

DEATH RECEPTOR 5 (DR5)-ARMORED CAR-T THERAPEUTIC**Cross Reference to Related Applications**

[01] This application claims priority to U.S. Provisional Application No. 63/612,572, filed December 20, 2023, the disclosure of which is incorporated by reference in its entirety.

Reference to Sequence Listing

[02] This application is being filed electronically and includes an electronically submitted sequence listing. The sequence listing is entitled "22-1946-WO_Sequence-Listing.xml" and was created on November 12, 2024, and has a size of 44,873 bytes. The sequence listing contained in this XML file is part of the specification and is herein incorporated by reference in its entirety.

Field of Invention

[03] This disclosure relates to the armoring of T cells with membrane-bound TRAILR2 (DR5) agonists and the treatment of cancer using armored chimeric antigen receptor T cells.

Technical Background

[04] Recent data suggest that CAR-T cells can be efficacious against solid tumors. A disialoganglioside 2 (GD2) CAR natural killer T cell (NKT) therapy has shown activity in neuroblastoma (Heczey *et al.*, "Anti-GD2 CAR-NKT cells in patients with relapsed or refractory neuroblastoma: an interim analysis" *Nature Medicine* 26(11):1686–90 (November 2020)). Additionally, GD2 CAR-T have demonstrated clinical efficacy and a manageable toxicity profile in pediatric neuroblastoma patients (Yu *et al.*, "GD2-specific chimeric antigen receptor-modified T cells for the treatment of refractory and/or recurrent neuroblastoma in pediatric patients" *Journal of Cancer Research and Clinical Oncology* 148(10):2643–52 (October 2022)). CAR-T therapy targeting mesothelin in combination with pembrolizumab demonstrated anti-tumor activity and safety in patients with malignant pleural mesothelioma (Adusumilli *et al.*, "A Phase I Trial of Regional Mesothelin-Targeted CAR T-cell Therapy in Patients with Malignant Pleural Disease, in Combination with the Anti-PD-1 Agent Pembrolizumab" *Cancer Discov*, 11(11):2748-63 (November 2021)). Interim analysis of a phase I clinical trial of CLDN18.2 targeting CAR-Ts demonstrated these CAR-T were well tolerated and had promising antitumor efficacy compared to other therapeutic approaches utilized in the third line setting of gastric cancer (Qi *et al.*, "Claudin18.2-specific CAR T cells in gastrointestinal cancers: phase 1 trial interim results" *Nature Medicine* 28:1189–98 (May 2022)). Other clinical studies are underway evaluating safety and efficacy of CAR-T therapies in a variety of solid tumor indications including several clinical trials of CAR-Ts to

GPC3 (Zheng *et al.*, “Glypican-3: A Novel and Promising Target for the Treatment of Hepatocellular Carcinoma” *Front. Oncol.*, 12:824208 (February 2022)), CLDN6 (Mackensen *et al.*, “BNT211: a phase I/II trial to evaluate safety and efficacy of CLDN6 CAR-T cells and vaccine-mediated in vivo expansion in patients with CLDN6-positive advanced solid tumors” *J Immunother Cancer* 2021;9(Suppl 2): Abstract 958) and PSMA (Narayan *et al.*, “PSMA-targeting TGFβ-insensitive armored CAR T cells in metastatic castration-resistant prostate cancer: a phase 1 trial” *Nat Med* 28(4):724-34 (April 2022)). However, identification of new targets, optimized CAR-T design and manufacturing, are needed to succeed in the solid tumor setting.

[05] CAR T cells and adoptive cell therapy (ACT) have shown promising results in treating hematological malignancies, but their therapeutic efficacy is not similarly echoed in solid tumors. A main challenge is the tumor microenvironment (TME) created by solid tumors, which prevent anti-tumor T cells from functioning properly. The role of myeloid-derived suppressor cells (MDSCs) in the TME has been increasingly appreciated for their association with poor clinical responses to immunotherapies and their abilities to inhibit T cell function. An agonistic antibody targeting TRAILR2 (also known as DR5), a death receptor expressed by MDSCs, has shown clinical benefits in cancer patients by reducing the numbers of MDSCs. However, the reduction in MDSCs was only transient even after repeated dosing of the antibody.

[06] Additional CAR T cell-based therapies are needed to augment the armamentarium of effective cancer treatments especially in solid tumor settings, and these new CAR T cell therapies must be devised that effectively treat cancer while minimizing the risk of developing dangerous inflammatory responses, such as CRS.

SUMMARY

[07] This disclosure describes compositions and methods for using CAR T cells to treat cancer, in particular, the armoring of CAR-T cells with a membrane-bound DR5 agonist. The DR5 agonist can confer more efficacious and sustained anti-tumor immunity, and as shown herein DR5 agonist-armored CAR-T therapy is a more superior therapeutic to DR5 agonistic antibody therapy or unarmored CAR-T therapy for solid cancers.

[08] In an aspect, this disclosure provides a TNF-related apoptosis-inducing ligand receptor 2 (TRAILR2) agonist comprising one or more TRAILR2 binding domains and a transmembrane domain. In some embodiments, the TRAILR2 agonist further comprises a linker, a hinge domain, one or more multimerization domains, and/or one or more

intracellular domains. In some embodiments of the TRAILR2 agonist, the one or more TRAILR2 binding domains comprises one or more sequences selected from SEQ ID NO: 1, 2, 23, 28, or 31-39 a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 1, 2, 23, 28, or 31-39, or any combination thereof. In some embodiments of the TRAILR2 agonist, the TRAILR2 agonist comprises an amino acid sequence having at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence of SEQ ID NO: 3, 12, 19, 22, 25, 27, or 30.

[009] In another aspect, this disclosure provides an isolated nucleic acid sequence encoding a TNF-related apoptosis-inducing ligand receptor 2 (TRAILR2) agonist, wherein the TRAILR2 agonist comprises one or more TRAILR2 binding domains and a transmembrane domain. In certain embodiments, the nucleic acid sequence encodes a TRAILR2 agonist with an amino acid sequence as set forth in SEQ ID NO: 3, 12, 19, 22, 25, 27, or 30. In some embodiments, the isolated nucleic acid sequence is as set forth in SEQ ID NO: 8, 11, 18, 21, 24, 26, or 29. In some embodiments, the isolated nucleic acid sequence further comprises a chimeric antigen receptor (CAR).

[010] In another aspect, this disclosure provides a vector comprising the isolated nucleic acid sequence encoding a TNF-related apoptosis-inducing ligand receptor 2 (TRAILR2) agonist as disclosed herein.

[011] In another aspect, this disclosure provides for a cell comprising the TRAILR2 agonist as disclosed herein, the isolated nucleic acid sequence as disclosed herein, or the vector as disclosed herein.

[012] In another aspect, this disclosure provides methods of treating cancer, comprising administering an effective amount of the cells or cell populations as disclosed herein comprising the the TRAILR2 agonist as disclosed herein.

[013] In another aspect, this disclosure provides a pharmaceutical composition comprising the TRAILR2 agonist as disclosed herein, the isolated nucleic acid as disclosed herein, the vector as disclosed herein, or the cells as disclosed herein, and a pharmaceutically acceptable excipient

[014] In another aspect, this disclosure provides methods of treating a disease or condition in a subject in need thereof, comprising administering to the subject the TRAILR2 agonist as disclosed herein, the isolated nucleic acid as disclosed herein, the vector as disclosed herein, or the cell as disclosed herein, or the pharmaceutical composition as disclosed herein.

[015] In another aspect, this disclosure provides for the use of the TRAILR2 agonist as disclosed herein, the isolated nucleic acid as disclosed herein, the vector as disclosed herein, or the cell as disclosed herein, or the pharmaceutical composition as disclosed herein for the manufacture of a medicament for treating a disease or condition in a subject in need thereof. In certain embodiments the disease is cancer.

[016] In another aspect, this disclosure provides for the use of a TNF-related apoptosis-inducing ligand receptor 2 (TRAILR2) agonist for the manufacture of a medicament for treating cancer in a patient, wherein the TRAILR2 agonist comprises one or more TRAILR2 binding domains and a transmembrane domain. In some embodiments, the TRAILR2 agonist further comprises a linker, a hinge domain, one or more multimerization domains, and/or one or more intracellular domains. In certain embodiments of the use, the one or more TRAILR2 binding domains comprises one or more sequences selected from SEQ ID NO: 1, 2, 23, 28, or 31-39, a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 1, 2, 23, 28, or 31-39, or any combination thereof. In certain embodiments of the use, the TRAILR2 agonist comprises an amino acid sequence having at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence of SEQ ID NO:3, 12, 19, 22, 25, 27, or 30.

[017] In another aspect, this disclosure provides a population of cells for the treatment of cancer in a patient, wherein the population of cells comprises TRAILR2 agonist comprising one or more TRAILR2 binding domains and a transmembrane domain. In certain embodiments of the population of cells, the TRAILR2 agonist further comprises a linker, a hinge domain, one or more multimerization domains, and/or one or more intracellular domains. In certain embodiments of the population of cells, the one or more TRAILR2 binding domains comprises one or more sequences selected from SEQ ID NO: 1, 2, 23, 28, or 31-39, a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 1, 2, 23, 28, or 31-39, or any combination thereof. In certain embodiments of the population of cells, the TRAILR2 agonist comprises an amino acid sequence having at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence of SEQ ID NO:3, 12, 19, 22, 25, 27, or 30.

[018] These and other features and advantages of the present disclosure will be more fully understood from the following detailed description taken together with the accompanying claims. It is noted that the scope of the claims is defined by the recitations therein and not by the specific discussion of features and advantages set forth in the present description.

BRIEF DESCRIPTION OF THE DRAWINGS

[019] The accompanying drawings are included to provide a further understanding of the methods and compositions of the disclosure. The drawings illustrate one or more embodiment(s) of the disclosure, and together with the description serve to explain the principles and operation of the disclosure.

[020] **FIG. 1** shows a schematic for DR5 agonists disclosed herein.

[021] **FIG. 2A – 2C** show that membrane-bound anti-DR5 (α DR5) can be expressed on the surface on T cells without affecting fold expansion or CD4/CD8 ratio. **FIG. 2A** shows representative dot plots showing the expression of α DR5 on primary human T cells transduced with a lentiviral α DR5 construct. α DR5 is detected by incubating the cells with recombinant DR5-Fc, followed by staining with BV421-anti-Fc antibody. UT: untransduced cells. **FIG. 2B** shows the expansion of UT and α DR5 cells over time. **FIG. 2C** shows the CD4/CD8 ratio of UT and α DR5 cells.

[022] **FIG. 3A – 3B** show that membrane-bound anti-DR5 (α DR5) induces death of sensitive targets. **FIG. 3A** shows an xCELLigence assay showing real-time killing of the PLC/PRF/5 tumor cells by indicated treatments. Equal number of T cells was added to the tumor cells (E:T = 1:1). Soluble α DR5 was used at 10 nM. Dotted line indicates the beginning of treatments. **FIG. 3B** shows flow cytometry-based killing assay showing the killing of a TRAIL-sensitive cell line, Colo 205 and a TRAIL-resistant cell line, SNU-398 by indicated treatments on day 3. UT and α DR5 cells were added at E:T = 1:1. Soluble α DR5 was used at 10 nM. The comparison of membrane-bound and soluble DR5 agonist demonstrates that the two modalities have a similar effect on sensitive target cells.

[023] **FIG. 4A – 4B** show that anti-DR5 (α DR5) synergizes with a CAR (anti-GPC3 CAR) to induce death of cells expressing the CAR target (GPC3) and being sensitive to DR5 agonism. **FIG. 4A and 4B** provide xCELLigence assays showing real-time killing of the PLC/PRF/5 parental or DR5 KO tumor cells by indicated treatments. T cells were added at E:T = 1:3. Soluble α DR5 was used at 10 nM. Suboptimal dose of T cells expressing either the CAR or the α DR5 resulted in a partial killing of target cells, that was greatly improved by co-expression of the CAR and α DR5 demonstrating in vitro synergy between the two modalities against the parental target cell line. Target cells knocked out for DR5 were insensitive to α DR5-induced cell death while retaining sensitivity to CAR-induced death, demonstrating the specificity of the α DR5 moiety.

[024] **FIG. 5** shows that DR5 agonist (α DR5) mediated tumor killing does not involve T cell activation. Parental or DR5 KO PLC/PRF/5 tumor cells were treated as described in

FIG. 4. Cell culture supernatant was collected after 24 hours and the concentration of IFN- γ analyzed by MSD. IFN- γ was produced by T cells upon CAR-induced activation and can be used as an indicator of T cell activation. α DR5-induced killing did not result in production of IFN- γ , indicating that expression of the surface agonist is sufficient to induce apoptosis of target cells and does not require T cell activation.

