

ANTI-DINITROPHENOL CHIMERIC ANTIGEN RECEPTORS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. 62/969,931 filed February 4, 2020 entitled "ANTI-DINITROPHENOL CHIMERIC ANTIGEN RECEPTORS" which is incorporated by reference in its entirety.

REFERENCE TO SEQUENCE LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled SCRI269WOSEQLIST, created February 2, 2021, which is approximately 43 Kb in size. The information in the electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0003] Embodiments provided herein include methods and compositions comprising anti-dinitrophenol chimeric antigen receptors (CARs). Some embodiments include nucleic acids encoding such CARs, polypeptides encoded by such nucleic acids, cells comprising such polypeptides, and methods utilizing such cells. Some embodiments also include the use of dinitrophenol (DNP) and derivatives thereof.

BACKGROUND OF THE INVENTION

[0004] Immunotherapy using adoptive cell transfer of chimeric antigen receptor (CAR) bearing T-cells is an effective approach to treat cancer. The structure of a CAR includes an antigen binding domain, a spacer domain, a transmembrane domain, and one or more co-stimulatory activation domains. CAR T cells may be prepared from T cells obtained from a patient or from a donor. In some instances, CARs function by binding to a specific antigen on a cell surface, which causes lysis of the antigen-bearing cell. Although considerable research has focused on the design of CAR ligand binding domains, which target desired cell surface antigens with reduced toxicity, there remains a need for additional CAR T cell-mediated therapies.

SUMMARY OF THE INVENTION

[0005] Some embodiments of the methods and compositions provided herein include a nucleic acid encoding a chimeric antigen receptor (CAR), wherein the CAR comprises: a ligand binding domain which specifically binds to a dinitrophenol (DNP) moiety; a spacer; a transmembrane domain; and an intracellular signaling domain.

[0006] In some embodiments, the ligand binding domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence set forth in any one of SEQ ID NOs:01-12. In some embodiments, the ligand binding domain comprises the amino acid sequence set forth in any one of SEQ ID NOs:01-12.

[0007] In some embodiments, the ligand binding domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence set forth in SEQ ID NOs:01, 02, 09, or 10. In some embodiments, the ligand binding domain comprises the amino acid sequences set forth in SEQ ID NOs:01, 02, 09, or 10.

[0008] In some embodiments, the spacer is selected from the group consisting of: a short spacer having a length of 12 consecutive amino acid residues or less but, preferably not 1 amino acid in length, a medium spacer having a length of 119 consecutive amino acid residues or less but, preferably not 1 amino acid in length, and a long spacer having a length greater than 119 consecutive amino acid residues.

[0009] In some embodiments, the spacer is selected from the group consisting of: a short spacer comprising an IgG4 hinge domain, a medium spacer comprising an IgG4 hinge-CH3 domain, and a long spacer comprising an IgG4 hinge -CH2-CH3 domain.

[0010] In some embodiments, the spacer is a long spacer having a length of at least 229 consecutive amino acid residues.

[0011] In some embodiments, the transmembrane domain comprises a CD28 transmembrane domain.

[0012] In some embodiments, the intracellular signaling domain comprises a portion of CD3 zeta and/or a portion of 4-1BB.

[0013] Some embodiments also include a polynucleotide encoding a selectable gene, a cell surface selectable marker, or a cleavable linker. In some embodiments, the selectable gene comprises a dihydrofolate reductase double mutant (DHFRdm). In some embodiments, the cell surface selectable marker is selected from the group consisting of a

truncated EGFR (EGFRt), a truncated Her2 (Her2tG), and a truncated CD19 (CD19t). In some embodiments, the cleavable linker comprises a ribosome skip sequence is selected from the group consisting of P2A, T2A, E2A and F2A.

[0014] Some embodiments of the methods and compositions provided herein include a vector comprising any one of the nucleic acids provided herein. In some embodiments, the vector comprises a lentiviral vector.

[0015] Some embodiments of the methods and compositions provided herein include a method for preparing a population of cells for an infusion, comprising: introducing the nucleic acid of any one of the nucleic acids provided herein encoding an anti-DNP CAR into a cell; and culturing the cell under conditions suitable to obtain a population of cells sufficient for an infusion.

[0016] Some embodiments of the methods and compositions provided herein include a CAR encoded by any one of the nucleic acids provided herein.

[0017] Some embodiments of the methods and compositions provided herein include a cell comprising any one of the CARs provided herein.

[0018] In some embodiments, the cell is derived from a CD4⁺ T cell, a CD8⁺ T cell, a precursor T cell, or a hematopoietic stem cell. In some embodiments, the CD8⁺ T cell is a CD8⁺ cytotoxic T lymphocyte cell selected from the group consisting of a naïve CD8⁺ T cell, a central memory CD8⁺ T cell, an effector memory CD8⁺ T cell, and a bulk CD8⁺ T cell. In some embodiments, the central memory CD8⁺ T cell is positive for CD45RO and CD62L. In some embodiments, the CD4⁺ cell is a CD4⁺ helper T lymphocyte cell selected from the group consisting of a naïve CD4⁺ T cell, a central memory CD4⁺ T cell, an effector memory CD4⁺ T cell, and a bulk CD4⁺ T cell. In some embodiments, the naïve CD4⁺ T cell is positive for CD45RA and CD62L, and negative for CD45RO.

[0019] In some embodiments, the cell is *ex vivo*. In some embodiments, the cell is *in vivo*.

[0020] In some embodiments, the cell is mammalian. In some embodiments, the cell is human.

[0021] Some embodiments of the methods and compositions provided herein include a composition comprising: any one of the CARs provided herein; and a DNP moiety attached to a target cell, wherein the CAR is specifically bound to the DNP moiety.

[0022] In some embodiments, the DNP moiety is attached to the target cell via an antibody or antigen binding fragment thereof that binds to the target cell.

[0023] In some embodiments, the DNP moiety is attached to the target cell via a folate moiety.

[0024] In some embodiments, the DNP moiety is attached to a cell surface of the target cell via a lipid integrated into the cell surface. In some embodiments, the lipid comprises a polar head group and a hydrophobic group. In some embodiments, the polar head comprises a group selected from choline, phosphatidylcholine, phosphocholine, sphingomyelin, aphosphoethanolamine, a sugar residue, phosphatidyl serine, phosphatidyl inositol, a piperidine, or a trimethylarseno-ethyl-phosphate. In some embodiments, the hydrophobic group comprises an aliphatic chain or a terpenoid moiety. In some embodiments, the hydrophobic group comprises an ether linkage, wherein the ether linkage is between the polar head group and the aliphatic chain. In some embodiments, the aliphatic chain comprises a C₁₀₋₂₀ alkyl chain. In some embodiments, the lipid is a phospholipid ether (PLE).

[0025] In some embodiments, the target cell is a cancer cell. In some embodiments, the cancer cell is selected from the group consisting of a breast cancer cell, brain cancer cell, colon cancer cell, renal cancer cell, pancreatic cancer cell, and ovarian cancer cell.

[0026] In some embodiments, the target cell is *ex vivo*. In some embodiments, the target cell is *in vivo*.

[0027] In some embodiments, the target cell is mammalian. In some embodiments, the target cell is human.

[0028] Some embodiments of the methods and compositions provided herein include a method of treating or ameliorating a cancer in a subject comprising: administering any one of anti-DNP CAR T cells provided herein to the subject in combination with a composition comprising a DNP moiety.

[0029] In some embodiments, the cell is administered prior to administration of the composition. In some embodiments, the cell is administered subsequent to administration of the composition. In some embodiments, the cell is co-administered with the composition.

[0030] In some embodiments, the composition is adapted to target the cancer.

[0031] In some embodiments, the DNP moiety is attached to an antibody of antigen binding fragment thereof which specifically binds to the cancer.

[0032] In some embodiments, the DNP moiety is attached to a folate.

[0033] In some embodiments, the DNP moiety is attached to a lipid. In some embodiments, the lipid comprises a polar head group and a hydrophobic group. In some embodiments, the polar head comprises a group selected from choline, phosphatidylcholine, phosphocholine, sphingomyelin, aphosphoethanolamine, a sugar residue, phosphatidyl serine, phosphatidyl inositol, a piperidine, or a trimethylarseno-ethyl-phosphate. In some embodiments, the hydrophobic group comprises an aliphatic chain or a terpenoid moiety. In some embodiments, the hydrophobic group comprises an ether linkage, wherein the ether linkage is between the polar head group and the aliphatic chain. In some embodiments, the aliphatic chain comprises a C₁₀₋₂₀ alkyl chain. In some embodiments, the lipid is a PLE.

[0034] In some embodiments, the cancer comprises a target cell selected from the group consisting of a breast cancer cell, brain cancer cell, colon cancer cell, renal cancer cell, pancreatic cancer cell, and ovarian cancer cell.

[0035] In some embodiments, the cell is autologous to the subject.

[0036] In some embodiments, the subject is mammalian. In some embodiments, the subject is human.

[0037] Some embodiments of the methods and compositions provided herein include use of any one of anti-DNP CAR T cells provided herein in combination with a composition comprising a DNP moiety to treat a cancer in a subject.

[0038] Some embodiments of the methods and compositions provided herein include use of any one of anti-DNP CAR T cells provided herein in combination with a composition comprising a DNP moiety in the manufacture of a medicament to treat a cancer in a subject.

[0039] Some embodiments of the methods and compositions provided herein include any one of anti-DNP CAR T cells provided herein for use in a medicament.

BRIEF DESCRIPTION OF THE DRAWINGS

[0040] FIG. 1 depicts an embodiment of a DNP phospholipid ether (DNP-PLE) which includes: (i) a DNP moiety; (ii) a polyethylene glycol (PEG) moiety; (iii) a polar head moiety; and (iv) a hydrophobic tail moiety.

[0041] FIG. 2A depicts flow cytometry data for control MDA-MB-231 cells and control MDA-MB-231 cells incubated with an anti-DNP-Alexa Fluor 488 antibody.

[0042] FIG. 2B depicts flow cytometry data for control MDA-MB-231 cells, control MDA-MB-231 cells incubated with an anti-DNP-Alexa Fluor 488 antibody, and MDA-MB-231 cells labelled with 5 μ M DNP-PLE and stained with the anti-DNP-Alexa Fluor 488 antibody.

[0043] FIG. 2C depicts flow cytometry data for control MDA-MB-231 cells, control MDA-MB-231 cells incubated with an anti-DNP-Alexa Fluor 488 antibody, and MDA-MB-231 cells labelled with 500 nM DNP-PLE and stained with the anti-DNP-Alexa Fluor 488 antibody.

[0044] FIG. 2D depicts flow cytometry data for control MDA-MB-231 cells, control MDA-MB-231 cells incubated with an anti-DNP-Alexa Fluor 488 antibody, and MDA-MB-231 cells labelled with 50 nM DNP-PLE and stained with the anti-DNP-Alexa Fluor 488 antibody.

[0045] FIG. 2E depicts a histogram plot for the flow cytometry data shown in FIG. 2A-FIG. 2D.

[0046] FIG. 3A depicts confocal images of control MDA-MB-231 cells incubated with anti-DNP-Alexa Fluor 488 antibody.

[0047] FIG. 3B depicts confocal images of MDA-MB-231 cells incubated with 5 μ M DNP-PLE.

[0048] FIG. 3C depicts confocal images of MDA-MB-231 cells incubated with 5 μ M DNP-PLE and stained with anti-DNP-Alexa Fluor 488 antibody.

