abstract, para [0001], para [0110]	1-6, 8, 11, (13-15)/11, 18, 23, 27, 29-37
Y	WO 2017/172981 A2 (UNIVERSITY OF SOUTHERN CALIFORNIA) 05 October 2017 (05.10.2017) Abstract, para [00584]	5, 6
Y	WO 2016/174852 A1 (DENSO CORP) 03 November 2016 (03.11.2016) p.3 ln.19-22; p.92 ln.16-20	30

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

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

13 September 2019

Date of mailing of the international search report

22 OCT 2019

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/35096

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 7, 9, 10, 16, 17, 19, 20, 22, 24-26, 28, 38-41
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

-----Please see continuation on extra sheet.-----

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-6, 8, 11, 13-15(in part), 18, 23, 27, 29-37 limited to SEQ ID NOs: 4118, 4192, 4038, 12602, 4107, 4064, 28, 4027, 4067.

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/35096

Continuation of Box No. III, Observations where unity of invention is lacking

Groups I+: Claims 1-6, 8, 11-15, 18, 21, 23, 27, 29-37, 42-43, drawn to a recombinant polynucleotide encoding at least one chimeric antigen receptor (CAR). The composition will be searched to the extent that the CAR encompasses BCMA binding domain (SEQ ID NOs: 4118, 4192), TCR chain (SEQ ID NO: 4038), transmembrane domain (SEQ ID NO: 12602), backbone (SEQ ID NO: 4107), CD3z (SEQ ID NO: 4064), linker IgCL (SEQ ID NOs: 28, 4027), costimulatory module (SEQ ID NO: 4067). It is believed that claims 1-6, 8, 11, 13-15(in part), 18, 23, 27, 29-37, encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass SEQ ID NOs: 4118, 4192, 4038, 12602, 4107, 4064, 28, 4027, 4067. Additional CAR(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected CAR(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a CAR encompasses CD19 binding domain (SEQ ID NO: 4121, 4195), TCR chain (SEQ ID NO: 4039), transmembrane domain (SEQ ID NO: 12603), backbone (SEQ ID NO: 4112), CD3z (SEQ ID NO: 4065), linker IgCL (SEQ ID NOs: 29, 4028), costimulatory module (SEQ ID NO: 4068) (claims 1-6, 8, 11, (13-15)(in part), 18, 23, 27, 29-37).

The inventions listed as Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

No technical features are shared between the amino acids sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features of including: a recombinant polynucleotide encoding at least one chimeric antigen receptor (CAR); these shared technical features are previously disclosed by WO 2017/070608 to Eureka Therapeutics Inc. (hereinafter 'Eureka').

Eureka teaches (instant claim 1) at least one recombinant polynucleotide encoding at least one first generation or next generation chimeric antigen receptor (CAR), the at least one recombinant polynucleotide (Abstract "The present application provides antibody-TCR chimeric constructs comprising an antibody moiety that specifically binds to a target antigen fused to a TCRM capable of recruiting at least one TCR-associated signaling module. Also provided are methods of making and using these constructs."; para [0210] "In some embodiments, there is provided nucleic acid encoding an abTCR according to any of the abTCRs described herein."); 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 triggers the activation or proliferation of the lymphocyte (para [0016] "The present application in one aspect provides a construct (such as an isolated construct) comprising an antibody moiety (such as a Fab-like antigen-binding module) fused to a T cell receptor module (said construct also referred to herein as an "antibody-TCR chimeric molecule," or "abTCR"); para [0017] "In some embodiments, there is provided an abTCR (such as an isolated abTCR) that specifically binds to a target antigen, wherein the abTCR comprises ... a first T cell receptor domain (TCRD) comprising a first transmembrane domain of a first TCR subunit"; para [0147] "The TCRM comprises a) a first polypeptide chain comprising a first T cell receptor domain (TCRD) comprising a first transmembrane domain; para [0192] "Nucleic acid molecules encoding the abTCRs are also contemplated. In some embodiments, according to any of the abTCRs described herein, there is provided a nucleic acid (or a set of nucleic acids) encoding the abTCR."; para [0004] "Each T lymphocyte expresses a unique TCR on the cell surface"; para [0045] "FIG. 2 shows a conventional model for the assembly of the TCR-CD3 complex."; Fig. 2 shows that the TCR is expressed on the surface of lymphocytes; para [0101] "Target-specific activation was observed for multiple different T cell subsets transduced to express an abTCR"; para [0244] "In any of some such embodiments described herein, the abTCR effector cell has a greater rate of proliferation compared to a corresponding CAR effector cell"; (b) optionally a polynucleotide linker (para [0017] "In some embodiments, the first polypeptide chain further comprises a first peptide linker between the first antigen-binding domain and the first TCRD."); (c) a second nucleic acid domain optionally linked to the first nucleic acid domain, wherein the second nucleic acid domain encodes the one or more non-natural TCR (T-cell receptor) antigen binding domain(s) (para [0017] "In some embodiments, there is provided an abTCR (such as an isolated abTCR) that specifically binds to a target antigen, wherein the abTCR comprises: a) a first polypeptide chain comprising a first antigen-binding domain comprising VH and CRI antibody domains ... and b) a second polypeptide chain comprising a second antigen-binding domain comprising VL and CL antibody domains"; [0192] "Nucleic acid molecules encoding the abTCRs are also contemplated. In some embodiments, according to any of the abTCRs described herein, there is provided a nucleic acid (or a set of nucleic acids) encoding the abTCR.").

As said technical features were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the groups.

Groups I+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.