[025] **FIG. 6A – 6B** show *in vivo* synergy between a CAR and a DR5-agonist. PLC/PRF/5 cells were implanted s.c. in the flank of NSG mice, and when the tumor size reached approximately 150 mm³, 0.8E6 CAR-T cells were dosed i.v. and tumor size was measured twice a week. This data demonstrates that a suboptimal dose of GPC3 CAR-T cells partially delayed tumor growth. DR5 agonist (α DR5) expressing T cells were not able to control tumor growth, in line with the fact that the α DR5 does not have an intracellular domain resulting in T cell activation, proliferation, and persistence. In contrast, cells co-expressing the GPC3 CAR and the DR5 agonist (α DR5) were able to efficiently control tumor growth and induced complete regression in 6 out of 10 mice.

[026] **FIG. 7A - 7B** shows that membrane-bound DR5 agonist *in vivo* efficacy is DR5 specific. PLC/PRF/5 cells parental (FIG. 7A) or DR5 KO (FIG. 7B) were implanted s.c. in the flank of NSG mice, and when the tumor size reached approximately 150 mm³, 2E6 CAR-T cells were dosed i.v. and tumor size was measured twice a week. This data demonstrate that DR5 armored CAR T cells provide additional tumor killing over CAR only if DR5 is expressed, demonstrating *in vivo* specificity.

[027] **FIG. 8** shows that membrane-bound DR5 armoring is effective in TGF- β rich environment. The DR5-sensistive cell line HUH7 overexpressing TGF- β cells was implanted s.c. in the flank of NSG mice, and when the tumor size reached approximately 100 mm³, 5E6 CAR-T cells were dosed i.v. and tumor size was measured twice a week. This data demonstrates that unarmored CAR-T cells were not able to efficiently control tumor growth, while cells co-expressing the CAR and the DR5 agonist (α DR5) had an enhanced efficacy compared to unarmored CAR-T cells.

[028] **FIG.9** shows that expression of membrane-bound DR5 agonist results in killing of tumor associated macrophages (TAMs). TAMs were cocultured with UT or membrane-bound DR5 expressing T cells at an effector:target ratio of 3:1. After 3 days the number of viable TAMs was evaluated by flow cytometry. Data show the average number of TAMs recovered as a percentage of the number recovered upon coculture with UT (n=9 TAMs donors).

[029] Skilled artisans will appreciate that elements in the Figures are illustrated for simplicity and clarity and have not necessarily been drawn to scale. For example, the dimensions of some of the elements in the Figures can be exaggerated relative to other elements to help improve understanding of the embodiment(s) of the present disclosure.

DETAILED DESCRIPTION

[030] The disclosure relates to methods, cells, and compositions for preparing cell populations and compositions for immune cell therapy. In particular, provided are methods, cells, and compositions for preparing cell populations comprising a membrane-bound DR5 agonist, and methods of making and use of said populations.

[031] As utilized in accordance with the present disclosure, unless otherwise indicated, all technical and scientific terms shall be understood to have the same meaning as commonly understood by one of ordinary skill in the art. The following references provide one of skill with a general definition of many of the terms used in this disclosure: Singleton, *et al.*, Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger, *et al.* (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991). As used herein, the following terms have the meanings ascribed to them below, unless specified otherwise.

[032] Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[033] As used herein, the terms "comprise" and "include" and variations thereof (e.g., "comprises," "comprising," "includes," and "including") will be understood to indicate the inclusion of a stated component, feature, element, or step or group of components, features, elements or steps but not the exclusion of any other component, feature, element, or step or group of components, features, elements, or steps. Any of the terms "comprising," "consisting essentially of," and "consisting of" may be replaced with either of the other two terms, while retaining their ordinary meanings.

[034] As used herein, the singular forms "a," "an" and "the" include plural referents unless the context clearly indicates otherwise.

[035] As used herein, ranges and amounts can be expressed as "about" a particular value or range. The term "about" also includes the exact amount. For example, "about 5%" means "about 5%" and also "5%." The term "about" can also refer to $\pm 10\%$ of a given value or

range of values. Therefore, about 5% also means 4.5% - 5.5%, for example. Unless otherwise clear from context, all numerical values provided herein are modified by the term “about.”

[036] Percentages disclosed herein can vary in amount by ± 10 , 20, or 30% from values disclosed and remain within the scope of the contemplated disclosure.

[037] Unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values herein that are expressed as ranges can assume any specific value or sub-range within the stated ranges in different embodiments of the disclosure, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

[038] As used herein, the terms “or” and “and/or” can describe multiple components in combination or exclusive of one another. For example, “x, y, and/or z” can refer to “x” alone, “y” alone, “z” alone, “x, y, and z,” “(x and y) or z,” “x or (y and z),” or “x or y or z.”

[039] Units, prefixes, and symbols are denoted in their *Système International de Unites* (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, nucleotide sequences are written left to right in 5' to 3' orientation. Amino acid sequences are written left to right in amino to carboxy orientation. The headings provided herein are not limitations of the various aspects of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

[040] As used herein, the term “polypeptide” refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term “polypeptide” refers to any chain or chains of two or more amino acids. Thus, peptides, dipeptides, tripeptides, oligopeptides, “protein,” “amino acid chain,” or any other term used to refer to a chain or chains of two or more amino acids are included within the definition of “polypeptide,” and the term “polypeptide” can be used instead of, or interchangeably with any of these terms.

[041] A “protein” as used herein can refer to a single polypeptide, i.e., a single amino acid chain as defined above, but can also refer to two or more polypeptides that are associated, e.g., by disulfide bonds, hydrogen bonds, or hydrophobic interactions, to produce a multimeric protein.

[042] An “isolated” substance, e.g., isolated nucleic acid, is a substance that is not in its natural milieu, though it is not necessarily purified. For example, an isolated nucleic acid is a nucleic acid that is not produced or situated in its native or natural environment, such as a

cell. An isolated substance can have been separated, fractionated, or at least partially purified by any suitable technique.

[043] As used herein, the term “polynucleotide” includes a singular nucleic acid as well as multiple nucleic acids, and refers to an isolated nucleic acid molecule or construct, e.g., messenger RNA (mRNA) or plasmid DNA (pDNA). The term “nucleic acid” includes any nucleic acid type, such as DNA or RNA. “Conservative amino acid substitutions” refer to substitutions of an amino acid residue with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine).

[044] The percent identity between two sequences is a function of the number of identical positions shared by the sequences (i.e., % homology = # of identical positions/total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm, as described in the non-limiting examples below.

[045] The percent identity between two nucleotide sequences can be determined using the GAP program in the GCG software package (freely available), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. The percent identity between two nucleotide or amino acid sequences can also be determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4: 11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (J. Mol. Biol. (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package, using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

[046] The nucleic acid and protein sequences described herein can further be used as a “query sequence” to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the NBLAST and XBLAST

programs (version 2.0) of Altschul, *et al.* (1990) J. Mol. Biol. 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, word length = 12 to obtain nucleotide sequences homologous to the nucleic acid molecules described herein. BLAST protein searches can be performed with the XBLAST program, score = 50, word length = 3 to obtain amino acid sequences homologous to the protein molecules described herein. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) Nucleic Acids Res. 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used.

[047] As used herein, the term "vector" can refer to a nucleic acid molecule as introduced into a host cell, thereby producing a transformed host cell. A vector can include nucleic acid sequences that permits it to replicate in a host cell, such as an origin of replication. A vector can also include one or more selectable marker gene(s) and other genetic elements known in the art. Specific types of vector envisioned here can be associated with or incorporated into viruses to facilitate cell transformation. The term "vector," as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid," which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, also included are other forms of expression vectors, such as viral vectors (e.g., lentiviral vectors, replication defective retroviruses, adenoviruses and adeno-associated viruses), or transposons (e.g. DNA transposons or retrotransposons) which serve equivalent functions. In certain embodiments, the CARs and/or antibodies or antigen binding fragments thereof are encompassed by and/or delivered to a cell and or patient using a virus, a lentivirus, an adenovirus, a retrovirus, an

adeno-associated virus (AAV), a transposon, a DNA vector, a mRNA, a lipid nanoparticle (LNP), or a CRISPR-Cas System. In an embodiment, lentiviral vectors are used.

[048] A “transformed” cell, or a “host” cell, is a cell into which a nucleic acid molecule has been introduced by molecular biology techniques. All techniques by which a nucleic acid molecule can be introduced into such a cell, including transfection with viral vectors, transformation with plasmid vectors, and introduction of naked DNA by electroporation, lipofection, and particle gun acceleration are contemplated herein. In certain embodiments, cells are transformed by one or more techniques using a virus, a lentivirus, an adenovirus, a retrovirus, an adeno-associated virus (AAV), a transposon, a DNA vector, a mRNA, a lipid nanoparticle (LNP), and a CRISPR-Cas System.

[049] As used herein, the term “affinity” refers to a measure of the strength of the binding of an antigen or target (such as an epitope) to its cognate binding domain (such as a paratope). As used herein, the term “avidity” refers to the overall stability of the complex between a population of epitopes and paratopes (*i.e.*, antigens and antigen binding domains).

[050] The term “epitope” refers to a site on an antigen to which a chimeric antigen receptor, immunoglobulin, or antibody specifically binds, *e.g.*, as defined by the specific method used to identify it. Epitopes can be formed both from contiguous amino acids (usually a linear epitope) or non-contiguous amino acids juxtaposed by tertiary folding of a protein (usually a conformational epitope). Epitopes formed from contiguous amino acids are typically, but not always, retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in a unique spatial conformation.

[051] In one aspect, this disclosure provides for a TNF-related apoptosis-inducing ligand receptor 2 (TRAILR2) agonist comprising one or more TRAILR2 binding domains and a transmembrane domain.

[052] The TRAILR2 protein is encoded by a member of the TNF-receptor superfamily gene, and contains an intracellular death domain. In some instances, it may also be known as TNFRSF10B; CD262, DR5, KILLER, KILLER/DR5, TRAILR2, TRICK2, TRICK2A, TRICK2B, TRICKB, or ZTNFR9. This receptor can be activated by tumor necrosis factor-related apoptosis inducing ligand (TNFSF 10/TRAIL/APO-2L), and transduces an apoptotic signal.

[053] As used herein, the terms “DR5 agonist” or “ α DR5” refers to a TRAIL receptor 2 (TRAILR2) agonist. The α DR5 agonist comprises an optimized multivalent DR5 agonist that

is potent in triggering cell death in TRAIL-sensitive cell lines. In some embodiments, the DR agonists is expressed on T cells as a membrane-bound, monomeric protein. In some embodiments, the TRAILR2 agonist as disclosed herein comprises one or more TRAILR2 binding domains having the sequence of SEQ ID NO:1, 2, 23, 28, or 31-39. In some embodiments, the TRAILR2 agonist as disclosed herein comprises one or more TRAILR2 binding domains having at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence of SEQ ID NO: 1, 2, 23, 28, or 31-39, or any combination thereof. In some embodiments, the TRAILR2 agonist comprises an amino acid sequence having at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence of SEQ ID NO:3, 12, 19, 22, 25, 27, or 30. In some embodiments, the TRAILR2 agonist comprises a nucleic acid sequence having at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence of SEQ ID NO:8, 11, 18, 21, 24, 26, or 29.

[054] >G6 binding domain sequence

[055] SRLDAPSKIEVKDVTDTTALITWAKPWVDPPLWGIELTYGIKDVPGDRTTIDLQQKHTAYSIGNLKPD
TEYEVSLISFDPYGMRSKPAKITFKTGL (SEQ ID NO:1)

[056] >G6Tz Amino Acid Sequence

[057] MPLLLLLLPLLWAGALASRLDAPSKIEVKDVTDTTALITWAKPWVDPPLWGIELTYGIKDVPGDRTTID
LQQKHTAYSIGNLKPDTEYEVSLISFDPYGMRSKPAKITFKTGLGGSGGTLTTTPAPRPPTPAPTIASQPLSLRP
EACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCRVKFSRSADAPA (SEQ ID NO:3)

[058] > CD33 signal peptide

[059] MPLLLLLLPLLWAGALA (SEQ ID NO:4)

[060] >G4S linker

[061] GGSGG (SEQ ID NO:5)

[062] >CD8 alpha hinge and transmembrane

[063] TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYC
(SEQ ID NO:6)

[064] >CD8 alpha hinge

[065] TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO:15)

[066] >CD8 alpha transmembrane

[067] DIYIWAPLAGTCGVLLLSLVITLYC (SEQ ID NO:40)

[068] >truncated CD3 zeta

[069] RVKFSRSADAPA (SEQ ID NO:7)

[070] >G6Tz DNA Sequence

[071] atgcctttgttgctgctgctgcctcttctttgggctggcgCGCTAGCctccagactcgacgctccgagt
aaaatcgaagttaaagacgttaccgatacaaccgcgttgatcacctgggctaagccatgggtcgatccccacct
ctgtggggcatagaattgacctacggtattaaggatgtgcctggggatcggacgacgatcgatcttcaacagaaa
catactgcgtactctattggcaacctgaaacccgataccgagtatgaagtttcaacttatctcattcgatccgtat
ggtagaggtcaaaacccgcgaaaatcacgtttaagaccggacttggtggaagcggtggaacactcactacgacc
ccgccccagaccgccaactcccgtccaaccatagcatctcagcctttgagtttgcgaccggaagcttgaga
cctgccgcggaggggctgtacacactcgcggtcttgatttcgctgcgacatatatatatgggcgccactcgcc
gggactgtggcggtgttgcttctgagtttggtcattactttgtactgcaggggtgaaattctctcgctcagctgat
gcgccagct (SEQ ID NO:8)