[0049] FIG. 3D depicts confocal images of MDA-MB-231 cells incubated with 1 μ M DNP-PLE and stained with anti-DNP-Alexa Fluor 488 antibody.

[0050] FIG. 4 depicts a schematic of a second generation long CAR cassette for an anti-DNP CAR.

[0051] FIG. 5A depicts confocal images of control MDA-MB-231 cells co-cultured with anti-DNP CAR H9 cells.

[0052] FIG. 5B depicts confocal images MDA-MB-231 cells stained with 5 μ M DNP-PLE and co-cultured with anti-DNP CAR H9 cells.

DETAILED DESCRIPTION

[0053] Embodiments provided herein include methods and compositions comprising anti-dinitrophenol chimeric antigen receptors (CARs). Some embodiments include nucleic acids encoding such CARs, polypeptides encoded by such nucleic acids, cells comprising such polypeptides, and methods utilizing such cells. Some embodiments also include the use of dinitrophenol (DNP) and derivatives thereof.

[0054] While the adoptive transfer of transgene modified T cells has been successful in select settings, such as CD19 B cell lineage malignancies, these therapies have proved difficult to genericize to other cancer types because of the lack of a single target antigen that is present on all forms of cancer but not normal, healthy cells. The development of CAR T cell therapies, which treat the multitude of cancers afflicting humans, has been hampered by the onerous task of identifying and vetting tens of hundreds of antigens presented on cancer cells, e.g., CAR targets. In some embodiments described herein, this problem is addressed by using a DNP phospholipid ether (DNP-PLE), which associates with tumor cells and displays a unique target molecule for T cells bearing DNP-specific CARS to interact with and thereby lyse the DNP-presenting tumor cells. By this approach, one can eliminate the need to identify and validate tens of hundreds of unique antigen specific CARs.

[0055] Some embodiments provided herein include CARs comprising a specificity or a selected affinity or avidity for a DNP moiety. In some embodiments a DNP moiety is joined to a molecule, which can be associated with or bound to the surface of a tumor cell. In some embodiments, the molecule is a PLE. In some embodiments, the molecule can include an antibody or antigen-binding fragment thereof which specifically binds to a cell. In some embodiments, methods of redirected anti-tumor T cell reactivity can then be performed using said DNP-PLE, or other DNP-molecule, and T cells having said CARS. In some embodiments, the DNP-PLE is a synthetic molecule having a structure designed to integrate into the plasma membrane of tumor cells in a manner that allows for the molecule's DNP moiety to be adjacent to the outer leaflet of the plasma membrane and displayed in the extracellular space in an orientation or proximity that allows for a desired interaction with a T cell bearing a CAR having a desired affinity, specificity, or avidity for the DNP moiety. Similarly, in some embodiments, the CARs described herein have a unique structure designed to allow the anti-DNP ligand binding domain of the receptor to be displayed on the T cell plasma membrane in an orientation

or proximity that allows for a desired interaction or avidity with the DNP-PLE-bearing tumor cells. Thus, in some embodiments, the DNP-PLE molecule and CARs described herein imbue therapeutically important attributes and the use of such DNP-PLE molecules with or without the anti-DNP specific CARs as a medicament are contemplated. Stated differently, any one or more of the CARs and the DNP-PLE molecules described herein are useful for the treatment or amelioration of a human disease or condition, such as a cancer.

[0056] Additional embodiments relate to CARs that target and interact with a DNP moiety joined to a PLE molecule, which can be constitutively expressed, or placed under regulated control in a cell, preferably a T cell, nucleic acids encoding said CARs, cells having said nucleic acids and CARs, preferably T cells, and methods of making and using these compositions to treat a disease such as cancer in humans. Some embodiments of the methods and compositions provided herein include aspects disclosed in WO 2018/148224; WO 2019/156795; WO 2019/144095; U.S. 2019/0224237; and PCT/US2019/044981, which are each hereby expressly incorporated herein by reference in its entirety.

Definitions

[0057] As used herein, “nucleic acid” or “nucleic acid molecule” refers to polynucleotides, such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), oligonucleotides, fragments generated by the polymerase chain reaction (PCR), or fragments generated by any of ligation, scission, endonuclease action, or exonuclease action. Nucleic acid molecules can be composed of monomers that are naturally-occurring nucleotides (such as DNA or RNA), or analogs of naturally-occurring nucleotides (e.g., enantiomeric forms of naturally-occurring nucleotides), or a combination of both. Modified nucleotides can have alterations in sugar moieties and/or in pyrimidine or purine base moieties. Sugar modifications include, for example, replacement of one or more hydroxyl groups with halogens, alkyl groups, amines, and azido groups, or sugars can be functionalized as ethers or esters. Moreover, the entire sugar moiety can be replaced with sterically and electronically similar structures, such as aza-sugars or carbocyclic sugar analogs. Examples of modifications in a base moiety include alkylated purines and pyrimidines, acylated purines or pyrimidines, or other well-known heterocyclic substitutes. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Analogous of phosphodiester linkages include phosphorothioate,

phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilidate, or phosphoramidate, and the like. The term “nucleic acid molecule” also includes “peptide nucleic acids,” which comprise naturally-occurring or modified nucleic acid bases attached to a polyamide backbone. Nucleic acids can be either single stranded or double stranded. “Coding for” is used herein to refer to the 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 macromolecules such as a defined sequence of amino acids. Thus, a gene codes for a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. A “nucleic acid sequence coding for a polypeptide” includes all nucleotide sequences that are degenerate versions of each other and that code for the same amino acid sequence. “Specific” or “Specificity” can refer to the characteristic of a ligand for a binding partner or alternatively, a binding partner for a ligand, and can include complementary shape, charge or hydrophobic specificity for binding. Specificity for binding can include stereospecificity, regioselectivity and/or chemoselectivity. In some embodiments, a method of making a nucleic acid encoding a chimeric antigen receptor is provided such that a nucleic acid encoding a chimeric antigen receptor is generated that is specific for a DNP moiety joined to or associated with a PLE molecule, which can be associated or joined to a tumor cell.

[0058] A “vector” or “construct” is a nucleic acid used to introduce heterologous nucleic acids into a cell that can also have regulatory elements to provide expression of the heterologous nucleic acids in the cell. Vectors include but are not limited to plasmid, minicircles, yeast, or viral genomes. In some embodiments, the vectors are plasmid, minicircles, viral vectors, DNA or mRNA. In some embodiments, the vector is a lentiviral vector or a retroviral vector. In some embodiments, the vector is a lentiviral vector.

[0059] “Chimeric receptor” as used herein refers to a synthetically designed receptor comprising a ligand binding domain of an antibody or other protein sequence that binds to a target molecule and is linked via a spacer domain to one or more intracellular signaling domains of a T cell or other receptors, such as a costimulatory domain. Chimeric receptors can also be referred to as artificial T cell receptors, chimeric T cell receptors, chimeric immunoreceptors, or chimeric antigen receptors (CARs). These receptors can be used to graft the specificity of a monoclonal antibody or binding fragment thereof or other ligand

binding domain onto a T-cell, wherein transfer of the required coding sequences is facilitated by viral vectors, such as a retroviral vector or a lentiviral vector. CARs are e.g., genetically engineered T-cell receptors designed to redirect T-cells to target cells that express or display the specific cell-surface antigen to which the CAR is directed. T-cells can be removed from a subject and modified so that they express receptors that are specific for a desired antigen by a process called “adoptive cell transfer.” The T-cells are then reintroduced into the patient, wherein they recognize, target and bind to molecules displaying the antigen presented on cells. These CARs are engineered receptors that graft a selected specificity onto an immune receptor cell. The term chimeric antigen receptors or “CARs” is also considered by some investigators to include the antibody or antibody fragment, the spacer, signaling domain(s), and transmembrane region. Due to the surprising effects of modifying the different components or domains of the CAR described herein, such as the epitope binding region (for example, antibody fragment, scFv, or portion thereof), spacer, transmembrane domain, and/ or signaling domain), the components of the CAR are frequently distinguished throughout this disclosure in terms of independent elements.

[0060] A “single-chain variable fragment,” (scFv) is a fusion protein that can have variable regions of the heavy (VH) and light chains (VL) of immunoglobulins, connected with a short linker peptide of ten to 25 amino acids. The short linker peptide can comprise 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 amino acids or any number of amino acids within a range defined by any two aforementioned values. The linker is usually rich in glycine for flexibility, as well as, serine or threonine for solubility, and can either connect the N-terminus of the VH with the C-terminus of the VL, or vice versa. This protein retains the specificity of the original immunoglobulin, despite removal of the constant regions and the introduction of the linker. The scFv can be specific for an antigen. “Antigen” or “Ag” as used herein, refers to a molecule that provokes an immune response. This immune response can involve either antibody production, or the activation of specific immunologically-competent cells, or both. An antigen can be generated, synthesized, produced recombinantly or can be derived from a biological sample. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a biological fluid such, for example, blood, plasma or ascites fluid. In some embodiments herein, a composition is provided, wherein the composition comprises cells, preferably T cells, manufactured by any one of the alternative methods herein.

In some embodiments, the cells, preferably T cells, comprise a chimeric antigen receptor, wherein the chimeric antigen receptor comprises a scFv that is specific for a DNP moiety joined to or associated with a PLE molecule, which can be associated or joined to a tumor cell.

[0061] “Antigen specific binding domains” can include protein or protein domains that specifically bind to an epitope on a protein at a low or high binding affinity (fM to mM binding capacity). In some embodiments, the fusion protein comprises a protein or portion thereof that modulate an immune response. In some embodiments, the protein comprises an antigen specific binding domain.

[0062] Several types of “spacers” are described herein. With regard to CARs, a spacer for a CAR refers to a polypeptide spacer, which spacer length is configured to or is selected for its ability to promote an increase in binding or interaction with a chimeric antigen receptor or to reduce or minimizes an adverse side effect associated with CAR T cell therapy. In some embodiments, a short spacer domain of a CAR has 12 or about 12 amino acids or less but greater than 1 amino acid and comprises all or a portion of a IgG4 hinge region sequence or variant thereof. In some embodiments, an intermediate (medium) spacer domain of a CAR has 119 or about 119 amino acids or less but greater than 1 amino acid and comprises all or a portion of a IgG4 hinge region sequence and a CH3 region or variant thereof. In some embodiments, a long spacer domain of a CAR has 229 or about 229 amino acids or less but greater than 1 amino acid and comprises all or a portion of a IgG4 hinge region sequence, a CH2 region, and a CH3 region or variant thereof. In some embodiments, the spacer length or sequence or both are selected based on a desired avidity or interaction with a DNP moiety joined to or associated with a PLE molecule, which can be associated or joined to a tumor cell.

[0063] A “transmembrane domain” is a region of a protein that is hydrophobic that can reside in the bilayer of a cell to anchor a protein that is embedded to the biological membrane. Without being limiting, the topology of the transmembrane domain can be a transmembrane alpha helix. In some embodiments of the method of making genetically modified T-cells, which have a chimeric antigen receptor, the vector comprises a sequence encoding a transmembrane domain. In some embodiments of the method, the transmembrane domain comprises a CD28 transmembrane sequence or a fragment thereof that is a length of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 amino acids or a length within a range defined by any two of the aforementioned lengths. In some embodiments

of the method, the CD28 transmembrane sequence or fragment thereof comprise 28 amino acids in length. In some embodiments, the chimeric receptor comprises a transmembrane domain. The transmembrane domain provides for anchoring of the chimeric receptor in the membrane.