[072] >G6 Monomer CD8a Hinge GPI Anchor DNA Sequence

[073] atgccgctgcttctgctgcttccgctgctgtgggcccggagcgctagcctctcggttgagcgaccatcc
aagattgaggtgaaagacgtgaccgacaccaccgcctgatcacctgggctaagccgtgggtggatccacccct
ctttggggcattgagctaacctacggtatcaaggacgtgccaggggacaggacgactatcgacctgcagcagaag
cacactgcttacagcatcggcaacctgaaacccgacacggagtacgaggtatctctgatctcgtttgatccgtac
ggaatgcgcagcaagcctgccaaagatcacgttcaaaacccggcctgggcggcggggctctaccaccactccggcc
ccacggcccccaacccccccccctaccatcgcttcccagccactgagcctgcgaccgaggcttgctcgccctgcc
gctggggggcgccgtgcacactcgcggcctcgattttgcatgtgatctcgagcccaacaagggttccgggaccacc
agtgggactaccgcctgctgtctggccacacctgcttcacctgaccggcttggtgggcactctggtgactatg
ggcctgctgacg (SEQ ID NO:11)

[074] >G6 Monomer CD8a Hinge GPI Anchor Amino Acid Sequence

[075] MPLLLLLLPLLWAGALASRLDAPSKIIEVKDVTDTTALITWAKPWVDPPLWGIELTYGIKDVPGDRTTID
LQQKHTAYSIGNLKPDTYEYVSLISFDPYGMRSKPAKITFKTGLGGGGSTTTPAPRPPTPAPTIASQPLSLRPEA
CRPAAGGAVHTRGLDFACDLEPNKSGTTSGTTRLLSGHTCFTLTGLLGLVTMGLLT (SEQ ID NO:12)

[076] G6 Monomer CD8a Hinge GPI Anchor Individual Components**[077]** >CD33 Signal Peptide

[078] MPLLLLLLPLLWAGALA (SEQ ID NO:13)

[079] >G6 Monomer

[080] SRLDAPSKIIEVKDVTDTTALITWAKPWVDPPLWGIELTYGIKDVPGDRTTIDLQQKHTAYSIGNLKPDT
TEYEVSLISFDPYGMRSKPAKITFKTGL (SEQ ID NO:2)

[081] >Gly/Ser Linker

[082] GGGGS (SEQ ID NO:14)

[083] >CD8 alpha Hinge

[084] TTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO:15)

[085] >CD55 GPI Anchor

[086] PNKSGTTSGTTRLLSGHTCFTLTGLLGLVTMGLLT (SEQ ID NO:16)

[087] >CD55 GPI Anchor Post-processing

[088] PNKSGTTS- (GPI Anchor) (SEQ ID NO:17)

[089] >G6 Monomer 2xG4S GPI Anchor DNA Sequence

[090] atgccgctgcttctgctgcttccgctgctgtgggccggagcgctagcctctcggttggacgcaccatcc
aagattgaggtgaaagacgtgaccgacaccaccgcctgatcacctgggctaagccgtgggtggatccacccct
ctttggggcattgagctaacctacggtatcaaggacgtgccaggggacaggacgactatcgacctgcagcagaag
cacactgcttacagcatcggcaacctgaaacccgacacggagtacgaggtatctctgatctcgtttgatccgtac
ggaatgctgcagcaagcctgccaaagatcacgttcaaaacccggcctggcgcggggggctcgggcggtggcggatcc
cccaacaagggctccgggaccacttccggaactaccgcctgctgtccggccacacctgctttacactgaccggc
ctcttgggcacctggtgacctgggcctgctgacc (SEQ ID NO:18)

[091] >G6 Monomer 2xG4S GPI Anchor Amino Acid Sequence

[092] MPLLLLLLPLLWAGALASRLDAPSKIIEVKDVTDTTALITWAKPWVDPPLWGIELTYGIKDVPGRDRTTID
LQQKHTAYSIGNLKPDTYEYVSLISFDPYGMRSKPAKITFKTGLGGGSGGGGSPNKSGTTSSTTRLLSGHTCF
TLTGLLGLVTMGLLT (SEQ ID NO:19)

[093] >2xG4S Linker

[094] GGGGSGGGGS (SEQ ID NO:20)

[095] >G6 Tandem 2 CD8a Hinge GPI Anchor DNA Sequence

[096] atgccgctgcttctgctgcttccgctgctgtgggccggagcgctagccagccgtttggacgccccctca
aagattgaggtgaaaggacgtaaccgacaccaccgcctgatcacctgggccaagccgtgggtggaccgcccccg
ctttggggcatcgagctcacatacggcatcaaatgtgtccaggtgatcgaccaccattgacttgcagcagaag
cacactgcgtactccatcggaaatctcaagcccgacaccgagtagcaagtcagcctaatactcgtttgatccctat
ggaatgcgttctaaacctgctaagatcacgttcaaaactggactgggtgggtggcggttctgggggaggcggatcc
tcccgcctcgacgcaccctccaagatagaagttaaggacgtgaccgacaccaccgcctgatcacctgggctaaa
ccgtgggtggacccccccccactttggggcattgagctgacctacggcatcaaggatgtgccccgggaccgcaca
actattgacctgcagcagaagcacacggcctacagcatcggcaacctgaagcccgacacggagtacgaggtgtcg
ctcatctccttcgacccctatggtatgctgctccaagcctgccaaagatcacgttcaagaccggtctggcgcgcg
ggctctaccaccactccggccccacggcccccaacccccgcccctaccatcgcttcccagccactgagcctgcga
cccgaggttgtaggcctgccgctggggcgccgtgcacactcgggcctcgattttgcattgtgatctcgagccc
aacaagggttccgggaccaccagtgaggactaccgcctgctgtctggccacacctgcttcacctgaccggccttg
ttgggcactctggtgactatgggcctgctgacg (SEQ ID NO:21)

[097] >G6 Tandem 2 CD8a Hinge GPI Anchor Amino Acid Sequence

[098] MPLLLLLLPLLWAGALASRLDAPSKIIEVKDVTDTTALITWAKPWVDPPLWGIELTYGIKDVPGRDRTTID
LQQKHTAYSIGNLKPDTYEYVSLISFDPYGMRSKPAKITFKTGLGGGSGGGGSSRLDAPSKIIEVKDVTDTTALI
TWAKPWVDPPLWGIELTYGIKDVPGRDRTTIDLQQKHTAYSIGNLKPDTYEYVSLISFDPYGMRSKPAKITFKTG
LGGGSGTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDLEPNKSGTTSSTTRLLSGHTCFT
LTGLLGLVTMGLLT (SEQ ID NO:22)

[099] >G6 Tandem 2

[0100] SRLDAPSKIEVKDVTDTTALITWAKPWVDPPLWGIELTYGIKDVPGDRTTIDLQQKHTAYSIGNLKPD
TEYEVSLISFDPYGMRSKPAKITFKTGLGGGSGGGSSRLDAPSKIEVKDVTDTTALITWAKPWVDPPLWGIE
LTYGIKDVPGDRTTIDLQQKHTAYSIGNLKPDTEYEVSLISFDPYGMRSKPAKITFKTGL (SEQ ID NO:23)

[0101] >G6 Tandem 2 2xG4S GPI Anchor DNA Sequence

[0102] atgccgctgcttctgctgcttccgctgctgtgtggcgagcgctagcctctcggttggaacgcaccatcc
aagattgaggtgaaagacgtgaccgacaccaccgcctgatcacctgggctaagccgtgggtggatccacccct
ctttggggcattgagctaacctacggtatcaaggacgtgccaggggacaggacgactatcgacctgcagcagaag
cacactgcttacagcatcggcaacctgaaacccgacacggagtacgaggtatctctgatctcgtttgatccgtac
ggaatgcgagcaagcctgccaagatcacgttcaaaacggcctgggcggcggggctcgggcggtggcggtatcc
agccgctggacgctccttccaaatcgaagtgaaggatgtgactgataccaccgcctcatcacctgggcgaag
ccgtgggtcgatcctccccgctatggggcattgagttgacctatggcatcaaggacgtgcccggcgaccgcacc
acaattgacttgcagcagaagcacactgcctactctatcggaatctgaagcccgacaccgagtatgaggtgagc
cttatatccttcgacccgtacggtatgcgttcaaagcctgccaagatcacgttcaagaccggtctgggtggggga
ggaagtgggtgggggtggttcgcccacaagggtccgggaccacttccggaactaccgcctgctgtccggccac
acctgctttacactgaccggcctcttaggcaccctggtgacctgggcctgctgacc (SEQ ID NO:24)

[0103] >G6 Tandem 2 2xG4S GPI Anchor DNA Sequence

[0104] MPLLLLLLPLLWAGALASRLDAPSKIEVKDVTDTTALITWAKPWVDPPLWGIELTYGIKDVPGDRTTID
LQQKHTAYSIGNLKPDTEYEVSLISFDPYGMRSKPAKITFKTGLGGGSGGGSSRLDAPSKIEVKDVTDTTALI
TWAKPWVDPPLWGIELTYGIKDVPGDRTTIDLQQKHTAYSIGNLKPDTEYEVSLISFDPYGMRSKPAKITFKTG
LGGGSGGGSPNKGSGTTSGTTRLLSGHTCFTLTGLLGLVTMGLLT (SEQ ID NO:25)

[0105] >G6 Tandem 3 CD8a Hinge GPI Anchor DNA Sequence

[0106] atgccgctgcttctgctgcttccgctgctgtgtggcgagcgctagcctctcggttggaacgcaccatcc
aagattgaggtgaaagacgtgaccgacaccaccgcctgatcacctgggctaagccgtgggtggatccacccct
ctttggggcattgagctaacctacggtatcaaggacgtgccaggggacaggacgactatcgacctgcagcagaag
cacactgcttacagcatcggcaacctgaaacccgacacggagtacgaggtatctctgatctcgtttgatccgtac
ggaatgcgagcaagcctgccaagatcacgttcaaaacggcctgggcggcggggctcgggcggtggcggtatcc
agccgctggacgctccttccaaatcgaagtgaaggatgtgactgataccaccgcctcatcacctgggcgaag
ccgtgggtcgatcctccccgctatggggcattgagttgacctatggcatcaaggacgtgcccggcgaccgcacc
acaattgacttgcagcagaagcacactgcctactctatcggaatctgaagcccgacaccgagtatgaggtgagc
cttatatccttcgacccgtacggtatgcgttcaaagcctgccaagatcacgttcaagaccggtctgggcggaggc
ggctctggcgaggcggtatccagtcgccttgacgcaccttccaagatagaagtcaaggacgtgacagatactacc
gccctgatcacctgggcaaagccgtgggtggaccgcctccctgtggggcattgagctcacttacggcatcaag
gacgtaccaggagacaggaccaccatcgacctgcagcagaagcacacggcgtactccatcggaacctgaaaccg
gacaccgagtatgaggtgtctctgatttccctcgatccctatggaatgcgctccaagcccgctaagatcacgttc
aagaccggtctgggcggcgggggtctaccaccactccggccccaaggcccccaaccccgccctaccatcgct
tcccagccactgagcctgcgacccgaggcttgtcggcctgccgctggggcgcgctgcacactcgcgccctcgat
tttgcattgtgatctcgagcccaacaagggtccgggaccaccagtgaggactaccgcctgctgtctggccacacc
tgcttaccctgaccggcttgttgggcactctggtgactatgggcctgctgacg (SEQ ID NO:26)

[0107] >G6 Tandem 3 CD8a Hinge GPI Anchor Amino Acid Sequence

[0108] MPLL LLL LPLLWAGALASRLDAPSKIEVKDVTDTTALITWAKPWVDPPLWGIELTYGIKDVPGRDRTID
LQQKHTAYSIGNLKPDTHEYEVSLISFDPYGMRSKPAKITFKTGLGGGGSGGGSSRLDAPSKIEVKDVTDTTALI
TWAKPWVDPPLWGIELTYGIKDVPGRDRTIDLQQKHTAYSIGNLKPDTHEYEVSLISFDPYGMRSKPAKITFKTG
LGGGGSGGGSSRLDAPSKIEVKDVTDTTALITWAKPWVDPPLWGIELTYGIKDVPGRDRTIDLQQKHTAYSIG
NLKPDTHEYEVSLISFDPYGMRSKPAKITFKTGLGGGGSTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT
RGLDFACDLEPNKSGTTS GTTRLLSGHTCTLTGLLGLTVTMGLLT (SEQ ID NO:27)