[0064] “Co-stimulatory domain,” or “intracellular signaling domain” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a signaling moiety that provides to T cells a signal which, in addition to the primary signal provided by for instance the CD3 zeta chain of the TCR/CD3 complex, mediates a T cell response, including, but not limited to, activation, proliferation, differentiation, or cytokine secretion, and the like. A co-stimulatory domain can include all or a portion of, but is not limited to, CD27, CD28, 4-1BB, OX40, CD30, CD40, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, or a ligand that specifically binds with CD83. In some embodiments, the co-stimulatory domain is an intracellular signaling domain that interacts with other intracellular mediators to mediate a cell response including any one or more of activation, proliferation, differentiation or cytokine secretion.

[0065] A “marker sequence,” as described herein, encodes a protein that is used for selecting or tracking a protein or cell that has a protein of interest. In the embodiments described herein, the fusion protein provided can comprise a marker sequence that can be selected in experiments, such as flow cytometry. In some embodiments, the marker is the protein Her2tG, CD19t, or EGFRt.

[0066] A “ribosome skip sequence” as described herein refers to a sequence that during translation, forces the ribosome to “skip” the ribosome skip sequence and translate the region after the ribosome skip sequence without formation of a peptide bond. Several viruses, for example, have ribosome skip sequences that allow sequential translation of several proteins on a single nucleic acid without having the proteins linked via a peptide bond. As described herein, this is the “linker” sequence. In some embodiments of the nucleic acids provided herein, the nucleic acids comprise a ribosome skip sequence between the sequence for the chimeric antigen receptor and the sequence of the marker protein, such that the proteins are co-expressed and not linked by a peptide bond. In some embodiments, the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence. In some embodiments, the ribosome skip sequence is a T2A sequence. In some embodiments, there are ribosome skip sequences between the two chimeric

antigen receptors and a second ribosome skip sequence between one of the chimeric antigen receptors and the marker.

[0067] As used herein, 2,4-Dinitrophenol (2,4-DNP or simply DNP) is an organic compound with the formula $\text{HOC}_6\text{H}_3(\text{NO}_2)_2$, and has its plain and ordinary meaning when read in light of the specification. DNP is used as an antiseptic, non-selective bioaccumulating pesticide, herbicide, among others. It is a chemical intermediate in the production of sulfur dyes, wood preservatives, and picric acid. In some embodiments herein, DNP is a target moiety on a lipid that is recognized and bound by a chimeric antigen receptor. In some embodiments, the hapten is DNP or derivatives thereof. In some embodiments, the lipid is a phospholipid, such as a phospholipid ether (PLE).

[0068] “Lipid” as described herein, is a class of organic compounds that comprise carbon chains, fatty acids or a fatty acid derivative that is typically insoluble in water but can integrate into or mix with hydrophobic or organic solvents. Without being limiting, lipids can include fats, waxes, fat soluble vitamins, monoglycerides, diglycerides, triglycerides, sphingolipids, cerebroside, ceramides, or phospholipids. Described herein are amphiphilic lipids that can have a polar head group and a hydrophobic moiety or hydrophobic group. “Hydrophobic group” or hydrophobic moiety, as described herein, is a molecule or a part of a molecule that is repelled from a mass of water and tends to be non-polar. This can include alkanes, oils or fats. Without being limiting, lipids can be glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids or polyketides. In the embodiments herein a complex is provided, wherein the complex comprises a lipid. In some embodiments, the lipid comprises a polar head group and a hydrophobic moiety. In some embodiments, the hydrophobic moiety is a hydrophobic carbon tail. In some embodiments the hydrophobic carbon tail is saturated or unsaturated. In some embodiments, the hydrophobic carbon tail comprises 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 carbons or any number of carbons in between a range set forth in any aforementioned value. In some embodiments, the hydrophobic moiety is a steroid or a cholesterol. In some embodiments, the lipid comprises a glycerolipid, glycerophospholipid, sphingolipid, sterol lipid, prenol lipid, saccharolipid or polyketide. In some embodiments, the lipid is a phospholipid ether. In some embodiments, the lipid contains branched alkyl tails.

[0069] In some embodiments, the lipid is a sphingolipid. The sphingolipid can contain a backbone of sphingoid bases, such as a set of aliphatic amino alcohols that includes sphingosine. A sphingolipid with an R group consisting of a hydrogen atom only is a ceramide. Other common R groups include phosphocholine, yielding a sphingomyelin, or various sugar monomers or dimers, yielding cerebrosides and globosides, respectively. Cerebrosides and globosides are collectively known as glycosphingolipids. In some embodiments, the lipid is a glycosphingolipid.

[0070] As provided herein, the lipid comprises a polar head group and a hydrophobic group. In some embodiments, the hydrophobic group comprises a fatty acid such as an aliphatic chain. The fatty acid can be saturated or unsaturated. In some embodiments, the hydrophobic group comprises an alkyl, alkenyl or alkynyl group. In some embodiments, the hydrophobic group comprises a terpenoid lipid, such as a steroid or cholesterol. In some embodiments, the hydrophobic group comprises an ether linkage, wherein the ether linkage is between the polar head group and the aliphatic chain. In some embodiments, the lipid is a phospholipid ether. In some embodiments, the polar head comprises a choline, a phosphatidylcholine, sphingomyelin, phosphoethanolamine group, an oligosaccharide residue, a sugar residue, phosphatidyl serine or phosphatidyl inositol. In some embodiments, the sugar is a glycerol or sugar alcohol.

[0071] In some embodiments, the lipid is a single chain alkylphospholipid.

[0072] In some embodiments, the lipid comprises a structure of synthetic alkylphospholipids, such as edelfosine, perifosine or erucylphosphocholine. In some embodiments, the lipid is a lysophosphatidylcholine, edelfosine, erucylphosphocholine, D-21805 or perfisone. Such lipids are described for example, in van der Lui *et al* ("A new class of anticancer alkylphospholipids uses lipid rafts as membrane gateways to induce apoptosis in lymphoma cells" *Mol Cancer Ther* 2007; 6(8), 2007; hereby expressly incorporated by reference in its entirety). In some embodiments of the lipids described herein, a choline within the polar head group can be substituted with a piperidine moiety. In some embodiments, the lipid is an anticancer alkylphospholipid. Anticancer phospholipids are described by vander Lui *et al*. ("A new class of anticancer alkylphospholipids uses lipid rafts as membrane gateways to induce apoptosis in lymphoma cells" *Mol Cancer Ther* 2007; 6(8), 2007; hereby expressly incorporated by reference in its entirety).

[0073] In some embodiments, the lipids provided herein are synthetic and structurally related anti-tumor agents that interact with a cell membrane. These types of synthetic lipids are alkylphospholipids and are described by e.g., van Blitterswijk *et al.* (“Anticancer mechanisms and clinical application of alkylphospholipids” *Biochimica et Biophysica Acta* 1831 (2013)663-674; hereby incorporated by reference in its entirety herein). Without being limiting, the synthetic alkylphospholipids can include edelfosine, miltefosine, perifosine, erucylphosphocholine or Erufosine. In some embodiments, the lipid is edelfosine, miltefosine, perifosine, erucylphosphocholine or Erufosine. In some embodiments, the lipid is a stable analog of lysophosphatidylcholine. In some embodiments, the lipid is a thio-ether variant of edelfosine, or 1-hexadecylthio- 2-methoxymethyl-rac-glycero-3-phosphocholine. In some alternatives, the lipid is LysoPC, edelfosine, Ilmofosine, Miltefosine, Perifosine, Erucylphosphocholine, or Erufosine.

[0074] “Polar-head group” as described herein, is the hydrophilic group of a lipid, such as a phospholipid. “Phospholipids” as described herein are a specific class of lipids that can form lipid bilayers due to their amphiphilic characteristic. The phospholipid molecule comprises at least one hydrophobic fatty acid “tail” and a hydrophilic “head” or “polar-head group.” In the alternative herein, the phospholipid or phospholipid ether comprises a polar-head group. In some embodiments, the polar-head group comprises phosphocholine, a piperidine moiety or a trimethylarseno-ethyl-phosphate moiety. In some embodiments, the lipid comprises a target moiety, preferably DNP, and the CAR is joined to or is configured to join to said lipid through an interaction with said target moiety. In some embodiments, the lipid comprises a polar-head group (e.g., comprising an aromatic ring) and a carbon alkyl chain. In some embodiments herein, a complex is provided, wherein the complex comprises one or more of said lipids. In some embodiments, the lipid comprises a polar head group. In some embodiments, the lipid is a phospholipid ether. In some embodiments, the phospholipid ether comprises a target moiety, preferably DNP, and the CAR is joined to or is configured to join to said phospholipid ether through an interaction and/or binding with said target moiety. In some embodiments, the phospholipid ether comprises a polar-head group and a carbon alkyl chain. In some embodiments, the polar head group comprises a choline, a phosphatidylcholine, sphingomyelin, phosphoethanolamine group, an oligosaccharide residue, a sugar residue, phosphatidyl serine or phosphatidyl inositol. In some embodiments, the polar head group

comprises phosphocholine, a piperidine moiety or a trimethylarseno-ethyl-phosphate moiety. In some embodiments, the lipid is a phospholipid ether (PLE). In some embodiments, the sugar is a glycerol or sugar alcohol. In some embodiments, the polar head group comprises a sugar group. In some embodiments, the lipid comprises a mannose-containing head group. In some embodiments, the polar head group comprises sphingosine. In some embodiments, the polar head group comprises a glucose. In some embodiments, the polar head group comprises a di-, tri- or tetra-saccharide. In some embodiments, the lipid is a glucosylcerebroside. In some embodiments, the lipid is a lactosylceramide. In some embodiments, the lipid is a glycolipid. In some embodiments, the glycolipid comprises sugar units, such as n-glucose, n-galactose or N-actyl-n-glucosamine. In some embodiments, the lipid comprises a hydrocarbon ring such as a sterol.

[0075] In some embodiments, the polar head group of the lipid comprises glycerol or a sugar alcohol. In some embodiments, the polar head group of the lipid comprises a phosphate group. In some embodiments, the polar head group of the lipid comprises choline. In some embodiments, the lipid is a phosphatidylethanolamine. In some embodiments, the lipid is a phosphatidylinositol. In some embodiments, the lipid comprises a sphingoid base backbone. In some embodiments, the lipid comprises a sterol lipid, such as cholesterol or its derivatives. In some embodiments, the lipid comprises saccharolipids. In some embodiments, the polar head group comprises choline, phosphate or glycerol.

[0076] In some embodiments, the lipid is a glycolipid. In some embodiments, the lipid comprises a sugar. In some embodiments, the lipid is derived from sphingosine. In some embodiments, the lipid is a glycerol-glycolipid or a sphingo-glycolipid.

[0077] In some embodiments, the lipid is an ether lipid with branched hydrophobic chains.

[0078] “Terpenoid” as described herein, is a molecule that is derived from five carbon isoprene units. Steroids and sterols can be produced from terpenoid precursors. For example, steroids and cholesterol can be biosynthesized by terpenoid precursors.

[0079] “Phospholipid ether” (PLE) as described herein is a lipid in which one or more of the carbon atoms on a polar head group are bonded to an alkyl chain via an ether linkage, as opposed to the more common ester linkage. In some embodiments, the polar head group is a glycerol.

[0080] Several types of “spacers” are described herein and with regards to lipids, a lipid can comprise a spacer e.g., bound to the polar-head group of the lipid, which separates the target moiety, preferably DNP, from the lipid and a cell that is joined to or associates with said lipid so as to achieve a desired orientation or degree of freedom from steric hindrance. The spacer of the lipid can comprise a PEG spacer, a Hapten spacer, a small peptide or an alkane chain. In some embodiments, the hapten spacer comprises two haptens (hapten (2X) spacer). In some embodiments, the lipid comprises a hydrophobic group, such as an alkane chain. In some embodiments, the alkane chain can comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 carbons, or any number of carbons between a range defined by any two aforementioned values. In some embodiments, the PEG spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 PEG molecules, or any amount of PEG molecules that is within a range defined by any two aforementioned values. Preferably, the length and type of spacer is selected to display the target moiety, preferably DNP, in an orientation or proximity that allows for a desired affinity or avidity to an anti-DNP CAR displayed on a cell, such as a T cell.

[0081] T-cells” or “T lymphocytes” as used herein, can be from any mammalian species, preferably primate, including monkeys, dogs, and humans. In some embodiments, the T-cells are allogeneic (from the same species but different donor) as the recipient subject; in some embodiments the T-cells are autologous (the donor and the recipient are the same); in some embodiments the T-cells are syngeneic (the donor and the recipients are different but are identical twins).

[0082] In some embodiments, the T cells are preferably “memory” T cells (T_m cells) that are antigen-experienced. In some embodiments, the cell is a precursor T cell. In some embodiments, the precursor T cell is a hematopoietic stem cell. In some embodiments, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T cells, central memory CD8⁺ T cells, effector memory CD8⁺ T cells and bulk CD8⁺ T cells. In some embodiments, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells, and bulk CD4⁺ T cells.

[0083] “Cytotoxic T lymphocyte” (CTL), as used herein, refers to a T lymphocyte that expresses CD8 on the surface thereof (e.g., a CD8⁺ T-cell). In some embodiments, such

cells are preferably "memory" T-cells (T_M cells) that are antigen-experienced. In some embodiments, a cell for fusion protein secretion is provided. In some embodiments, the cell is a cytotoxic T lymphocyte. "Central memory" T-cell (or " T_{CM} ") as used herein, refers to an antigen experienced CTL that expresses CD62L, CCR-7 and/or CD45RO on the surface thereof, and does not express or has decreased expression of CD45RA, as compared to naïve cells. In some embodiments, a cell for fusion protein secretion is provided. In some embodiments, the cell is a central memory T-cell (T_{CM}).

[0084] In some embodiments, the central memory cells are positive for expression of CD62L, CCR7, CD28, CD127, CD45RO, and/or CD95, and may have decreased expression of CD54RA, as compared to naïve cells. "Effector memory" T-cell (or " T_{EM} ") as used herein refers to an antigen experienced T-cell that does not express or has decreased expression of CD62L on the surface thereof, as compared to central memory cells, and does not express or has a decreased expression of CD45RA, as compared to naïve cell. In some embodiments, a cell for fusion protein secretion is provided. In some embodiments, the cell is an effector memory T-cell. In some embodiments, effector memory cells are negative for expression of CD62L and/or CCR7, as compared to naïve cells or central memory cells, and may have variable expression of CD28 and/or CD45RA.

[0085] "Naïve T-cells" as used herein, refers to a non-antigen experienced T lymphocyte that expresses CD62L and/or CD45RA, and does not express CD45RO-, as compared to central or effector memory cells. In some embodiments, a cell for fusion protein secretion is provided. In some embodiments, the cell is a naïve T-cell. In some embodiments, naïve CD8+ T lymphocytes are characterized by the expression of phenotypic markers of naïve T-cells including CD62L, CCR7, CD28, CD127, and/or CD45RA.

[0086] "T-cells" or "T lymphocytes" as used herein can be from any mammalian, preferably primate, species, including monkeys, dogs, and humans. In some embodiments the T-cells are allogeneic (from the same species but different donor) as the recipient subject; in some embodiments the T-cells are autologous (the donor and the recipient are the same); in some embodiments the T-cells are syngeneic (the donor and the recipients are different but are identical twins).

[0087] "T cell precursors" as described herein refers to lymphoid precursor cells that can migrate to the thymus and become T cell precursors, which do not express a T cell

receptor. All T cells originate from hematopoietic stem cells in the bone marrow. Hematopoietic progenitors (lymphoid progenitor cells) from hematopoietic stem cells populate the thymus and expand by cell division to generate a large population of immature thymocytes. The earliest thymocytes express neither CD4 nor CD8, and are therefore classed as double-negative ($CD4^-CD8^-$) cells. As they progress through their development, they become double-positive thymocytes ($CD4^+CD8^+$), and finally mature to *single-positive* ($CD4^+CD8^-$ or $CD4^-CD8^+$) thymocytes that are then released from the thymus to peripheral tissues.

[0088] As described herein, “CD8 T-cells” or “killer T-cells” are T- lymphocytes that can kill cancer cells, cells that are infected with viruses or cells that are damages. CD8 T-cells recognize specific antigens, or a protein that is capable of stimulating an immune response and is produced by cancer cells or viruses. If the T-cell receptor of the CD8 T- cell recognizes the antigen, the CD8 T-cell can bind to the presented antigen and destroy the cell.

[0089] “Central memory T-cell” (T_{CM}) as used herein refers to an antigen experienced CTL that expresses CD62L or CCR-7 and CD45RO on the surface thereof and does not express or has decreased expression of CD45RA as compared to naïve cells. In some embodiments, central memory cells are positive for expression of CD62L, CCR7, CD28, CD127, CD45RO, and/or CD95, and have decreased expression of CD45RA as compared to naïve cells. “Effector memory” T-cell (or “ T_{EM} ”) as used herein refers to an antigen experienced T-cell that does not express or has decreased expression of CD62L on the surface thereof as compared to central memory cells, and does not express or has decreased expression of CD45RA as compared to naïve cell. In some embodiments, effector memory cells are negative for expression of CD62L and/or CCR7, as compared to naïve cells or central memory cells, and have variable expression of CD28 and/or CD45RA. “Effector T-cells” (T_E cells) as used herein, refers to antigen experienced cytotoxic T lymphocyte cells that do not express or have decreased expression of CD62L, CCR7, and/or CD28, and are positive for granzyme B and/or perforin, as compared to central memory or naïve T-cells. In some embodiments, a cell for fusion protein secretion is provided. In some embodiments, the cell is an effector T-cell. In some embodiments, the cell does not express or have decreased expression of CD62L, CCR7, and/or CD28, and are positive for granzyme B and/or perforin, as compared to central memory or naïve T-cells.

[0090] “Combination therapy” as described herein, refers to a therapy that uses more than one medication or modality for a therapy. Combination therapy, for example, can also refer to multiple therapies for a single disease, and often all the therapies are pharmaceutical product combinations. Combination therapy can also involve prescribing and administering separate drugs in which the dosage can also have more than one active ingredient. In some embodiments, a combination therapy is provided, wherein the combination therapy comprises administering a genetically modified immune cell for modifying a tumor microenvironment. In some embodiments, the combination therapy comprises administering a genetically modified immune cell for modulating the suppression of the immune response in a tumor microenvironment. In some embodiments, the combination therapy comprises administering a genetically modified immune cell for minimizing the proliferation of tumor and suppressive cells in a subject in need thereof e.g. a human. In some embodiments, the combination therapy comprises administering a genetically modified immune cell for increasing the efficiency of an anti-cancer therapy, anti-infection therapy, antibacterial therapy, anti-viral therapy, or anti-tumoral therapy in a subject in need thereof e.g., a human. In some embodiments, the combination therapy further comprises administration of an inhibitor. In some embodiments, the inhibitor is not an enzymatic inhibitor. In some embodiments, the inhibitor is an enzymatic inhibitor. In some embodiments, the combination therapy comprises administering a therapeutic dose of an inhibitor or an antibody or a binding fragment thereof. These antibodies or binding fragments thereof can be humanized in some embodiments. In some embodiments, the combination therapy can further comprise administering a CAR bearing T-cell to a subject in need e.g., a human.

[0091] “Subject” or “patient,” as described herein, refers to any organism upon which the embodiments described herein may be used or administered, e.g., for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Subjects or patients include, for example, animals. In some embodiments, the subject is mice, rats, rabbits, non-human primates, or humans. In some embodiments, the subject is a cow, sheep, pig, horse, dog, cat, primate or a human.

[0092] “Cancer,” as described herein, is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Subjects that can be addressed using the methods described herein include subjects identified or selected as

having cancer, including but not limited to colon, lung, liver, breast, renal, prostate, ovarian, skin (including melanoma), bone, leukemia, multiple myeloma, or brain cancer, etc. Such identification and/or selection can be made by clinical or diagnostic evaluation. In some embodiments, the tumor associated antigens or molecules are known, such as melanoma, breast cancer, brain cancer, squamous cell carcinoma, colon cancer, leukemia, myeloma, or prostate cancer. Examples include but are not limited to B cell lymphoma, breast cancer, brain cancer, prostate cancer, and/or leukemia. In some embodiments, one or more oncogenic polypeptides are associated with kidney, uterine, colon, lung, liver, breast, renal, prostate, ovarian, skin (including melanoma), bone, brain cancer, adenocarcinoma, pancreatic cancer, chronic myelogenous leukemia or leukemia.

[0093] In some embodiments, a method of treating, ameliorating, or inhibiting one or more of the aforementioned cancers in a subject is provided. In some embodiments, the cancer is breast, ovarian, lung, pancreatic, prostate, melanoma, renal, pancreatic, glioblastoma, neuroblastoma, medulloblastoma, sarcoma, liver, colon, skin (including melanoma), bone or brain cancer. In some embodiments, the subject that receives one of the therapies set forth herein e.g., DNP-PLE and T cells having a CAR with a selective avidity for DNP as described herein, is also selected to receive an additional cancer therapy, which can include a cancer therapeutic, radiation, chemotherapy, or a cancer therapy drug. In some embodiments, the cancer therapy drug provided comprises Abiraterone, Alemtuzumab, Anastrozole, Aprepitant, Arsenic trioxide, Atezolizumab, Azacitidine, Bevacizumab, Bleomycin, Bortezomib, Cabazitaxel, Capecitabine, Carboplatin, Cetuximab, Chemotherapy drug combinations, Cisplatin, Crizotinib, Cyclophosphamide, Cytarabine, Denosumab, Docetaxel, Doxorubicin, Eribulin, Erlotinib, Etoposide, Everolimus, Exemestane, Filgrastim, Fluorouracil, Fulvestrant, Gemcitabine, Imatinib, Imiquimod, Ipilimumab, Ixabepilone, Lapatinib, Lenalidomide, Letrozole, Leuprolide, Mesna, Methotrexate, Nivolumab, Oxaliplatin, Paclitaxel, Palonosetron, Pembrolizumab, Pemetrexed, Prednisone, Radium-223, Rituximab, Sipuleucel-T, Sorafenib, Sunitinib, Talc Intrapleural, Tamoxifen, Temozolomide, Temsirolimus, Thalidomide, Trastuzumab, Vinorelbine or Zoledronic acid.

[0094] “Tumor microenvironment” as described herein is a cellular environment, wherein a tumor exists. Without being limiting, the tumor microenvironment can include

surrounding blood vessels, immune cells, fibroblasts, bone marrow-derived inflammatory cells, lymphocytes, signaling molecules or the extracellular matrix (ECM).