[0109] >G6 Tandem 3

[0110] SRLDAPSKIEVKDVTDTTALITWAKPWVDPPLWGIELTYGIKDVPGDRTTIDLQQKHTAYSIGNLKPDT
TEYEVSLISFDPYGMRSKPAKITFKTGLGGGGSGGGSSRLDAPSKIEVKDVTDTTALITWAKPWVDPPLWGI
LTYGIKDVPGDRTTIDLQQKHTAYSIGNLKPDTTEYEVSLISFDPYGMRSKPAKITFKTGLGGGGSGGGSSRLDA
PSKIEVKDVTDTTALITWAKPWVDPPLWGIELTYGIKDVPGDRTTIDLQQKHTAYSIGNLKPDTTEYEVSLISF
DPYGMRSKPAKITFKTGL (SEQ ID NO:28)

[0111] >G6 Tandem 3 2xG4S GPI Anchor DNA Sequence

[0112] atgccgctgcttctgctgcttccgctgctgtggcgagcgctagcctctcggttggaacgacaccatcc
aagattgagggtgaaagacgtgaccgacaccacccgacctgatcacctgggctaagccgtgggtggatccacccct
ctttggggcattgagctaacctacggtatcaaggacgtgccaggggacaggacgactatcgacctgcagcagaag
cacactgcttacagcatcggcaacctgaaacccgacacggagtacgaggtatctctgatctcgtttgatccgtac
ggaatgcgacgacaagcctgccaaagatcacgttcaaaacccggcctggggcggggggctcgggcggtggcggatcc
agccgcctggacgctccttccaaaatcgaagtgaaggatgtgactgataccacgcacctcatcacctgggcgaag
ccgtgggtcgatcctccccgctatggggcattgagtgcacctatggcatcaaggacgtgccggcgacccgcacc
acaattgacttgcagcagaagcacactgcctactctatcggcaatctgaagcccgcacccgagtatgaggtgagc
cttatatccttcgaccgtaacggtatgcgttcaaagcctgccaaagatcacgttcaagaccggctcgggcggaggc
ggctctggcggaaggcggtaccagtcgccttgacgcaccttccaagatagaagtcaaggacgtgcagatactacc
gccttgatcacctgggcaaagccgtgggtggaccgcctccctctgggggcattgagctcacttacggcatcaag
gacgtaccaggagacaggaccaccatcgacctgcagcagaagcacacggcgctactccatcggcaacctgaaaccg
gacaccgagtatgaggtgtctctgatttctctcgatccctatggaatgcgctccaagcccgctaagatcacgttc
aagaccggctctggggggcgggcggtatccggcggaaggggtagccccaacaagggtctccgggaccacttccggaact
accgcctgctgtccggccacacctgctttacactgaccggcctcttgggcaccctggtgacctgggcctgctg
acc (SEQ ID NO:29)

[0113] >G6 Tandem 3 2xG4S GPI Anchor Amino Acid Sequence

[0114] MPLLLLLPLLLWAGALASRLDAPSKIEVKDVTDTTALITWAKPWVDPPLWGLIELTYGIKDVPGDRTTID
LQQKHTAYSIGNLKPDETEYEVSLISFDPYGMRSKPAKITFKTGLGGGSGGGGSSRLDAPSKIEVKDVTDTTALI
TWAKPWVDPPLWGLIELTYGIKDVPGDRTTIDLQQKHTAYSIGNLKPDETEYEVSLISFDPYGMRSKPAKITFKTG
LGGGSGGGGSSRLDAPSKIEVKDVTDTTALITWAKPWVDPPLWGLIELTYGIKDVPGDRTTIDLQQKHTAYSIG
NLKPDETEYEVSLISFDPYGMRSKPAKITFKTGLGGGSGGGGSPNKGSGTTSGTTRLLSGHTCFTLTGLLGLT
MGLLT (SEQ ID NO:30)

[0115] In some embodiments, the TRAILR2 binding domain in the constructs as disclosed herein can may be substituted with other known TRAILR2 binding domains or other binding domains targeting TRAILR2 (DR5). Examples of alternative TRAILR2 binding domains are

provided in SEQ ID Nos:31-39. These may be used as monomer or as tandem repeats of 2, 3 etc. as described above for G6. In certain embodiments, where tandem TRAILR2 binding domains are used, individual TRAILR2 binding domains can be connected by a peptide linker, for example G4S linkers.

[0116] As shown in the TRAILR2 binding sequences of SEQ ID Nos:31-39, the term “SS4” refers to a disulfide-stabilized version of the TRAILR2 binding domain. This is in contrast to the disulfide-free version of the TRAILR2 binding domain in the G6 constructs above. In certain embodiments, the TRAILR2 binding domains as disclosed herein may be on a disulfide-stabilized scaffold or on a disulfide-free scaffold.

[0117] >G6 SS4

[0118] SRLDAPSQIEVKDVTDTTALITWAKPWVDPPLWGCELTGKDVPGDRTTIDLQQKHTAYSIGNLKPD
TEYEVSLICFDPYGMRSKPAKETFTTGL (SEQ ID NO:31)

[0119] >G6 D49G

[0120] SRLDAPSKIEVKDVTDTTALITWAKPWVDPPLWGIELTYGKDVPGDRTTIGLQQKHTAYSIGNLKPD
TEYEVSLISFDPYGMRSKPAKITFKTGL (SEQ ID NO:32)

[0121] >G6 SS4 D49G

[0122] SRLDAPSQIEVKDVTDTTALITWAKPWVDPPLWGCELTGKDVPGDRTTIGLQQKHTAYSIGNLKPD
TEYEVSLICFDPYGMRSKPAKETFTTGL (SEQ ID NO:33)

[0123] >F4

[0124] SRLDAPSKIEVKDVTDTTALITWAKPWVDPPLWGIELTYGKDVPGDRTTIDLQQKHTAYSIGNLKPD
TEYEVSLISFDPYGLKSRPAKITFKTGL (SEQ ID NO:34)

[0125] >F4 SS4

[0126] SRLDAPSQIEVKDVTDTTALITWAKPWVDPPLWGCELTGKDVPGDRTTIDLQQKHTAYSIGNLKPD
TEYEVSLICFDPYGLKSRPAKETFTTGL (SEQ ID NO:35)

[0127] >F4mod1

[0128] SRLDAPSKIEVKDVTDTTALITWAKPWVDPPLWGIELTYGKDVPGDRTTIGLQQKHTAYSIGNLKPD
TEYEVSLISFDPYGLKSRPAKITFKTGL (SEQ ID NO:36)

[0129] >F4mod1 SS4

[0130] SRLDAPSQIEVKDVTDTTALITWAKPWVDPPLWGCELTGKDVPGDRTTIGLQQKHTAYSIGNLKPD
TEYEVSLICFDPYGLKSRPAKETFTTGL (SEQ ID NO:37)

[0131] >F4mod12

[0132] SRLDAPSKIEVKDVTDTTALITWAKPWVDPPLWGIELTYGKDVPGDRTTIGLQQKHNQYSIGNLKPD
TEYEVSLISFDPYGLKSRPAKITFKTGL (SEQ ID NO:38)

[0133] >F4mod12 SS4

[0134] SRLDAPSQIEVKDVTDTTALITWAKPWVDPPLWGCELTGDKDVPDRTTIGLQQKHNSYSIGNLKPD
TEYEVSLICFDPYGLKSRPAKETFTTGL (SEQ ID NO:39)

[0135] The term “transmembrane” domain refers to a domain that spans the cell membrane or inserts into a cell membrane. In some embodiments, a transmembrane domain can be between an extracellular portion a protein and an intracellular portion of a protein. In some embodiments, a transmembrane domain has a three-dimensional structure that is thermodynamically stable in a cell membrane. In certain embodiments, a transmembrane domain can range in length from about 15 amino acids to about 30 amino acids. In certain embodiments, a transmembrane domain can comprise an alpha helix, a beta barrel, a beta sheet, a beta helix, or any combination thereof. In certain embodiments, a transmembrane domain can be a single-pass transmembrane domain. In some embodiments, a transmembrane domain can include a hydrophobic alpha (α) helix that spans the cell membrane. In certain embodiments, the transmembrane domain comprises a transmembrane domain selected from the transmembrane domain of CD4, CD8 α , or CD28. In an embodiment, the transmembrane domain comprises a CD8 α transmembrane domain.

[0136] In some embodiments, the TRAILR2 agonist as disclosed herein further comprises a linker, a hinge domain, one or more multimerization domains, and/or one or more intracellular domains.

[0137] The term “linker” as used herein refers to any molecular assembly that joins or connects two or more proteins. The linker can be a molecule whose function is to act as a spacer between domains in the DR agonist molecule, or it can also be a molecule with additional function (i.e., a “functional moiety”). The terms “linked” and “fused” are used interchangeably. These terms refer to the joining together of two or more proteins by whatever means including chemical conjugation or recombinant means.

[0138] In some embodiments, the linker is a polypeptide. The linker should have a length, which is adequate to link two or more proteins in such a way that they assume the correct conformation relative to one another so that they retain the desired activity. In some embodiments, a polypeptide linker comprises 1 to about 1000 amino acids residues, 1 to about 50 amino acid residues, 1 to about 25 amino acid residues, 1 to about 20 amino acid residues, 1 to about 15 amino acid residues, 1 to about 10 amino acid residues, 1 to about 5 amino acid residues, 1 to about 3 amino acid residues. The DR5 agonist as disclosed herein further provides nucleic acids, such as DNA, RNA, or combinations of both, encoding a polypeptide linker sequence. The amino acid residues selected for inclusion in the linker should exhibit properties that do not interfere significantly with the activity or function of the

membrane-bound DR5 agonist. Thus, a polypeptide linker should on the whole not exhibit a charge which would be inconsistent with the activity or function of the membrane-bound DR5 agonist, or interfere with internal folding, or form bonds or other interactions with amino acid residues of the membrane-bound DR5 agonist as disclosed herein.

[0139] In some embodiments, a polypeptide linker possesses conformational flexibility. In some embodiments, the peptide linker is a flexible peptide linker. In some embodiments, the peptide linker comprises (GGSGG)_x (SEQ ID NO:9) or (GGGGS)_x (SEQ ID NO:10), wherein x can be 1, 2, 3, 4, or 5. In certain embodiments, the peptide linker comprises a (G_xS)_y sequence wherein x and y are integers, wherein x = 1, 2, 3 or 4, and wherein y = 1, 2, 3, 4, 5, 6, or 7. In some embodiments, the amino acid sequences of all peptide linkers present in the polypeptide multimer are identical. Alternatively, the amino acid sequences of all peptide linkers present in the polypeptide multimer may be different.

[0140] In some embodiments, the DR5 agonist can comprise a "hinge" domain. In certain embodiments, a hinge domain can comprise about 15-55 amino acids and is flexible, therefore allowing the two protein regions connected by a hinge domain to move independently. In certain embodiments, a hinge domain can comprise about 15 amino acids, about 20 amino acids, about 25 amino acids, about 30 amino acids, about 35 amino acids, about 40 amino acids, about 45 amino acids, about 50 amino acids, or about 55 amino acids. Non-limiting examples of a hinge domain can include immunoglobulin hinge/spacer domains, such as an IgG1 hinge domain, an IgG2 hinge domain, an IgG3 hinge domain, an IgG4 hinge domain, an IgG4P hinge domain (an IgG4 hinge domain comprising a S241P mutation), or a CD8α hinge domain, or a CD28 hinge domain.

[0141] The term "multimerization domain" as used herein, refers to a protein domain that preferentially interacts with another protein domain, directly or indirectly, wherein the interaction of the protein domains efficiently promotes multimerization (i.e., the formation of a dimer, trimer, tetramer, or higher order multimers, which may be a homodimer, heterodimer, homotrimer, heterotrimer, homomultimer, heteromultimer). For example, multimerization may be due to one or more types of molecular forces, including covalent bonds (e.g., disulfide bonds or bridges), ionic bonds, metallic bonds, electrostatic interactions, salt bridges, dipole-dipole forces, hydrogen bonding, Van der Waals forces, hydrophobic interactions, or any combination thereof. A multimer is stable under appropriate conditions (e.g., physiological conditions, in an aqueous solution suitable for expressing, purifying, or storing recombinant or engineered proteins, or under conditions for non-denaturing or non-reducing electrophoresis). Exemplary multimerization domains may

comprise one or more of a coiled-coil domain, a zinc finger motif, a leucine zipper motif, a helix-turn-helix, a helix-loop-helix, and/or combinations thereof.