Certain nucleic acids encoding CARs

[0095] Some embodiments of the methods and compositions provided herein include one or more nucleic acids encoding a CAR specific for a DNP moiety associated with or joined to a PLE molecule. In some embodiments, the DNP moiety is associated or joined to an antibody or a folate. In some embodiments, the DNP moiety is in an orientation or proximity on an extracellular cell surface that allows for a desired affinity or avidity to the anti-DNP CAR, such as a T cell or a component of said CAR, such as a ligand binding domain. In some embodiments, the CAR comprises a selected ligand binding domain, which binds to a DNP moiety, a selected spacer of a desired length, a transmembrane domain; and an intracellular signaling domain(s) and, preferably said CAR is displayed on the surface of a T cell in an orientation that promotes or obtains a desired affinity or avidity to DNP-PLE, which may be associated with or joined to a tumor cell.

[0096] In some embodiments, one or more nucleic acids encode a ligand binding domain of one or more CARs set forth herein, which comprises an scFv domain comprising a VH sequence, and VL sequence derived from an antibody. In some embodiments, the VH and VL sequences are joined together via a linker. TABLE 1 lists example embodiments for a series of CARs, and TABLE 2 lists sequences for components of certain CARs. Example embodiments of VH sequences, VL sequences and linkers encoded by one or more of the nucleic acids set forth herein are listed in TABLE 2. In some embodiments, the one or more nucleic acids encoding a ligand binding domain of one or more CARs set forth herein encodes a ligand binding domain that comprises an amino acid sequence having at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to the amino acid sequence set forth in any one of SEQ ID NOs:01-12. In some embodiments, the one or more nucleic acids encode a ligand binding domain that comprises the amino acid sequence set forth in any one of SEQ ID NOs:01-12. In some embodiments, the one or more nucleic acids encode a ligand binding domain that comprises an amino acid sequence having at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to the amino acid sequence set forth in SEQ ID NOs:01, 02, 09, or 10.

TABLE 1

Ligand binding domain derived from scFv	Spacer	Transmembrane domain	Co-stimulatory domain
VH-linker-VL of Ab-1 (1BAF)	short	CD28tm	41BB and CD3-zeta
VH-linker-VL of Ab-1 (1BAF)	medium	CD28tm	41BB and CD3-zeta
VH-linker-VL of Ab-1 (1BAF)	long	CD28tm	41BB and CD3-zeta
VL-linker-VH of Ab-1 (1BAF)	short	CD28tm	41BB and CD3-zeta
VL-linker-VH of Ab-1 (1BAF)	medium	CD28tm	41BB and CD3-zeta
VL-linker-VH of Ab-1 (1BAF)	long	CD28tm	41BB and CD3-zeta
VH-linker-VL of Ab-2 (XC)	short	CD28tm	41BB and CD3-zeta
VH-linker-VL of Ab-2 (XC)	medium	CD28tm	41BB and CD3-zeta
VH-linker-VL of Ab-2 (XC)	long	CD28tm	41BB and CD3-zeta
VL-linker-VH of Ab-2 (XC)	short	CD28tm	41BB and CD3-zeta
VL-linker-VH of Ab-2 (XC)	medium	CD28tm	41BB and CD3-zeta
VL-linker-VH of Ab-2 (XC)	long	CD28tm	41BB and CD3-zeta
VH-linker-VL of Ab-3	short	CD28tm	41BB and CD3-zeta
VH-linker-VL of Ab-3	medium	CD28tm	41BB and CD3-zeta
VH-linker-VL of Ab-3	long	CD28tm	41BB and CD3-zeta
VL-linker-VH of Ab-3	short	CD28tm	41BB and CD3-zeta
VL-linker-VH of Ab-3	medium	CD28tm	41BB and CD3-zeta
VL-linker-VH of Ab-3	long	CD28tm	41BB and CD3-zeta

TABLE 2

SEQ ID NO	Sequence
SEQ ID NO:01 VH of anti DNP scFv (Ab-1; 1BAF)	DVQLQESGPGLVKPSQSQSLTCTVTGYSITSDYAWNWIQFP GNKLEWMGYMSYSGSTRYNPSLRSRISITRDTSKNQFFLQLK SVTTEDTATYFCARGWPLAYWGQGTQVSVSEAKTTPPSVYP LAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVH TFPAVLQSDLYTLSSSVTPSSPRPSETVTCNVAHPASSTKVD KKIVPRDC
SEQ ID NO:02 VL of anti DNP scFv (Ab-1; 1BAF)	QIVLTQSPAIMASAPGEKVTMTCSASSSVYYMYWYQKPGSS PRLLIYDTSNLASGVPVRFSGSGSGTSYSLTISRMEAEDAATY YCQQWSSYPPIFGVGTKLELKRADAAPTIVSIFPPSSEQLTSG

SEQ ID NO	Sequence
	GASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSK DSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFN RNEC
SEQ ID NO:03 VH of anti DNP scFv (Ab-2; XC)	QQLEQSGGGAEGGLVKPGGSLELCKASGFSLSSSYCICWVR QAPGKGLEWIGCIYAGSSGSTYYASWVNGRFTLSRDIDQSTG CLQLNSLTAADTAMYYCARAPYSSGWVLYFNLWGPGLVIV
SEQ ID NO:04 VL of anti DNP scFv (Ab-2; XC)	AQVLTQTPSPVSAAVGGTVTISCQSSSESVYGNSRLAWYQQK PQSPKLLIYYASTLASGVPSRFGSGSGTQFTLTISDLECD DAASYCQGGYYSGNLDALAF
SEQ ID NO:05 VH of anti DNP scFv (Ab-3)	QQLEQSGGGAEGGLVKPGGSLELCKASGFSLSSSYCICWVR QAPGKGLEWIGCIYAGSSGSTYYASWVNGRFTLSRDIDQSTG CLQLNSLTAADTAMYYCARAPYSSGWVLYFNLWGPGLVIV
SEQ ID NO:06 VL of anti DNP scFv (Ab-3)	PGATFAQVLTQTPSPVSAAVGGTVTISCQSSSESVYGNSRLAW YQQKPGQSPKLLIYYASTLASGVPSRFGSGSGTQFTLTISDL ECDDAASYCQGGYYSGNLDALAFGGGTEVVVRG
SEQ ID NO:7 VL-linker-VH of anti DNP scFv (Ab-3)	PGATFAQVLTQTPSPVSAAVGGTVTISCQSSSESVYGNSRLAW YQQKPGQSPKLLIYYASTLASGVPSRFGSGSGTQFTLTISDL ECDDAASYCQGGYYSGNLDALAFGGGTEVVVRGGGGGSG GGGSGGGGSGCQQLEQSGGGAEGGLVKPGGSLELCKASG FSLSSSYCICWVRQAPGKGLEWIGCIYAGSSGSTYYASWVNGR FTLSRDIDQSTGCLQLNSLTAADTAMYYCARAPYSSGWVLY FNLWGPGLVIVSS
SEQ ID NO:08 VH-linker-VL of anti DNP scFv (Ab-3)	QCQQLEQSGGGAEGGLVKPGGSLELCKASGFSLSSSYCICW VRQAPGKGLEWIGCIYAGSSGSTYYASWVNGRFTLSRDIDQ STGTGCLQLNSLTAADTAMYYCARAPYSSGWVLYFNLWGPGL VIVSSGGGGSGGGGSGGGGSPGATFAQVLTQTPSPVSAAVGG TVTISCQSSSESVYGNSRLAWYQQKPGQSPKLLIYYASTLASG VPSRFGSGSGTQFTLTISDLECDAAASYCQGGYYSGNLDA LAFGGGTEVVVRG
SEQ ID NO:09 VH-linker-VL of anti DNP scFv (1BAF)	DVQLQESGPGLVKPSQSQSLTCTVTGYSITSDYAWNWRQFP GNKLEWMGYMSYSGSTRYNPSLRSRISITRDTSKNQFFLQ LKVSTTETDATTYFCARGWPLAYWGQGTQVSVSEAKTTPPSV YPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSG VHTFPAVLQSDLYTLSSSVTPSSPRPSETVTCNVAHPASSTK VDKKIVPRDCGGGGSGGGGSGGGGSIQVLTQSPAIMSASPGEK VTMTCSASSSVYYMYWYQQKPGSSPRLLIYDTSNLASGVPV RFSGSGSGTSYSLTISRMEAEADAATYYCQQWSSYPPIFGV GTKLELKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFY PKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTL TKDEYERHNSYTCEATHKTSTSPIVKSFNRECEC

SEQ ID NO	Sequence
SEQ ID NO:10 VL-linker-VH of anti DNP scFv (1BAF)	QIVLTQSPAIMASAPGEKVTMTCSASSSVYYMYWYQQKPGSS PRLLIYDTSNLASGVVRFSGSGSGTSYSLTISRMEAEDAATY YCQQWSSYPPIITFGVGTKLELKRADAAPTVSIFPPSSEQLTSG GASVVCFLNNFYPKDINVKWKIDGSRQNGVLNSWTDQDSK DSTYSMSSTLTTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRN ECGGGGSGGGSGGGGSDVQLQESGPGLVKPSQSLSLTCTV TGYSITSDYAWNWRQFPGNKLEWMGYMSYSGSTRYNPSLR SRISITRDTSKNQFFLQLKSVTTEDTATYFCARGWPLAYWGQ GTQVSVSEAKTTPPSVYPLAPGSAQAQNSMVTLGCLVKGYFP EPVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTPSSPRPS ETVTCNV AHPASSTKVDKKIVPRDC
SEQ ID NO:11 VH-linker-VL of anti DNP scFv (XC)	QQLEQSGGGAEGGLVKPGGSLELCKASGFSLSSSYICWVR QAPGKGLEWIGCIYAGSSGSTYYASWVNGRFTLSRDIDQSTG CLQLNSLTAADTAMYYCARAPYSSGWVLYFNLWGPGLVIV SSGGGGSGGGSGGGGSAQVLQTTPSPVSAAVGGTVTISCQS SESVYGNSRLAWYQQKPGQSPKLLIYYASTLASGVPSRFKGS GSGTQFTLTISDLECDAAASYCQGGYYSGNLDALAF
SEQ ID NO:12 VL-linker-VH of anti DNP scFv (XC)	AQVLTQTTPSPVSAAVGGTVTISCQSSSESVYGNSRLAWYQQK GQSPKLLIYYASTLASGVPSRFKGS GSGTQFTLTISDLECDAA ASYCQGGYYSGNLDALAFGGGTEVVVRGGGGSGGGGSG GGGQQLEQSGGGAEGGLVKPGGSLELCKASGFSLSSSYIC CWVRQAPGKGLEWIGCIYAGSSGSTYYASWVNGRFTLSRDI DQSTGCLQLNSLTAADTAMYYCARAPYSSGWVLYFNLWGP GTLVIV
SEQ ID NO:13 VH-linker-VL of anti DNP scFv (1BAF)	gacgtacaactgcaagaatcaggtccgggactgggtcaaacctagccagctcagtccttgacgtg caccgtgacgggctatagataacgagtgattacgcttggaaattggattcggcagttccaggca taagcttgagtgatgggctatatgtcttactcggctcaactaggtataatccgagccttcggctc ggatttctatcacaagagatcagtaagaatcagtttttcttcaactcaaaagcgtcaccacggga ggataccgccacataattctgtgctaggggatggcctcttgcttattgggggcaagggaacaaagt gtctgtttccgaagccaaacaacgcccccttcagttctatccgctggcaccgggaagcgcggca caaaccaatagtgatggaacgctcggatgtctcgtcaaggggtatttcccgagcctgtgacagtg acatggaaattctggagtgcttagcagcggagtagatacttccggcagtgacttcaatccgattgta cacgctctcctctagtgttacagttccaagctctccacgacctagtgagacggttacatgtaacgtc gcgcatccggcctcttccactaaagtggataaaaagattgtgcccagggaactgcggcggaagg ggctctggcgcgaggatctgggggagggggcagccaaattgtgtgacccagtcgccggc cataatgtccgctctcctggcgagaaggttactatgactgtctcagcctcctcagtggtgtattat gtactggtatcaaaaaagccgggctcttccccccggctccttatatacagacagagtaactggc aagtggcgtgcctgttagatttctgggtccggctctggaacttcatactccctgacaattagccga atggaagccgagagcgcggcgacatactactccagcaatggtcatcctatccgcctatcacittt ggagtagggaccaaattggagtgaaagcgggctgatgcggctccacagtagtattttccctcc gtccagtgaaacttaactccggggagcctccgtgtgtgttcttctgaaacttttacccgaaa gatataaatgtcaagtgaagatcgacggctcagagcgccaaaacggggtactcaactatgga cagatcaggatagtaaaagattcaacttacagtagtcttagtaccctgacactgacgaaagatgaat

SEQ ID NO	Sequence
	acgaaagacataatagttatacctgtgaagctacacataagacttcaacctctcctattgtaaaatca ttcaaccgaaacgaatgt
SEQ ID NO: 14 VL-linker-VH of anti DNP scFv (1BAF)	caaattgtgttgaccagtcctccggccataatgtccgcttctcctggcgagaagggtactatgactt gctcagcctcctccagtggtattatagtactggatcaacaaaagccgggctcttccccccggct ccttatatacgacacgagtaatctggcaagtggtgctgctgttagattttctgggtccggctctgga acttcatactccctgacaattagccgaatggaagccgaggacgctggcgacatactactgcccagc aatggctatccctatccgctatcacttttggagtagggaccaaattggagttgaagcgggctgatg cggctcccacagttagtattttccctccgtccagtgacaacttacctccgggggagcctccgttgt ttgctttctgaacaacttttaccgaaagataaaatgtcaagtggaagatcgacgggctcagagcg ccaaaacgggggtactcaactcatggacagatcaggatagtaagattcaacttacagtatgtctag tacctgacactgacgaaagatgaatacgaaagacataatagttatacctgtgaagctacacataa gacttcaacctctcctattgtaaaatcattcaaccgaaacgaatgtggcggagggggctctggcg gctggaggatctgggggagggggagcgcagctacaactgcaagaatcaggctccgggactgggtc aaacctagccagctcagtccttgacgtgcacgtgacgggctatagataacagtgattacgct tggaattggattcggcagttccaggcaataagcttgagtgatgggctatagtcttactccggct caactaggatataatccgagccttggctcggatttctatcacaagagatacagagtaagaatcagtt ttttctcaactcaaaagcgtcaccacggaggataccgacataatttctgtctaggggatggcct cttgcttattgggggcaaggggacacaagtgctgtttccgaagccaaaacaacgccccttcagtc tatccgtctggcaccgggaagcgcggcacaaccaatagtatgtaacgctcggatgtctcgtca aggggtattttccgagcctgtgacagtgacatggaattctgggagtgtagcagcggagtagcata ctttccggcagtagtcaatccgattgtacacgctcctctagtgttaacagttccaagctctccac gacctagtgagaccgttacatgtaacgtcgcgcatccggcctctccactaaagtggataaaaaag attgtgccaggggactgc
SEQ ID NO: 15 VH-linker-VL of anti DNP scFv (XC)	cagcagctggagcagtcggaggaggagccgaaggaggcctgggtcaagcctgggggatccc tggaactctgctgcaaaagcctctggattctccctcagtagtagctactgcatatgttgggtccgcca ggctccagggaaggggctggagtgatcggtgatttatgctgtagtagtgtagcacttact acgcgagctgggtgaatggccgattcactctctccagagacattgaccagagcacagggtgcta caactgaacagctctgacagccgcgacacggccatgtattactgtgcgagagccccctatagta gtggctgggtcctctactttaacttggggggccaggcaccctgggtcattgtctcctcaggcggag ggggctctggcgccggaggatctgggggagggggcagcgcccaagtgctgacccagactcc atcgctgtgtctgagctgtggaggcagtcaccatcagttgacagtcagtgagagtggtta tggtaacagccgcttagcctggtatcagcagaaaccagggcagtcctccaagctcctgatctatta tgcatccactctggcatctgggtcccttcgcggttcaaaaggcagtggtgctgggacacagttcac tctcaccattagcgacctggagtgtagcatgctgcctcttactactgtcaaggcggttattatagt gtaatcttgatgctgctgttct
SEQ ID NO: 16 VL-linker-VH of anti DNP scFv (XC)	gccccagtgctgaccagactccatcgctgtgtctgcagctgtggaggcagatcaccatca gttgccagtcagtgagagtggttatggttaacagccgcttagcctggtatcagcagaaaccagg cagctcccaagctcctgaictattatgcatccactctggcatctgggtcccttcgcggttcaaa gcagtgatctgggacacagttcactctcaccattagcgacctggagtgtagcatgctgctctt actactgcaaggcggttattatagtggttaacttgatgcgcttcttccggcggaggggaccgaggt ggtggtcagaggtggcgagggggctctggcgccggaggatctgggggagggggcagcca gcagctggagcagtcggaggaggagccgaaggaggcctgtgaagcctgggggatccctg gaactctgctgcaaaagcctctggattctcctcagtagtagctactgcatatgttgggtccgagg

SEQ ID NO	Sequence
	ctccaggggaaggggctggagtgatcgatgcattatgctggtagtagtggtagcacttactac gcgagctgggtgaatggccgattcactctctccagagacattgaccagagcacagggtgctaca actgaacagctcgacagccgcccggacacggccatgtattactgtgcgagagccccctatagtagtg gctgggtcctctactttaactgtggggcccaggcaccctgggtcattgtc
SEQ ID NO:17 VH-linker-VL of anti DNP scFv (Ab-3)	ccagggtgccacattgcccaggtctgacccagactccatcgctgtgtctgcagctgtgggagg cacagtcaccatcagttgccagtcagtgagagtggttatggtaacagccgcttagcctggatca gcagaaaccaggggcagctctccaagctcctgatctattatgcatccactctggcatctggggtccc ttcgcggttcaaaggcagtgatctgggacacagttcactctcaccattagcgacctggagtgga cgatgctgctcttactactgtcaaggcggttattatagtggtaatcttgatcgctgttctggcggg agggaccgaggtgggtgctcagaggtggcgagggggctctggcggcgaggagatctggggga gggggcagccagtgctcagcagctggagcagtcggaggaggagccgaaggaggcctgggtca agcctgggggatccctggaaactctgctgcaaaagcctctggattctccctcagtagtagctactgca tatgttgggtccgcccaggtccagggaaggggctggagtgatcgatgattatgctggtagt agtggtagcacttactacgcgagctgggtgaatggccgattcactctcaccagagacattgacca gagcacaggtgctacaactgaacagctcgacagccgcccggacacggccatgtattactgtcg agagccccctatagtagtggtggtcctctactttaactgtggggcccaggcaccctgggtcatt gtctctca
SEQ ID NO:18 anti DNP scFv VH-linker-VL of anti DNP scFv (Ab-3)	cagtgctcagcagctggagcagtcggaggaggagccgaaggaggcctggtaagcctgggg gateccctggaaactctgctgcaaaagcctctggattctccctcagtagtagctactgcatatgttgggt ccgccaggtccagggaagggtggtgagtgatcgatgcatttatgctggtagtagtgtagc acttactacgcgagctgggtgaatggccgattcactctctccagagacattgaccagagcacagg ttgctacaactgaacagctcgacagccgcccggacacggccatgtattactgtgcgagagccccct atagtagtggtgggtcctctactttaactgtggggcccaggcaccctgggtcattgtctcctcagg cggagggggctctggcggcggaggatctggggaggggggcagcccagggtgccaatttggc caagtgtgacccagactccatcgctgtgtctgcagctgtggaggacagtcaccatcagttg ccagtcagtgagagtggttatggtaacagccgcttagcctgtatcagcagaaccaggggcag ctccaaagctcctgatctattatgcatccactctggcatctgggggtcccttcgcggttcaaaggcag tgatctgggacacagttcactctcaccattagcgacctggagtgtagcagtgctgctcttactac tgtcaaggcggttattatagtggtaatcttgatgcgctgtcttcggcggagggaccgaggtggtg gtcagaggt
SEQ ID NO:19 linker	GGGSGGGSGGGSGGGG
SEQ ID NO:20 Long spacer: IgG4hinge- CH2(L235D)-CH3	ESKYGPPCPPCPAPEFDGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYR VSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTKAKGQ PREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSC SVMHEALHNHYTQKSLSLGLK
SEQ ID NO:21 Medium spacer: IgG4 hinge-CH3	ESKYGPPCPPCPGQPREPQVYTLPPSQEEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTV DKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLK
SEQ ID NO:22	ESKYGPPCPPCP

SEQ ID NO	Sequence
Short spacer: IgG4 hinge	
SEQ ID NO:23 CD28tm	MFWVLVVVGGVLACYSLLVTVAFIIFWV
SEQ ID NO:24 4-1BB	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL
SEQ ID NO:25 CD3 zeta	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG RDPENMGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGER RRGKGHDGLYQGLSTATKDTYDALHMQALPPR
SEQ ID NO:26 T2A	GGGEGRGSLLTCGDVEENPGP
SEQ ID NO:27 GM-CSF receptor ss to EGFRt	MLLLVTSLLLCELPHPAFLIPRKVCNGIGIGEFKDSLSINATN IKHFKNCTISGDLHILPVAFRGDSFTHTPPLDPQELDILKTVK EITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGGQFSLAVVS LNITSLGLRSLKEISDGDVHISGNKNLCYANTINWKKLFGTSG QKTKIISNRGENSCKATGQVCHALCSPEGCWGPEPRDCVSC RNVSRGRECVDKCNLLEGEPREFVENSECIQCHPECLPQAM NITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVMGENNTLVW KYADAGHVCHLCHPNCTYGCTGPGLEGCPNGPKIPSIATG MVGALLLLLVVALGIGLFM
SEQ ID NO:28 DHFRdm	MVGSLNCIVAVSQNMGIGKNGDFWPPLRNESRYFQRM TTT SSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVLSRELKEP PQGAHFLSRSLDDALKLTEQPELANKVDMVWIVGGSSVYK EAMNHPGHLKLFVTRIMQDFESDTFFPEIDLEKYKLLPEYPG VLSDVQEEKGIKYKFEVYEKND
SEQ ID NO:29 GM-CSF signal Sequence	MLLLVTSLLLCELPHPAFLIP

[0097] In some embodiments, the one or more nucleic acids described herein comprise a spacer, wherein the spacer is a short spacer having 12 consecutive amino acid residues or less, and not less than 1 amino acid residue. In some embodiments, the one or more nucleic acids described herein encode a spacer, which is a medium spacer having 119 consecutive amino acid residues or less but not less than one amino acid. In some embodiments, the one or more nucleic acids described herein encode a spacer, which is a long spacer having greater than 119 consecutive amino acid residues. In some embodiments, the long spacer has 229 consecutive amino acid residues or less and not less than 1 amino acid. In some embodiments, nucleic acids described herein encode a spacer selected from the group consisting of a short spacer comprising an IgG4 hinge domain, a medium spacer comprising

an IgG4 hinge-CH3 domain, and a long spacer comprising an IgG4 hinge -CH2-CH3 domain. In some embodiments, the nucleic acids described herein encode a spacer, which is a long spacer.