[0142] The term “intracellular domain” refers to a domain capable of potentiating or modulating the response of immune effector cells (*i.e.*, capable of initiating the response of immune effector cells). In certain embodiments an intracellular domain refers to the combination of a costimulatory domain (e.g. a costimulatory domain from 4-1BB or CD28). Costimulatory domains can include sequences, for example, a costimulatory domain from one or more of CD28, 4-1BB, OX-40, ICOS, CD27, GITR, CD2, IL-2R β and MyD88/CD40. In certain embodiments, an intracellular domain comprises a costimulatory domain selected from CD28, 4-1BB, OX-40, ICOS, CD27, GITR, CD2, IL-2R β and MyD88/CD40. In some embodiments, the intracellular domain, can include variants of a costimulatory domain one or more of CD28, 4-1BB, OX-40, ICOS, CD27, GITR, CD2, IL-2R β and MyD88/CD40. In certain embodiments, a costimulatory domain variant is selected from a costimulatory domain variant of CD28, 4-1BB, OX-40, ICOS, CD27, GITR, CD2, IL-2R β and MyD88/CD40. The choice of intracellular domain influences the phenotype and metabolic signature of cells. For example, a CD28 intracellular domain yields a potent, yet short-lived, effector-like phenotype, with high levels of cytolytic capacity, interleukin-2 (IL-2) secretion, and glycolysis. By contrast, a 4-1BB intracellular domain tends to expand and persist longer *in vivo*, have increased oxidative metabolism, are less prone to exhaustion, and have an increased capacity to generate central memory T cells. In some embodiments, the intracellular domain comprises a costimulatory domain or a portion thereof.

Cells

[0143] In some embodiments, this disclosure provides cell populations comprising a TNF-related apoptosis-inducing ligand receptor 2 (TRAILR2) agonist as disclosed herein. The population of cells comprising a TRAILR2 agonist and/or an isolated nucleic acid sequence encoding a TRAILR2 agonist can comprise a variety of cell types, such as lymphocytes. Particular types of cells that can be used include T cells, Natural Killer (NK) cells, Natural Killer T (NKT) cells, Invariant Natural Killer T (iNKT) cells, alpha beta T cells, gamma delta T cells, viral-specific T (VST) cells, cytotoxic T lymphocytes (CTLs), tumor infiltrating lymphocytes, and regulatory T cells (Tregs). In some embodiments, the cells are autologous. In certain embodiments, the cells are allogeneic. In other embodiments, the cells may be from a genetically similar, but non-identical donor (allogeneic).

[0144] In some embodiments, the population of cells can also include expanded populations, and/or the engineered T cells. In some embodiments, the population of cells can comprise

total T cells, CD4-positive T cells, CD8-positive T cells, regulatory T cells, gamma-delta T cells, mucosal associated invariant T (MAIT) T cells, natural killer (NK) cells, or natural killer T (NKT) cells. T cells are broadly divided into cells expressing CD4 on their surface (also referred to as CD4-positive cells) and cells expressing CD8 on their surface (also referred to as CD8-positive cells). T cells appropriate for use according to the methods provided herein are mononuclear lymphocytes derived from bone marrow (BM), peripheral blood (PB), or cord blood (CB) of a human donor. These cells could be collected directly from BM, PB, or CB or after mobilization or stimulation via administration of growth factors and/or cytokines such as granulocyte-colony stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF) to allogeneic or autologous donors. Those skilled in the art would appreciate that there are many established protocols for isolating peripheral blood mononuclear cells (PBMC) from peripheral blood. Isolation of PBMC can be aided by density-gradient separation protocols, usually employing a density-gradient centrifugation technique using Ficoll®-Hypaque or Histopaque® for separating lymphocytes from other elements in the blood. Preferably, PBMC isolation is performed under sterile conditions. Isolation of PBMC can also use negative selection kits. Alternatively, cell elutriation methods may be employed to separate mononuclear cell populations. In some embodiments, the population of cells are human cells. In certain embodiments, the population of cells are human primary immune cells.

[0145] “Autologous,” as used herein, refers to cells from the same subject. In some embodiments, the cells of the disclosure are autologous.

[0146] “Allogeneic,” as used herein, refers to cells of the same species that differ genetically to the cell in comparison. In some embodiments, the cells of the disclosure are allogeneic.

[0147] “Syngeneic,” as used herein, refers to cells of a different subject that are genetically identical to the cell in comparison. In some embodiments, the cells of the disclosure are syngeneic.

[0148] “Xenogeneic,” as used herein, refers to cells of a different species to the cell in comparison. In some embodiments, the cells of the disclosure are xenogeneic.

[0149] An “immune effector cell,” is any cell of the immune system that has one or more effector functions (e.g., cytotoxic cell killing activity, secretion of cytokines, induction of ADCC and/or CDC). The illustrative immune effector cells contemplated herein are T lymphocytes, in particular cytotoxic T cells (CTLs; CD8+ T cells), TILs, and helper T cells (HTLs; CD4+ T cells).

[0150] “Modified T cells” refer to T cells that have been modified by the introduction of a polynucleotide encoding an engineered CAR contemplated herein. Modified T cells include both genetic and non-genetic modifications (e.g., episomal or extrachromosomal).

[0151] As used herein, the term “genetically engineered” or “genetically modified” refers to the addition of extra genetic material in the form of DNA or RNA into the total genetic material in a cell.

[0152] The terms, “genetically modified cells,” “modified cells,” and, “redirected cells,” are used interchangeably.

[0153] In some embodiments, the cell compositions and methods of this disclosure further include populations of cells comprising a genetically engineered or chimeric antigen receptor. Chimeric antigen receptors (CARs), also known as chimeric T cell receptors, artificial T cell receptors, and chimeric immunoreceptors, are engineered receptors, which graft specificity onto an immune effector cell. In some embodiments, at least about 90%, at least about 95%, or at least about 99% of cells in the population of cells express the CAR. In general, a chimeric antigen receptor is a transmembrane protein having a target-antigen binding domain that is fused via a spacer and a transmembrane domain to a signaling endodomain. When the CAR binds its target antigen, an activating signal is transmitted to the T cell.

[0154] In certain embodiments, a polynucleotide that encodes a chimeric antigen receptor is introduced to the population of cells comprising a TRAILR2 agonist. In one embodiment, a nucleic acid vector encoding the chimeric antigen receptor or genetically engineered receptor is introduced into the population of cells comprising a TRAILR2 agonist whereby the T cells express the chimeric antigen receptor. In certain embodiments, the CAR can bind any target for use in immunotherapy.

[0155] In some embodiments, the DR5 agonist is used to armor a CAR-T cell population. The term “chimeric antigen receptor” or “CAR,” as used herein, refers to an engineered antigen-binding polypeptide, comprising an antigen-binding domain, a transmembrane domain, and one or more intracellular domains (e.g. costimulatory domains). In some embodiments, a CAR can optionally comprise a spacer domain and/or a flexible hinge domain to provide conformational freedom to facilitate binding to the target antigen on the target cell. In some embodiments, a CAR can optionally comprise an armoring domain comprising a nucleic acid sequence encoding an armoring molecule. Expression of a CAR on the surface of a cell, e.g., an immune cell, allows the cell to target and bind a particular antigen. In some embodiments, the CAR is expressed by an immune cell, e.g., a T cell. In some embodiments, the antigen binding domain comprises an Fab, Fab', F(ab')₂, Fd, Fv, single-chain fragment variable

(scFv), single chain antibody, VHH, vNAR, nanobody (single-domain antibody), or any combination thereof. In some embodiments, the transmembrane domain comprises a transmembrane domain selected from the transmembrane domain of CD4, CD8 α , or CD28. In some embodiments, the one or more intracellular domains comprises a costimulatory domain or a portion thereof. In some embodiments, the intracellular domain comprises a costimulatory domain or a portion thereof. In some embodiments, the intracellular domain comprises a costimulatory domain of CD3z or variants thereof. For instance, the CD3z costimulatory domain variants may contain only 1 or 2 functional immunoreceptor tyrosine-based activation motifs (ITAMs) of the three ITAMs present in wild-type CD3z. In some embodiments, the intracellular domain comprises a costimulatory domain selected from the group consisting of a CD3zeta costimulatory domain, CD28 costimulatory domain, a CD27 costimulatory domain, a 4-1BB costimulatory domain, an ICOS costimulatory domain, an OX-40 costimulatory domain, a GITR costimulatory domain, a CD2 costimulatory domain, an IL-2R β costimulatory domain, a MyD88/CD40 costimulatory domain, and any combination thereof. A CAR can further comprise a "hinge" or "spacer" domain. Non-limiting examples of hinge/spacer domains include immunoglobulin hinge/spacer domains, such as an IgG1 hinge domain, and IgG2 hinge domain, an IgG3 hinge domain, an IgG4 hinge domain, an IgG4P hinge domain (an IgG4 hinge domain comprising a S241P mutation), or a CD8a hinge domain, or a CD28 hinge domain.

CAR construct design

[0156] CAR constructs of the present disclosure can have several components, many of which can be selected based upon a desired or refined function of the resultant CAR construct. In addition to an antigen binding domain, CAR constructs can have a spacer domain, a hinge domain, a signal peptide domain, a transmembrane domain, and one or more costimulatory domains. Selection of one component over another (i.e., selection of a specific co-stimulatory domain from one receptor versus a co-stimulatory domain from a different receptor) can influence clinical efficacy and safety profiles.

[0157] *Antigen binding domain:* Antigen binding domains contemplated herein can include antibodies or one or more antigen-binding fragments thereof. In an embodiment, a CAR construct targets any molecule useful in an immunotherapy. In certain embodiments, the antigen binding domain comprises a single chain variable fragment (scFv) containing light and heavy chain variable regions from one or more antibodies specific for molecule useful in an immunotherapy that are either directly linked together or linked together via a flexible linker (e.g., a repeat of G4S having 1, 2, 3 or more repeats).

[0158] *Spacer domain:* A CAR construct can have a spacer domain to provide conformational freedom to facilitate binding to the target antigen on the target cell. The optimal length of a spacer domain may depend on the proximity of the binding epitope to the target cell surface. For example, proximal epitopes can require longer spacers and distal epitopes can require shorter ones. Besides promoting binding of the CAR to the target antigen, achieving an optimal distance between a CAR cell and a cancer cell may also help to sterically occlude large inhibitory molecules from the immunological synapse formed between the CAR cell and the target cancer cell. A CAR can have a long spacer, an intermediate spacer, or a shorter spacer. Long spacers can include a CH2CH3 domain (~220 amino acids) of immunoglobulin G1 (IgG1) or IgG4 (either native or with modifications common in therapeutic antibodies, such as a S228P mutation), whereas the CH3 region can be used on its own to construct an intermediate spacer (~120 amino acids). Shorter spacers can be derived from segments (< 60 amino acids) of CD28, CD8 α , CD3 or CD4. Short spacers can also be derived from the hinge regions of IgG molecules. These hinge regions may be derived from any IgG isotype and may or may not contain mutations common in therapeutic antibodies such as the S228P mutation mentioned above.

[0159] *Hinge domain:* A CAR can also have a hinge domain. The flexible hinge domain is a short peptide fragment that provides conformational freedom to facilitate binding to the target antigen on the tumor cell. It may be used alone or in conjunction with a spacer sequence. The terms “hinge” and “spacer” are often used interchangeably – for example, IgG4 sequences can be considered both “hinge” and “spacer” sequences (i.e., hinge/spacer sequences).

[0160] *Signal peptide:* A CAR construct can further include a sequence comprising a signal peptide. Signal peptides function to prompt a cell to translocate the CAR to the cellular membrane. Examples include an IgG1 heavy chain signal polypeptide, Ig kappa or lambda light chain signal peptides, colony stimulating factor 2 receptor subunit alpha or granulocyte-macrophage colony-stimulating factor receptor subunit alpha (CSF2RA, also known as, GM-CSFR2, GM-CSF-R-alpha, CD116, or CSFR2) signal peptide, a CD8a signal polypeptide, or a CD33 signal peptide.

[0161] *Transmembrane domain:* A CAR construct can further include a sequence comprising a transmembrane domain. The transmembrane domain can include a hydrophobic α helix that spans the cell membrane. The properties of the transmembrane domain have not been as meticulously studied as other aspects of CAR constructs, but they can potentially affect CAR expression and association with endogenous membrane proteins. Transmembrane domains can be derived, for example, from CD4, CD8 α , or CD28.

[0162] *Costimulatory domain:* A CAR construct can further include one or more sequences that form a co-stimulatory domain. A co-stimulatory domain is a domain capable of potentiating or modulating the response of immune effector cells. Co-stimulatory domains can include sequences, for example, from one or more of CD3zeta (or CD3z), CD28, 4-1BB, OX-40, ICOS, CD27, GITR, CD2, IL-2R β and MyD88/CD40. The choice of co-stimulatory domain influences the phenotype and metabolic signature of CAR cells. For example, CD28 co-stimulation yields a potent, yet short-lived, effector-like phenotype, with high levels of cytolytic capacity, interleukin-2 (IL-2) secretion, and glycolysis. By contrast, T cells modified with CARs bearing 4-1BB costimulatory domains tend to expand and persist longer in vivo, have increased oxidative metabolism, are less prone to exhaustion, and have an increased capacity to generate central memory T cells.

Methods of Treatment

[0163] Cells comprising the DR5 agonist as disclosed herein may be administered alone or as a pharmaceutical composition with a diluent and/or other components associated with cytokines or cell populations. Briefly, pharmaceutical compositions of the disclosure can include, for example cells comprising the DR5 agonist described herein (with or without a CAR), with one or more pharmaceutically or physiologically acceptable carrier, diluent, or excipient. Such compositions can comprise buffers such as neutral buffered saline, buffered saline, and the like; sulfates; carbohydrates such as glucose, mannose, sucrose, or dextrans, mannitol; proteins, polypeptides, or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); and preservatives. The pharmaceutical compositions of the disclosure may be adapted to the treatment (or prophylaxis).

[0164] In some embodiments, the present disclosure provides a method of treatment that include administering to a subject in need thereof an effective amount of the DR5 agonist as disclosed herein. As used herein, an "effective amount" of the the DR5 agonist as disclosed herein (or a pharmaceutical formulation), refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

[0165] In some embodiments, the population of cells as described herein are used in methods of treating a cancer in a subject in need thereof. In some embodiments, the cancer is selected from bladder cancer, bone cancer, brain cancer, breast cancer, bronchial cancer, cervical cancer, colon cancer, colorectal cancer, endometrial cancer, esophageal cancer, gallbladder cancer, head and neck cancer, kidney cancer, liver cancer, lung cancer, melanoma, mesothelioma, nasal cavity cancer, nasopharyngeal cancer, neuroblastoma, non-small cell

lung cancer, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, small cell lung cancer, small intestine cancer, soft tissue sarcoma, stomach cancer, testicular cancer, throat cancer, thyroid cancer, uterine cancer, and metastatic forms thereof. However, the cancers listed herein are not intended to be limiting.

[0166] "Immunotherapy" refers to the treatment of a subject afflicted with, or at risk of contracting or suffering a recurrence of, a disease by a method comprising inducing, enhancing, suppressing or otherwise modifying the immune system or an immune response.

[0167] An "immune response" is as understood in the art, and generally refers to a biological response within a vertebrate against foreign agents or abnormal, e.g., cancerous cells, which response protects the organism against these agents and diseases caused by them. An immune response is mediated by the action of one or more cells of the immune system (for example, a T lymphocyte, B lymphocyte, natural killer (NK) cell, macrophage, eosinophil, mast cell, dendritic cell or neutrophil) and soluble macromolecules produced by any of these cells or the liver (including antibodies, cytokines, and complement) that results in selective targeting, binding to, damage to, destruction of, and/or elimination from the vertebrate's body of invading pathogens, cells or tissues infected with pathogens, cancerous or other abnormal cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues. An immune reaction includes, e.g., activation or inhibition of a T cell, e.g., an effector T cell, a Th cell, a CD4⁺ cell, a CD8⁺ T cell, or a Treg cell, or activation or inhibition of any other cell of the immune system, e.g., NK cell.

[0168] The terms "treatment" or "treat," as used herein, refer to both therapeutic treatment and prophylactic or preventative measures, and when used in the context of treating cancer can refer to reducing disease pathology, reducing or eliminating disease symptoms, promoting increased survival rates, and/or reducing discomfort. For example, treating can refer to the ability of a therapy when administered to a subject, to reduce disease symptoms, signs, or causes. Treating also refers to mitigating or decreasing at least one clinical symptom and/or inhibition or delay in the progression of the condition and/or prevention or delay of the onset of a disease or illness. Those in need of treatment include subjects having cancer as well as those prone to having cancer or those in cancer is to be prevented. In some embodiments, the methods, compositions, and combinations disclosed herein can be used for the treatment of cancer. In other embodiments, those in need of treatment include subjects having a tumor as well as those prone to have a tumor or those in which a tumor is to be prevented. In certain embodiments, the methods, compositions, and combinations disclosed herein can be used for the treatment of tumors. In certain embodiments, the methods,

compositions, and combinations disclosed herein can be used for the treatment of solid tumors. In other embodiments, treatment of a tumor includes inhibiting tumor growth, promoting tumor reduction, or both inhibiting tumor growth and promoting tumor reduction.

[0169] In some embodiments, the populations of cells provided herein can be administered as a pharmaceutical composition comprising a therapeutically effective amount of population of cells as a therapeutic agent (i.e., for therapeutic applications).

[0170] As used herein, the term an “effective amount” or a “therapeutically effective amount” of an administered therapeutic substance, such as a CAR T cell comprising the DR5 agonist disclosed herein, is an amount sufficient to carry out a specifically stated or intended purpose, such as treating or treatment of cancer. An “effective amount” can be determined empirically in a routine manner in relation to the stated purpose.

[0171] In some embodiments, the cells comprising a DR5 agonist as provided herein are CAR-modified cells of, such as CAR T cells, and may be administered alone or as a pharmaceutical composition with a diluent and/or other components associated with cytokines or cell populations. Briefly, pharmaceutical compositions of the disclosure can include, for example CAR T cells comprising a membrane-bound DR5 agonist as described herein, with one or more pharmaceutically or physiologically acceptable carrier, diluent, or excipient. Such compositions can comprise buffers such as neutral buffered saline, buffered saline, and the like; sulfates; carbohydrates such as glucose, mannose, sucrose, or dextrans, mannitol; proteins, polypeptides, or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); and preservatives. The pharmaceutical compositions of the disclosure may be adapted to the treatment (or prophylaxis).

[0172] The terms “pharmaceutical composition” or “therapeutic composition,” as used herein, refer to a compound or composition capable of inducing a desired therapeutic effect when properly administered to a subject. In some embodiments, the disclosure provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of the population of cells of the disclosure.

[0173] The terms “pharmaceutically acceptable carrier” or “physiologically acceptable carrier,” as used herein, refer to one or more formulation materials suitable for accomplishing or enhancing the delivery of one or more populations of cells the disclosure.

[0174] The term “subject” is intended to include human and non-human animals, particularly mammals. In certain embodiments, the subject is a human patient.

[0175] The terms "administration" or "administering," as used herein, refer to providing, contacting, and/or delivering a compound or compounds by any appropriate route to achieve the desired effect. Any acceptable route of administration is contemplated, such as, without limitation, administration intravenous (e.g., intravenous infusion), parenteral, or subcutaneous routes of administration. Administration may include, but is not limited to, oral, sublingual, parenteral (e.g., intravenous, subcutaneous, intracutaneous, intramuscular, intraarticular, intraarterial, intrasynovial, intrasternal, intrathecal, intralesional, or intracranial injection), transdermal, topical, buccal, rectal, vaginal, nasal, ophthalmic, via inhalation, and implants.

[0176] In a particular embodiment, the methods of treating as disclosed herein can also include one or more therapeutic components, such as an anticancer antibody and/or a chemotherapeutic component. For example, it is contemplated that a treatment regimen can additionally include additional therapeutics (e.g., chemotherapies and/or biologics). Contemplated additional therapeutics can include without limitation: cisplatin/gemcitabine or methotrexate, vinblastine, ADRIAMYCIN™ (doxorubicin), cisplatin (MVAC), carboplatin-based regimen, or single-agent taxane or gemcitabine, temozolomide, or dacarbazine, vinflunine, docetaxel, paclitaxel, nab-paclitaxel, Vemurafenib, Erlotinib, Afatinib, Cetuximab, Bevacizumab, Erlotinib, Gefitinib, and/or Pemetrexed. Further examples include drugs targeting DNA damage repair systems, such as poly (ADP-ribose) polymerase 1 (PARP1) inhibitors and therapeutics inhibiting WEE1 protein kinase activity, ATR protein kinase activity, ATM protein kinase activity, Aurora B protein kinase activity, and DNA-PK activity. Additional therapeutic options may also include, but are not limited to: 1) combination regimens such as: AD (doxorubicin, dacarbazine); AIM (doxorubicin, ifosfamide, mesna); MAID (mesna, doxorubicin, ifosfamide, dacarbazine); ifosfamide, epirubicin, mesna; gemcitabine and docetaxel; gemcitabine and vinorelbine; gemcitabine and dacarbazine; doxorubicin and olaratumab; methotrexate and vinblastine; tamoxifen and sulindac; vincristine, dactinomycin, cyclophosphamide; vincristine, doxorubicin, cyclophosphamide; vincristine, doxorubicin, cyclophosphamide with ifosfamide and etoposide; vincristine, doxorubicin, ifosfamide; cyclophosphamide topotecan; or ifosfamide, doxorubicin; and/or 2) single agents, such as: cisplatin or other metallic compounds, 5-FU/capecitabine (Xeloda®), cetuximab (Erbix®), cemiplimab (Libtayo®), pembrolizumab (MK-3475), panitumumab (Vectibix®), dacomitinib (PF-00299804), gefitinib (ZD1839, Iressa), doxorubicin, ifosfamide, epirubicin, gemcitabine, dacarbazine, temozolomide, vinorelbine, eribulin, trabectedin, pazopanib, imatinib, sunitinib, regorafenib, sorafenib, nilotinib, dasatinib, interferon, toremifene, methotrexate, irinotecan, topotecan, paclitaxel,

nab-paclitaxel (abraxane), docetaxel, bevacizumab, temozolomide, sirolimus (Rapamune®), everolimus, temsirolimus, crizotinib, ceritinib, or palbociclib. Examples of additional immunotherapies can include, for example, MEDI-0680, durvalumab (Imfinzi®; MEDI-4736), pembrolizumab (Keytruda®) and nivolumab (Opdivo®), cemiplimab (Libtayo®), atezolizumab (Tecentriq®), and avelumab (Bavencio®). CTLA-4 inhibitors (for example, ipilimumab (Yervoy®)) are another class of drugs that can boost the immune response. In some instances, cytokine therapy (such as, interferon-alpha and interleukin-2) can be used to boost the immune system. Examples of interferon and interleukin-based treatments can include, but are not limited to, aldesleukin (proleukin®), interferon alpha-2b (INTRON®), and PEGylated interferon alpha-2b (Sylvatron®; PEG-INTRON®, PEGASYS).

[0177] In some embodiments, this disclosure provides a method for treating cancer comprising: (a) obtaining a population of cells from a donor; (b) genetically modifying the population of cells to express the DR5 agonist as disclosed herein; (c) expanding the genetically modified population of cells; and (d) administering the expanded genetically modified population of cells to a patient. In some embodiments, the method for treating cancer further comprises administering CAR-modified cells of, such as CAR T cells to the patient.

[0178] The term “expanding” in the method of the disclosure refers to the process of increasing the number of cells in a cell culture. In the expanding step, cells are fed and culture media is replaced at regular intervals, in one embodiment according to a feed regimen. The specific timings and amounts of media added in a particular feed regimen will depend on the cell number and the levels of metabolites in the culture.

[0179] As used herein, the term “proliferation” refers to an increase in cell division, either symmetric or asymmetric division of cells. In particular embodiments, “proliferation” refers to the symmetric or asymmetric division of T cells. “Increased proliferation” occurs when there is an increase in the number of cells in a treated sample compared to cells in a non-treated sample.

[0180] In particular embodiments, the genetically modified population of cells undergoes at least about a 50-fold expansion, at least about a 500-fold expansion, at least about a 5000-fold expansion, at least about a 250,000-fold expansion, at least about a 500,000-fold expansion, at least about a 10^6 fold expansion, at least about a 10^7 fold expansion, at least about a 10^8 fold expansion, at least about a 10^9 fold expansion, or at least about a 10^{10} fold expansion during culturing. In particular embodiments, the population of expanded cells is resistant to replicative senescence. Furthermore, these cells are not functionally exhausted

following long-term expansion and can be directed to carry out cytotoxic function through engagement of their TCRs by a T cell engager antibody or through engagement of a chimeric antigen receptor (CAR), or through a natural or genetically-introduced TCR.

[0181] In some embodiments, the population of cells is cultured in a culture medium that includes supportive cytokine(s), but does not include a primary immune cell stimulus. In certain embodiments, the primary immune cells undergo expansion during culturing in the absence of feeder cells or stimulation through CD3 and/or their antigen receptor. The ability of the disclosed methods to generate immune cells in the absence of extensive T cell re-stimulation or feeder cells advantageously eliminates the issues of scaling up the methods and producing dysfunctional populations of immune cells.

[0182] In some embodiments, the methods disclosed herein provide populations of expanded cells (including human CD8⁺ T cells, human CD4⁺ T cells, or human natural killer T cells) comprising a membrane-bound DR5 agonist that have the ability to proliferate for substantial periods of time in the absence of re-stimulation through their T cell receptors (TCRs), expanding millions of fold in long-term culture. In particular embodiments, the population of cells is cultured for at least 5 days, at least 10 days, at least 15 days, at least 20 days, at least 30 days, at least 40 days, at least 50 days, at least 60 days, at least 70 days, at least 80 days, at least 90 days, at least 100 days, at least 150 days, at least 200 days, at least 300 days, or at least 400 days.

[0183] The term “stimulation” refers to a primary response induced by binding of a stimulatory molecule (e.g., a TCR/CD3 complex) with its cognate ligand thereby mediating a signal transduction event including, but not limited to, signal transduction via the TCR/CD3 complex.