[0098] In some embodiments, the transmembrane domain comprises a CD28 transmembrane domain.

[0099] In some embodiments, the nucleic acids described herein encode an intracellular signaling domain comprises a portion of CD3 zeta and/or a portion of 4-1BB.

[0100] Some embodiments also include a polynucleotide encoding a selectable gene, a cell surface selectable marker, or a cleavable linker. In some embodiments, the selectable gene comprises a dihydrofolate reductase double mutant (DHFRdm). In some embodiments, the cell surface selectable marker is selected from the group consisting of a truncated EGFR (EGFRt), a truncated Her2 (Her2tG), and a truncated CD19 (CD19t). In some embodiments, the cleavable linker comprises a ribosome skip sequence is selected from the group consisting of P2A, T2A, E2A and F2A.

[0101] Some embodiments of the methods and compositions provided herein include vectors comprising any one of the nucleic acids provided herein. In some embodiments, the vector comprises a lentiviral vector.

Certain CARs

[0102] Some embodiments of the methods and compositions provided herein include CARs, which specifically bind to a DNP moiety. In some embodiments, the DNP moiety is associated with a PLE molecule. In some embodiments, the DNP moiety is associated with an antibody or a folate. In some embodiments, the CAR is encoded by the any one or more of the nucleic acids provided herein.

[0103] In some embodiments, the ligand binding domain of the CARs described and used herein comprises an scFv domain comprising a VH sequence, and VL sequence derived from an antibody. In some embodiments, the VH and VL sequences are joined together via a linker. Examples of VH sequences, VL sequences and linkers used with one or more of the CARs described herein are listed in TABLE 2. In some embodiments, the ligand binding domain of a CAR set forth herein comprises an amino acid sequence having at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to the amino acid sequence set

forth in any one of SEQ ID NOs:01-12. In some embodiments, the ligand binding domain of a CAR described herein comprises the amino acid sequence set forth in any one of SEQ ID NOs:01-12. In some embodiments, the ligand binding domain of a CAR set forth herein comprises an amino acid sequence having at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to the amino acid sequence set forth in SEQ ID NOs:01 or 02. In some embodiments, the ligand binding domain of a CAR set forth herein comprises an amino acid sequence having at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to the amino acid sequence set forth in SEQ ID NOs:01, 02, 09, or 10.

[0104] In some embodiments, the CARs described herein comprise a spacer, which is a short spacer having 12 consecutive amino acid residues or less, and not less than 1 amino acid residue. In some embodiments, the CARs described herein comprise a spacer, which is a medium spacer having 119 consecutive amino acid residues or less and not less than 1. In some embodiments, the CARs described herein comprise a spacer, which is a long spacer having greater than 119 consecutive amino acid residues. In some embodiments, the CARs described herein comprise a long spacer having 229 consecutive amino acid residues or less but not less than 1. In some embodiments, the CARs described herein comprise a spacer selected from the group consisting of a short spacer comprising an IgG4 hinge domain, a medium spacer comprising an IgG4 hinge-CH3 domain, and a long spacer comprising an IgG4 hinge -CH2-CH3 domain. In some embodiments, the CARs described herein comprise spacer, which is a long spacer.

[0105] In some embodiments, the CARs described herein comprise a transmembrane domain, which comprises a CD28 transmembrane domain. In some embodiments, the CARs described herein comprise an intracellular signaling domain, which comprises a portion of CD3 zeta and/or a portion of 4-1BB.

[0106] In some embodiments, a DNP moiety is attached to an antibody or an antigen binding fragment thereof displayed on a CAR, which may be presented by a cell, such as a T cell.

[0107] In some embodiments, the DNP moiety is attached to a lipid. In some embodiments, the lipid comprises a polar head group and a hydrophobic group. In some embodiments, the polar head comprises a group selected from choline, phosphatidylcholine, phosphocholine, sphingomyelin, aphosphoethanolamine, a sugar residue, phosphatidyl serine,

phosphatidyl inositol, a piperidine, or a trimethylarseno-ethyl-phosphate. In some embodiments, the hydrophobic group comprises an aliphatic chain or a terpenoid moiety. In some embodiments, the hydrophobic group comprises an ether linkage, wherein the ether linkage is between the polar head group and the aliphatic chain. In some embodiments, the aliphatic chain comprises a C₁₀₋₂₀ alkyl chain. In some embodiments, the lipid is a PLE. An embodiment of a DNP moiety attached to a lipid is depicted in FIG. 1.

Certain cells comprising CARs

[0108] Some embodiments of the methods and compositions provided herein include cells comprising any one of the CARs provided herein and/or comprising any one of the nucleic acids encoding a CAR provided herein.

[0109] In some embodiments, the cell is derived from a CD4⁺ T cell, a CD8⁺ T cell, a precursor T cell, or a hematopoietic stem cell. In some embodiments, the CD8⁺ T cell is a CD8⁺ cytotoxic T lymphocyte cell selected from the group consisting of a naïve CD8⁺ T cell, a central memory CD8⁺ T cell, an effector memory CD8⁺ T cell, and a bulk CD8⁺ T cell. In some embodiments, the central memory CD8⁺ T cell is positive for CD45RO and CD62L. In some embodiments, the CD4⁺ cell is a CD4⁺ helper T lymphocyte cell selected from the group consisting of a naïve CD4⁺ T cell, a central memory CD4⁺ T cell, an effector memory CD4⁺ T cell, and a bulk CD4⁺ T cell. In some embodiments, the naïve CD4⁺ T cell is positive for CD45RA and CD62L, and negative for CD45RO.

[0110] In some embodiments, the cell is *ex vivo*. In some embodiments, the cell is *in vivo*. In some embodiments, the cell is mammalian. In some embodiments, the cell is human.

[0111] Some embodiments of the methods and compositions provided herein include preparing a population of cells comprising one or more of the CARs described herein. In some embodiments, the population of cells comprising a CAR described herein is incorporated into an infusion for administration to a subject in need e.g., a cancer patient. Some embodiments include introducing the nucleic acid of any one or more of the nucleic acids encoding a CAR or component thereof as provided herein. Some embodiments also include culturing the cell under conditions suitable to obtain a population of cells sufficient for an infusion.

Certain therapies

[0112] Some embodiments of the methods and compositions provided herein include methods of treating, inhibiting, or ameliorating a cancer in a subject. Some such methods include administering any one of the anti-DNP CAR T cells provided herein to the subject and optionally selecting said subject to receive such a therapy based on diagnostic or clinical evaluation or both. In some embodiments, the anti-DNP CAR T cell is administered to the subject in combination with a composition comprising a DNP moiety, such as DNP-PLE, or an anti-tumor antigen antibody or antigen binding fragment thereof comprising a DNP moiety.

[0113] In some embodiments, the cell is administered prior to administration of the DNP moiety, such as DNP-PLE, or an anti-tumor antigen antibody or antigen binding fragment thereof comprising a DNP moiety. In some embodiments, the cell is administered subsequent to administration of the DNP moiety, such as DNP-PLE, or an anti-tumor antigen antibody or antigen binding fragment thereof comprising a DNP moiety. In some embodiments, the cell is co-administered with the DNP moiety, such as DNP-PLE or an anti-tumor antigen antibody or antigen binding fragment thereof comprising a DNP moiety.

[0114] In some embodiments, the composition comprising a DNP moiety, such as DNP-PLE is adapted to or configured to target the cancer. Alternatively, in some embodiments, the DNP moiety is attached to an antibody or antigen binding fragment thereof, which specifically binds to the cancer.

[0115] Preferably, the DNP moiety is attached to a lipid. In some embodiments, the lipid comprises a polar head group and a hydrophobic group. In some embodiments, the polar head comprises a group selected from choline, phosphatidylcholine, phosphocholine, sphingomyelin, aphosphoethanolamine, a sugar residue, phosphatidyl serine, phosphatidyl inositol, a piperidine, or a trimethylarseno-ethyl-phosphate. In some embodiments, the hydrophobic group comprises an aliphatic chain or a terpenoid moiety. In some embodiments, the hydrophobic group comprises an ether linkage, wherein the ether linkage is between the polar head group and the aliphatic chain. In some embodiments, the aliphatic chain comprises a C₁₀₋₂₀ alkyl chain. In some embodiments, the lipid is a PLE.

[0116] In some embodiments, the cancer comprises a target cell selected from the group consisting of a breast cancer cell, brain cancer cell, colon cancer cell, renal cancer cell, pancreatic cancer cell, and ovarian cancer cell.

[0117] In some embodiments, the anti-DNP CAR T cell is autologous to the subject. In some embodiments, the subject is mammalian. In some embodiments, the subject is human.

Certain compositions, system and kits

[0118] Some embodiments of the methods and compositions provided herein include compositions comprising: any one of the anti-DNP CARs provided herein, and a DNP moiety attached to a target cell, in which the CAR is specifically bound to the DNP moiety.

[0119] In some embodiments, the DNP moiety is attached to the target cell via an antibody or antigen binding fragment thereof that binds to the target cell.

[0120] In some embodiments, the DNP moiety is attached to a cell surface of the target cell via a lipid integrated into the cell surface. In some embodiments, the lipid comprises a polar head group and a hydrophobic group. In some embodiments, the polar head comprises a group selected from choline, phosphatidylcholine, phosphocholine, sphingomyelin, aphosphoethanolamine, a sugar residue, phosphatidyl serine, phosphatidyl inositol, a piperidine, or a trimethylarseno-ethyl-phosphate. In some embodiments, the hydrophobic group comprises an aliphatic chain or a terpenoid moiety. In some embodiments, the hydrophobic group comprises an ether linkage, wherein the ether linkage is between the polar head group and the aliphatic chain. In some embodiments, the aliphatic chain comprises a C₁₀₋₂₀ alkyl chain. In some embodiments, the lipid is a PLE.

[0121] In some embodiments, the target cell is a cancer cell. In some embodiments, the cancer cell is selected from the group consisting of a breast cancer cell, brain cancer cell, colon cancer cell, renal cancer cell, pancreatic cancer cell, and ovarian cancer cell. In some embodiments, the target cell is *ex vivo*. In some embodiments, the target cell is *in vivo*. In some embodiments, the target cell is mammalian. In some embodiments, the target cell is human.

[0122] Some embodiments of the methods and compositions provided herein include systems and/or kits comprising any one of the nucleic acids encoding an anti-DNP CAR and a composition comprising a DNP moiety. In some embodiments, the DNP moiety

is attached to an antibody or antigen binding fragment thereof. In some embodiments, the DNP moiety is attached to a lipid.

EXAMPLES

Example 1—Anti-DNP antibodies binding to DNP-PLE-labelled cells

[0123] Adenocarcinoma cells, MDA-MB-231, were incubated overnight in the presence of complete media with either 50 nM, 500 nM or 5 μ M DNP-PLE. Integration of DNP-PLE into cell membranes was analyzed by contacting the cells with an anti-DNP Alexa Fluor 488 antibody and subsequent flow cytometry. In control cells, no shift was seen between untreated MDA-MB-231 cells and MDA-MB-231 cells stained with the anti-DNP-Alexa Fluor 488 antibody (FIG. 2A). For cells treated with 5 μ M DNP-PLE, there was a clear shift from the untreated control cells and treated cells (FIG. 2B); a smaller shift was observed between the untreated control cells and cells treated with 50 nM DNP-PLE (FIG. 2D); and an intermediate shift was observed between the untreated control cells and cells treated with 500 nM DNP-PLE (FIG. 2C). A histogram for the data in FIG. 2A-FIG. 2D is shown in FIG. 2E.

[0124] In a further study, MDA-MB-231 cells were incubated overnight with either 1 μ M or 5 μ M DNP-PLE, washed and imaged by confocal microscopy to confirm the location of DNP-PLE integration in the cells. Nuclei were stained with DAPI (i); cell surfaces were stained with wheat germ agglutinin (WGA) (ii); and DNP was stained with anti-DNP Alexa Fluor 488 antibody (iii). FIG. 3A depicts confocal images of control MDA-MB-231 cells incubated with anti-DNP-Alexa Fluor 488 antibody. FIG. 3B depicts confocal images of MDA-MB-231 cells incubated with 5 μ M DNP-PLE. FIG. 3C depicts confocal images of MDA-MB-231 cells incubated with 5 μ M DNP-PLE and stained with anti-DNP-Alexa Fluor 488 antibody. FIG. 3D depicts confocal images of MDA-MB-231 cells incubated with 1 μ M DNP-PLE and stained with anti-DNP-Alexa Fluor 488 antibody. Anti-DNP staining was localized to the cell surfaces (iii), confirming that DNP-PLE integrated at these sites (FIG. 3C, FIG. 3D). This study also confirmed that the DNP moiety was accessible for antibody binding.

Example 2—Generation of anti-DNP CARs

[0125] FIG. 4 shows the components of an example polynucleotide encoding an anti-DNP CAR which includes a leader sequence, a ligand binding domain comprising an anti-

DNP scFv, a spacer, a CD28 transmembrane domain, intracellular co-stimulatory domains including a 41BB domain and a CD3-zeta domain, a P2A sequence, a selectable marker including a DHFRdm, a T2A sequence, and a cell surface selectable marker including an EGFRt.

[0126] A series of polynucleotides encoding different anti-DNP CARs are prepared. Some polynucleotides encode CARs having different spacers, such as a long spacer, a medium spacer, or a short spacer. Some polynucleotides encode CARs having different anti-DNP ligand binding domains, such as different scFv derived from different anti-DNP antibodies (*See e.g.*, Brunger, A.T., *et al.*, (1991) *Journal of Molecular Biology*, 5:239-56 which is incorporated by reference in its entirety). Some ligand binding domains include a VH sequence, and a VL sequence. Some ligand binding domains include a VH sequence, and a VL sequence having a linker there between. TABLE 1 lists a series of CARs that are prepared, and TABLE 2 lists sequences for components of the CARs. For example, a CAR comprising a ligand binding domain for VH-linker-VL of Ab-1 (1BAF) with a long spacer includes a polynucleotide encoding the following amino acid sequences in a NH-COOH orientation: [SEQ ID NO:29, GM-CSF signal Sequence] [SEQ ID NO:01, VH of anti DNP scFv (Ab-1; 1BAF)] [SEQ ID NO:19, linker] [SEQ ID NO:02, VL of anti DNP scFv (Ab-1; 1BAF)] [SEQ ID NO:20, Long spacer: IgG4hinge-CH2(L235D)-CH3] [SEQ ID NO:23, CD28tm] [SEQ ID NO:24, 4-1BB] [SEQ ID NO:25, CD3 zeta] [P2A nucleic acid] [SEQ ID NO:28, DHFRdm] [SEQ ID NO:28, GM-CSF receptor ss to EGFRt].

Example 3—Preparation of anti-DNP CAR T cells

[0127] A polynucleotide encoding an anti-DNP CAR having a long spacer was transduced into H9 cells (CD4⁺ and CD3⁺ cutaneous T lymphocytes). Transduced cells were selected using methotrexate. The efficiency of transduction was determined using flow cytometry to quantify the presence of a EGFRt cell surface marker on transduced cells. Flow plots demonstrated a 92% positive anti-DNP CAR H9 population.

Example 4—Anti-DNP CARs binding to DNP-PLE-labelled cells

[0128] MDA-MB-231 cells were incubated overnight with 5 μ M DNP-PLE, washed, and co-cultured with anti-DNP CAR T cells. Cells were imaged by confocal

microscopy to confirm an interaction between the anti-DNP CAR T cells and DNP-labelled cells. Nuclei were stained with DAPI (i); cell surfaces were stained with wheat germ agglutinin (WGA) (ii); and DNP was stained with anti-DNP Alexa Fluor 488 antibody (iii; and iv). H9 CAR T cells were distinguished from MDA-MB-231 using an anti-CD3 antibody (red). Under each color image is a grey scale for each layer making up the full confocal image: nucleus (i), cell surface (ii), DNP-PLE (iii) and (iv) anti-DNP CAR H9 cells.

[0129] Control unlabeled MDA-MB-231 cells co-cultured with anti-DNP CAR H9 cells are shown in FIG. 5A which shows no binding between MDA-MB-231 and H9 cells. In FIG. 5A, the top left image shows full overlay confocal image of images (i) – (iv). DNP-PLE labelled MDA-MB-231 cells co-cultured with anti-DNP CAR H9 cells are shown in FIG. 5B which shows an interaction between MDA-MB-231 and H9 cells. In FIG. 5B, the top left image shows full overlay confocal image of images (i) – (iv). FIG. 5B showed a synapse formation between the cells, thus confirming recognition of the DNP exposed on the surface of the target cell by the anti-DNP CAR. This study confirmed that an anti-DNP CAR was capable of binding to DNP-labelled cells.

Example 5—*In vitro* activity of anti-DNP CARs

[0130] *In vitro* activity of anti-DNP CARs is measured using a chromium release assay, and a cytokine production assay. *See e.g.*, Gonzalez, S., Naranjo, A., Serrano, L. M., Chang, W.-C., Wright, C. L., & Jensen, M. C. (2004). Genetic engineering of cytolytic T lymphocytes for adoptive T-cell therapy of neuroblastoma. *The Journal of Gene Medicine*, 6(6), 704–711, hereby expressly incorporated by reference in its entirety.

[0131] For the chromium release assay, target cells are incubated with ^{51}Cr overnight. For target cells that receive the DNP-PLE, the DNP-PLE is also present in the media overnight with the ^{51}Cr . The following day the target cells are washed and seeded in a 96 well plate at a concentration 5000 cell per well. CD8+ anti-DNP CAR T cells, and mock T cell effectors (usually in day 8-16 of a rapid expansion protocol) are washed, seeded with the target cells in triplicate at various E:T ratios (30:1, 10:1, 3:1, 1:1), and allowed to co-incubate for 4 hours at 37°C. Also, to evaluate control ^{51}Cr release, each target cell line are seeded with media only and for maximum ^{51}Cr release each target cell line was seeded and lysed with 2% SDS. Control groups are done in sextuplicate. After co-incubation, the supernatant is harvested,

dispensed on LUMA plates, and allowed to dry overnight. The next day samples are run on the Top Count instrument. Percent-specific lysis is calculated by the following formula:

$$\frac{(\text{experimental } ^{51}\text{Cr release}) - (\text{control } ^{51}\text{Cr release})}{(\text{maximum } ^{51}\text{Cr release}) - (\text{control } ^{51}\text{Cr release})} \times 100$$

[0132] In the chromium release assay, the relative levels of lytic activity for various anti-DNP CAR T cells is determined against the DNP-labelled cells. Unlabeled control K562 cells do not induce lysis with the anti-DNP CAR T cells. A positive control includes the use of OKT3 cells which activates T cells through the TCR. Anti-DNP CAR T cells induces specific lysis of DNP-labeled cells.

[0133] A cytokine release assay is performed. For target cells that receive the DNP-PLE, the DNP-PLE are incubated overnight in media. The next day all target cells are harvested, washed, and seeded in a 96 well plate at a concentration of 5×10^4 cells per well. CD8+ anti-DNP CAR T cells and mock T cell effectors (usually in day 8-16 of a rapid expansion protocol) are washed and seeded (1×10^5 cells/well) with the target cells and are co-incubated for 24 hours at 37°C. After 24hr the supernatant is harvested and IFN-gamma, TNF-alpha, and IL-2 concentration in the supernatant are measured by using a Bio-Plex® 200 system (Bio-Rad). The levels of cytokines released by the anti-DNP CAR T cells is determined. DNP-labeled cells induce the release of IFN- γ , IL-2 and TNF- α in contact with the anti-DNP CAR T cells.

Example 6—*In vivo* targeting and integration of DNP-PLE

[0134] *In vivo* targeting and integration of DNP-PLE at tumor sites is tested. After a glioblastoma (U87 cells) tumor is established in a group of mice by intracranial injection, the mice receive an intravenous injection of DNP-PLE. Mice are sacrificed and brains are harvested at various time points post DNP-PLE injection. Specifically, mice having an orthotopic glioma xenograft are dosed intravenously with DNP-PLE, and the brains are evaluated over a period of 14 days. At 48 hr, the brain is prepared for histology. DAPI is also used to stain for the nucleus. An anti-DNP antibody with a fluorescent label is used to stain for availability of DNP-PLE that is integrated into the membrane of cells. The glioma tumor exhibits retention of DNP-PLE in excess compared to a tumor-free contra lateral hemisphere

of a subject. In a fluorescent image, tumor is very bright compared to the normal healthy tissue. This confirms the selective integration of DNP-PLE into tumor membranes with DNP moiety available for binding.

[0135] A similar study is performed in which mice receive flank tumors by injection with either adenocarcinoma cells (MDA-MB-231), or osteosarcoma cells (143B). After tumors are established in the groups of mice by subcutaneous injection, the mice receive an intravenous injection of DNP-PLE. Mice are sacrificed and tumors are harvested at various time points post DNP-PLE injection. The tumors are removed and immediately imaged for the presence of the DNP-PLE using an anti-DNP antibody with a fluorescent label. The three different types of cancer all show specific uptake of DNP-PLE, and a multiday day retention time of DNP-PLE.

Example 7—*In vivo* activity of anti-DNP CARs

[0136] *In vivo* activity of anti-DNP CARs is measured using a xenograft model. A neuroblastoma (Be2) or glioma (U87, U251T, or T98) tumor is established in mice by intracranial injection. Mice receive an intracranial injection of anti-DNP CAR T cells in combination with DNP-PLE. A control group receive an intracranial injection of the anti-DNP CAR T cells only. Mice receiving an intracranial injection of anti-DNP CAR T cells in combination with DNP-PLE have an increased survival rate, decreased tumor burden over time, and/or reduced tumor volume, compared to the control group.

[0137] A similar study is performed in which mice receive flank tumors by injection with an adenocarcinoma cells (MDA-MB-231). After tumors are established in the groups of mice by subcutaneous injection, the mice receive an intravenous injection of DNP-PLE, except for a control group. The mice receive a subsequent IV dose of anti-DNP CAR T cells. The mice that received DNP-PLE have an increased survival rate, decreased tumor burden over time, and/or reduced tumor volume, compared to the control group.

[0138] The term “comprising” as used herein is synonymous with “including,” “containing,” or “characterized by,” and is inclusive or open-ended and does not exclude additional, unrecited elements or method steps.

[0139] The above description discloses several methods and materials of the present invention. This invention is susceptible to modifications in the methods and materials, as well as alterations in the fabrication methods and equipment. Such modifications will become apparent to those skilled in the art from a consideration of this disclosure or practice of the invention disclosed herein. Consequently, it is not intended that this invention be limited to the specific embodiments disclosed herein, but that it cover all modifications and embodiments coming within the true scope and spirit of the invention.

[0140] All references cited herein, including but not limited to published and unpublished applications, patents, and literature references, are incorporated herein by reference in their entirety and are hereby made a part of this specification. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.