[0184] A “stimulatory molecule,” refers to a molecule on a T cell that specifically binds with a cognate stimulatory ligand.

[0185] A “stimulatory ligand,” as used herein, means a ligand that when present on an antigen presenting cell (e.g., an APC, a dendritic cell, a B-cell, and the like) can specifically bind with a cognate binding partner (referred to herein as a “stimulatory molecule”) on a T cell, thereby mediating a primary response by the T cell, including, but not limited to, activation, initiation of an immune response, proliferation, and the like. Stimulatory ligands include, but are not limited to CD3 ligands, e.g., an anti-CD3 antibody and CD2 ligands, e.g., anti-CD2 antibody, and peptides, e.g., CMV, HPV, EBV peptides.

[0186] The term, “activation” refers to the state of a T cell that has been sufficiently stimulated to induce detectable cellular proliferation. In particular embodiments, activation

can also be associated with induced cytokine production, and detectable effector functions. The term “activated T cells” refers to, among other things, T cells that are proliferating. Signals generated through the TCR alone are insufficient for full activation of the T cell and one or more secondary or costimulatory signals are also required. Thus, T cell activation comprises a primary stimulation signal through the TCR/CD3 complex and one or more secondary costimulatory signals. Costimulation can be evidenced by proliferation and/or cytokine production by T cells that have received a primary activation signal, such as stimulation through the CD3/TCR complex or through CD2.

[0187] A “costimulatory signal,” refers to a signal, which in combination with a primary signal, such as TCR/CD3 ligation, leads to T cell proliferation, cytokine production, and/or upregulation or downregulation of particular molecules (e.g., CD28).

[0188] A “costimulatory ligand,” refers to a molecule that binds a costimulatory molecule. A costimulatory ligand may be soluble or provided on a surface. A “costimulatory molecule” refers to the cognate binding partner on a T cell that specifically binds with a costimulatory ligand (e.g., anti-CD28 antibody).

[0189] As used herein, the terms “individual” and “subject” are often used interchangeably and refer to any animal that exhibits a symptom of a cancer that can be treated with the gene therapy vectors, cell-based therapeutics, and methods disclosed elsewhere herein. Suitable subjects (e.g., patients) include laboratory animals (such as mouse, rat, rabbit, or guinea pig), farm animals, and domestic animals or pets (such as a cat or dog). Non-human primates and, preferably, human patients, are included. Typical subjects include human patients that have a cancer, have been diagnosed with a cancer, or are at risk or having a cancer.

[0190] By “enhance” or “promote,” or “increase” or “expand” refers generally to the ability of a composition contemplated herein to produce, elicit, or cause a greater physiological response (i.e., downstream effects) compared to the response caused by either vehicle or a control molecule/composition. A measurable physiological response may include an increase in T cell expansion, activation, persistence, and/or an increase in cancer cell death killing ability, among others apparent from the understanding in the art and the description herein. An “increased” or “enhanced” amount is typically a “statistically significant” amount, and may include an increase that is 1.1, 1.2, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 or more times (e.g., 500, 1000 times) (including all integers and decimal points in between and above 1, e.g., 1.5, 1.6, 1.7, 1.8, etc.) the response produced by vehicle or a control composition.

[0191] By “decrease” or “lower,” or “lessen,” or “reduce,” or “abate” refers generally to the ability of composition contemplated herein to produce, elicit, or cause a lesser physiological

response (i.e., downstream effects) compared to the response caused by either vehicle or a control molecule/composition. A “decrease” or “reduced” amount is typically a “statistically significant” amount, and may include an decrease that is 1.1, 1.2, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 or more times (e.g., 500, 1000 times) (including all integers and decimal points in between and above 1, e.g., 1.5, 1.6, 1.7, 1.8, etc.) the response (reference response) produced by vehicle, a control composition, or the response in a particular cell lineage.

[0192] By “maintain,” or “preserve,” or “maintenance,” or “no change,” or “no substantial change,” or “no substantial decrease” refers generally to the ability of a composition contemplated herein to produce, elicit, or cause a lesser physiological response (i.e., downstream effects) in a cell, as compared to the response caused by either vehicle, a control molecule/composition, or the response in a particular cell lineage. A comparable response is one that is not significantly different or measurable different from the reference response.

Armoring

[0193] In some embodiments, an “armored” CAR T cell refers to a CAR construct comprising one or more armoring domains encoding one or more armoring molecules and/or an independent construct comprising one or more armoring domains encoding one or more armoring molecules (e.g. such that the transformed cell expresses the CAR protein along with the one or more armoring molecules, such as a membrane-bound DR5 agonist to modulate the cytokine milieu of the tissue microenvironment). An “armoring molecule” refers to a protein that counters immunosuppression of a cell in a tumor microenvironment when expressed on a surface of the cell or when excreted in a tumor microenvironment and can provide many additional benefits not described herein that allow for T cell survival in the immunosuppressive tumor microenvironment (TME). In some embodiments, expression of the armoring molecule can be inducible or constitutive. In some embodiments, the armoring molecule is expressed on the surface of the cell. In some embodiments, the armoring molecule is secreted outside of the cell to armor CAR T cells. Expression of the armoring molecule on the cell surface and/or excretion to the TME can improve efficacy and persistence for the CAR T cells. In certain embodiments, certain genes encoding armoring molecules can be knocked out or their expression effectively eliminated (for example, using CRISPR) in order to improve efficacy and persistence for the CAR T cells in the TME. In this context, such CAR T cells are also referred to as “armored CAR T cells”. The armoring molecule may be selected based on the tumor microenvironment and other elements of the innate and adaptive immune systems. In certain embodiments, the armoring molecule is selected from a DR5 agonist, a dominant negative TGFβ receptor type II, IL-7, IL-12, IL-15,

IL-18, a hybrid IL-4/IL-7 receptor, hybrid IL-7/IL-2 receptor, and HIF1 α dominant-negative. Furthermore, investigators reported modifying CAR-T cells to secrete PD-1-blocking single-chain variable fragments (scFv), which improved CAR-T cell anti-tumor activity in mouse models of PD-L1+ hematologic and solid tumors (Rafiq *et al.* Targeted delivery of a PD-1-blocking scFv by CAR-T cells enhances anti-tumor efficacy *in vivo*. Nat Biotechnol 36, 847–856 (2018)). In some embodiments, the armoring molecule comprises the membrane-bound DR5 agonist as disclosed herein. In certain embodiments, the armoring molecule comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1, 2, 23, 28, or 31-39. In some embodiments, the armoring molecule comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence set forth in SEQ ID NO: 3, 12, 19, 22, 25, 27, or 30.

Activation and Expansion of T Cells

[0194] Whether prior to or after genetic modification of the T cells to express a desirable CAR, the T cells can be activated and expanded generally using methods as described, for example, in U.S. Pat. Nos. 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; 6,867,041; and U.S. Patent Application Publication No. 20060121005.

[0195] Generally, the T cells of the disclosure are expanded by contact with a surface having attached thereto an agent that stimulates a CD3/TCR complex associated signal and a ligand that stimulates a costimulatory molecule on the surface of the T cells. In particular, T cell populations may be stimulated as described herein, such as by contact with an anti-CD3 antibody, or antigen-binding fragment thereof, or an anti-CD2 antibody immobilized on a surface, or by contact with a protein kinase C activator (e.g., bryostatin) in conjunction with a calcium ionophore. For costimulation of an accessory molecule on the surface of the T cells, a ligand that binds the accessory molecule is used. For example, a population of T cells can be contacted with an anti-CD3 antibody and an anti-CD28 antibody, under conditions appropriate for stimulating proliferation of the T cells. To stimulate proliferation of either CD4+ T cells or CD8+ T cells, an anti-CD3 antibody and an anti-CD28 antibody. Examples of an anti-CD28 antibody include 9.3, B-T3, XR-CD28 (Diaclone, Besangon, France) can be

used as can other methods commonly known in the art (Berg *et al.*, Transplant Proc. 30(8):3975-3977, 1998; Haanen *et al.*, J. Exp. Med. 190(9):1319-1328, 1999; Garland *et al.*, J. Immunol Meth. 227(1-2):53-63, 1999).

[0196] In certain embodiments, the primary stimulatory signal and the costimulatory signal for the T cell may be provided by different protocols. For example, the agents providing each signal may be in solution or coupled to a surface. When coupled to a surface, the agents may be coupled to the same surface (i.e., in “cis” formation) or to separate surfaces (i.e., in “trans” formation). Alternatively, one agent may be coupled to a surface and the other agent in solution. In one embodiment, the agent providing the costimulatory signal is bound to a cell surface and the agent providing the primary activation signal is in solution or coupled to a surface. In certain embodiments, both agents can be in solution. In another embodiment, the agents may be in soluble form, and then cross-linked to a surface, such as a cell expressing Fc receptors or an antibody or other binding agent which will bind to the agents. In this regard, see for example, U.S. Patent Application Publication Nos. 20040101519 and 20060034810 for artificial antigen presenting cells (aAPCs) that are contemplated for use in activating and expanding T cells in the present disclosure.

[0197] In one embodiment, the two agents are immobilized on beads, either on the same bead, i.e., “cis,” or to separate beads, i.e., “trans.” By way of example, the agent providing the primary activation signal is an anti-CD3 antibody or an antigen-binding fragment thereof and the agent providing the costimulatory signal is an anti-CD28 antibody or antigen-binding fragment thereof, and both agents are co-immobilized to the same bead in equivalent molecular amounts. In one embodiment, a 1:1 ratio of each antibody bound to the beads for CD4⁺ T cell expansion and T cell growth is used. In certain embodiments of the present disclosure, a ratio of anti CD3:CD28 antibodies bound to the beads is used such that an increase in T cell expansion is observed as compared to the expansion observed using a ratio of 1:1.

[0198] In further embodiments of the present disclosure, the cells, such as T cells, are combined with agent-coated beads, the beads and the cells are subsequently separated, and then the cells are cultured. In an alternative embodiment, prior to culture, the agent-coated beads and cells are not separated but are cultured together. In a further embodiment, the beads and cells are first concentrated by application of a force, such as a magnetic force, resulting in increased ligation of cell surface markers, thereby inducing cell stimulation.

[0199] Conditions appropriate for T cell culture include an appropriate media (e.g., Minimal Essential Media or RPMI Media 1640 or, X-vivo 15, (Lonza)) that may contain factors

necessary for proliferation and viability, including serum (e.g., fetal bovine or human serum), interleukin-2 (IL-2), IL-21, insulin, IFN- γ , IL-4, IL-7, GM-CSF, IL-10, IL-12, IL-15, TGF β , and TNF- α or any other additives for the growth of cells known to the skilled artisan. Other additives for the growth of cells include, but are not limited to, surfactant, plasmanate, and reducing agents such as N-acetyl-cysteine and 2-mercaptoethanol. Media can include RPMI 1640, AIM-V, DMEM, MEM, α -MEM, F-12, X-Vivo 15, and X-Vivo 20, Optimizer, with added amino acids, sodium pyruvate, and vitamins, either serum-free or supplemented with an appropriate amount of serum (or plasma) or a defined set of hormones, and/or an amount of cytokine(s) sufficient for the growth and expansion of T cells. Antibiotics, e.g., penicillin and streptomycin, are included only in experimental cultures, not in cultures of cells that are to be infused into a subject. The target cells are maintained under conditions necessary to support growth, for example, an appropriate temperature (e.g., 37°C) and atmosphere (e.g., air plus 5% CO₂). In one embodiment, the media is X-VIVO 15 serum-free media containing 1% (v/v) recombinant serum replacement (ITSE-A).

[0200] In one embodiment, the T cells are cultured in media containing between 10 and 300 IU/mL of recombinant human IL-2. In one embodiment, the T cells are cultured in media containing 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, or 300 IU/mL of recombinant human IL-2. In another embodiment, the T cells are cultured in media also containing between 0.1 and 0.3 U/mL of recombinant IL-21. In another embodiment, the T cells are cultured in media containing IL-2 and 0.1, 0.2, 0.5, 1, 2, 5, 10, 15, 20, 25, 30, 40, 50, 75, or 100 U/mL of recombinant human IL-21. In another embodiment, the T cells are culture in media containing IL-2 and 0.10, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19, 0.20, 0.21, 0.22, 0.23, 0.24, 0.25, 0.26, 0.27, 0.28, 0.29, or 0.30 U/mL of recombinant human IL-21. In one embodiment, the T cells are cultured in a media containing 40 IU/mL of recombinant human IL-2 and 0.24 U/mL of recombinant human IL-21.

[0201] In one embodiment of the present disclosure, the cells cultured for up to 14 days. In another embodiment, the mixture may be cultured for 4 days. The T cells can be agitated during any stage of culture. In one embodiment, the cells are agitated during cell culture in media containing IL-2 and IL-21. In certain embodiments, the T cells harvested on day 4 exhibit higher target independent killing activity compared to CAR-T cells harvested on day 6.

Vectors, Host Cells, and Pharmaceutical Compositions of the Disclosure

[0202] In some embodiments, the isolated polynucleotides of the present disclosure are present in a vector. As such, provided herein are vectors comprising the polynucleotides of the present disclosure. In some embodiments, the present disclosure is directed to a vector or a set of vectors comprising a polynucleotide encoding a membrane-bound DR5 agonist, as described herein. In other embodiments, the present disclosure is directed to a vector or a set of vectors comprising a polynucleotide encoding a membrane-bound DR5 agonist as disclosed herein and a CAR.

[0203] In some embodiments, the set of vectors comprises a first vector and a second vector, wherein the first vector comprises a nucleic acid sequence encoding a membrane-bound DR5 agonist disclosed herein, and the second vector comprises a nucleic acid sequence encoding a CAR. In other embodiments, the vector comprises both a nucleic acid sequence encoding a membrane-bound DR5 agonist as disclosed herein and a CAR.

[0204] Any vector known in the art can be suitable for the present disclosure. In some embodiments, the vector is a viral vector. In some embodiments, the vector is a retroviral vector, a DNA vector, a murine leukemia virus vector, an SFG vector, a plasmid, a RNA vector, an adenoviral vector, a baculoviral vector, an Epstein Barr viral vector, a papovaviral vector, a vaccinia viral vector, a herpes simplex viral vector, an adenovirus associated vector (AAV), a lentiviral vector, transposon, or any combination thereof. In certain embodiments, the membrane-bound DR5 agonist is encompassed by and/or delivered to a cell and or patient using a virus, a lentivirus, an adenovirus, a retrovirus, an adeno-associated virus (AAV), a transposon, a DNA vector, a mRNA, a lipid nanoparticle (LNP), or a CRISPR-Cas System.

[0205] In other embodiments, provided herein are host cells comprising a polynucleotide or a vector of the present disclosure. In some embodiments, the present disclosure is directed to host cells, e.g., *in vitro* cells, comprising a polynucleotide encoding a CAR or a TCR, as described herein. In some embodiments, the present disclosure is directed to host cells, e.g., *in vitro* cells, comprising a polynucleotide encoding a membrane-bound DR5 agonist, as disclosed herein. In other embodiments, the present disclosure is directed to *in vitro* cells comprising a membrane-bound DR5 agonist. In other embodiments, the present disclosure is directed to cells, *in vitro* cells, comprising a polypeptide encoded by a polynucleotide encoding a membrane-bound DR5 agonist and a CAR.

[0206] Any cell can be used as a host cell for the polynucleotides, the vectors, or the polypeptides of the present disclosure. In some embodiments, the cell can be a prokaryotic cell, fungal cell, yeast cell, or higher eukaryotic cells such as a mammalian cell. Suitable

prokaryotic cells include, without limitation, eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *Escherichia*, e.g., *E. coli*; *Enterobacter*; *Erwinia*; *Klebsiella*; *Proteus*; *Salmonella*, e.g., *Salmonella typhimurium*; *Serratia*, e.g., *Serratia Marcescens*, and *Shigella*; *Bacilli* such as *B. subtilis* and *B. licheniformis*; *Pseudomonas* such as *P. aeruginosa*; and *Streptomyces*. In some embodiments, the cell is a human cell.

[0207] Other embodiments of the present disclosure are directed to compositions comprising a polynucleotide described herein, a vector described herein, a polypeptide described herein, or cell described herein. In some embodiments, the composition comprises a pharmaceutically acceptable carrier, diluent, solubilizer, emulsifier, preservative and/or adjuvant. In some embodiments, the composition comprises an excipient. In one embodiment, the composition comprises a polynucleotide encoding a membrane-bound DR5 agonist. In another embodiment, the composition comprises a membrane-bound DR5 agonist encoded by a polynucleotide of the present disclosure, and wherein the polynucleotide further comprises a CAR. In another embodiment, the composition comprises a T cell comprising a polynucleotide encoding a membrane-bound DR5 agonist. In another embodiment, the composition comprises a cell (e.g., a T cell, e.g., a CAR-T cell) comprising a polynucleotide encoding a membrane-bound DR5 agonist as disclosed herein.

[0208] In other embodiments, the composition is formulated for parenteral delivery, for inhalation, or for delivery through the digestive tract, such as orally. The preparation of such pharmaceutically acceptable compositions is within the ability of one skilled in the art. In certain embodiments, buffers are used to maintain the composition at physiological pH or at a slightly lower pH, typically within a pH range of from about 5 to about 8. In certain embodiments, when parenteral administration is contemplated, the composition is in the form of a pyrogen-free, parenterally acceptable aqueous solution with or without additional therapeutic agents, in a pharmaceutically acceptable vehicle. In certain embodiments, the vehicle for parenteral injection is sterile distilled water with or without at least one additional therapeutic agent, is formulated as a sterile, isotonic solution, properly preserved. In certain embodiments, the preparation involves the formulation of the desired molecule with polymeric compounds (such as polylactic acid or polyglycolic acid), beads or liposomes, that provide for the controlled or sustained release of the product, which are then be delivered via a depot injection. In certain embodiments, implantable drug delivery devices are used to introduce the desired molecule.

Embodiments

[0209] In some embodiments, the present disclosure provides:

[0210] Embodiment 1. A TNF-related apoptosis-inducing ligand receptor 2 (TRAILR2) agonist comprising one or more TRAILR2 binding domains and a transmembrane domain.

[0211] Embodiment 2. The TRAILR2 agonist of embodiment 1, wherein the TRAILR2 agonist further comprises a linker, a hinge domain, one or more multimerization domains, and/or one or more intracellular domains.

[0212] Embodiment 3. The TRAILR2 agonist of either embodiment 1 or embodiment 2, wherein the one or more TRAILR2 binding domains comprises one or more sequences selected from SEQ ID NO:1, 2, 23, 28, or 31-39 a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 1, 2, 23, 28, or 31-39, or any combination thereof.

[0213] Embodiment 4. The TRAILR2 agonist of any one of embodiments 1 to 3, wherein the transmembrane domain comprises a CD8 α transmembrane domain, a CD4 transmembrane domain, or a CD28 transmembrane domain.

[0214] Embodiment 5. The TRAILR2 agonist of embodiment 4, wherein the transmembrane domain comprises a CD8 α transmembrane domain.

[0215] Embodiment 6. The TRAILR2 agonist of any one of embodiments 2 to 5, wherein the one or more multimerization domains comprises one or more of a coiled-coil motif, a zinc finger motif, a leucine zipper motif, a helix-turn-helix, a helix-loop-helix, and/or combinations thereof.

[0216] Embodiment 7. The TRAILR2 agonist of any one of embodiments 2 to 6, wherein the one or more intracellular domains comprises a 4-1BB domain, CD3-zeta domain, or a CD28 intracellular domain.

[0217] Embodiment 8. The TRAILR2 agonist of any one of embodiments 2 to 7, wherein the linker comprises (GGSGG) x (SEQ ID NO:9) or (GGGGS) x (SEQ ID NO:10), wherein x can be 1, 2, 3, 4, or 5.

[0218] Embodiment 9. The TRAILR2 agonist of any one of embodiments 2 to 8, wherein the hinge domain comprises a CD8 α hinge domain, a CD28 hinge domain, an IgG1 hinge domain, an IgG2 hinge domain, an IgG3 hinge domain, or an IgG4 hinge domain.

[0219] Embodiment 10. The TRAILR2 agonist of any one of embodiments 2 to 9, wherein the linker is located between the TRAILR2 binding domain and the transmembrane domain.

[0220] Embodiment 11. The TRAILR2 agonist of any one of embodiments 1 to 10, wherein the TRAILR2 agonist comprises an amino acid sequence having at least about 90%,

at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence of SEQ ID NO:3, 12, 19, 22, 25, 27, or 30.

[0221] Embodiment 12. An isolated nucleic acid sequence encoding a TNF-related apoptosis-inducing ligand receptor 2 (TRAILR2) agonist, wherein the TRAILR2 agonist comprises one or more TRAILR2 binding domains and a transmembrane domain.

[0222] Embodiment 13. The isolated nucleic acid sequence of embodiment 12, wherein the TRAILR2 agonist further comprises a linker, a hinge domain, one or more multimerization domains, and/or one or more intracellular domains.

[0223] Embodiment 14. The isolated nucleic acid sequence of either embodiment 12 or embodiment 13, wherein the one or more TRAILR2 binding domains comprises one or more sequences selected from SEQ ID NO: 1, 2, 23, 28, or 31-39, a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 1, 2, 23, 28, or 31-39, or any combination thereof.

[0224] Embodiment 15. The isolated nucleic acid sequence of any one of embodiments 12 to 14, wherein the transmembrane domain comprises a CD8 α transmembrane domain, a CD4 transmembrane domain, or a CD28 transmembrane domain.

[0225] Embodiment 16. The isolated nucleic acid sequence of embodiment 15, wherein the transmembrane domain comprises a CD8 α transmembrane domain.

[0226] Embodiment 17. The isolated nucleic acid sequence of any one of embodiments 12 to 16, wherein the one or more multimerization domains comprises one or more of a coiled-coil motif, a zinc finger motif, a leucine zipper motif, a helix-turn-helix, a helix-loop-helix, and/or combinations thereof

[0227] Embodiment 18. The isolated nucleic acid sequence of any one of embodiments 12 to 17, wherein the one or more intracellular domains comprises a 4-1BB domain, CD3-zeta domain, or a CD28 intracellular domain.

[0228] Embodiment 19. The isolated nucleic acid sequence of embodiment 18, wherein the one or more transmembrane domains comprises a 4-1BB domain.

[0229] Embodiment 20. The isolated nucleic acid sequence of any one of embodiments 13 to 19, wherein the linker comprises (GGSGG) x (SEQ ID NO:9) or (GGGGS) x (SEQ ID NO:10), wherein x can be 1, 2, 3, 4, or 5.

[0230] Embodiment 21. The isolated nucleic acid sequence of any one of embodiments 13 to 20, wherein the hinge domain comprises a CD8 α hinge domain, a CD28 hinge domain,

an IgG1 hinge domain, an IgG2 hinge domain, an IgG3 hinge domain, or an IgG4 hinge domain.

[0231] Embodiment 22. The isolated nucleic acid sequence of any one of embodiments 13 to 20, wherein the linker is located between the TRAILR2 binding domain and the transmembrane domain.

[0232] Embodiment 23. The isolated nucleic acid sequence of any one of embodiments 12 to 23, wherein the nucleic acid sequence encodes a TRAILR2 agonist with an amino acid sequence as set forth in SEQ ID NO: 3, 12, 19, 22, 25, 27, or 30, optionally wherein the nucleic acid sequence is as set forth in SEQ ID NO: 8, 11, 18, 21, 24, 26, or 29.

[0233] Embodiment 24. The isolated nucleic acid sequence of any one of embodiments 12 to 23 further comprising a CD33 signal peptide, and/or an inducible promotor, and/or a constitutive promotor.

[0234] Embodiment 25. The isolated nucleic acid sequence of any one of embodiments 12 to 24 further comprising a chimeric antigen receptor (CAR).

[0235] Embodiment 26. The isolated nucleic acid sequence of embodiment 25, wherein the TRAILR2 agonist and the CAR are operably linked under the control of a single promoter.

[0236] Embodiment 27. The isolated nucleic acid sequence of embodiment 25, wherein the TRAILR2 agonist and the CAR are operably linked by an internal ribosome entry site (IRES).

[0237] Embodiment 28. The isolated nucleic acid sequence of embodiment 25, wherein the TRAILR2 agonist and the CAR are linked by a nucleotide sequence encoding a cleavable peptide.

[0238] Embodiment 29. The isolated nucleic acid sequence of embodiment 28, wherein the cleavable peptide is a self-cleaving peptide (for example, T2A).

[0239] Embodiment 30. The isolated nucleic acid sequence of any one of embodiments 25 to 29, wherein the CAR comprises an antigen binding domain, a transmembrane domain, and one or more intracellular domains.

[0240] Embodiment 31. A vector comprising the isolated nucleic acid sequence of any one of embodiments 12 to 30.

[0241] Embodiment 32. The vector of embodiment 31, wherein the vector is a virus, a lentivirus, an adenovirus, a retrovirus, an adeno-associated virus (AAV), a transposon, a DNA vector, a mRNA, a lipid nanoparticle (LNP), or a CRISPR-Cas System.

- [0242] Embodiment 33. The vector of either embodiment 31 or embodiment 32, wherein the vector is a lentivirus.
- [0243] Embodiment 34. A cell comprising the TRAILR2 agonist of any one of embodiments 1 to 11, the isolated nucleic acid sequence of any one of embodiments 12 to 30, or the vector of any one of embodiments 31 to 33.
- [0244] Embodiment 35. The cell of embodiment 34, wherein the cell is an autologous cell or an allogenic cell.
- [0245] Embodiment 36. The cell of either embodiment 34 or embodiment 35, wherein the cell is selected from T cells, Natural Killer (NK) cells, Natural Killer T (NKT) cells, Invariant Natural Killer T (iNKT) cells, alpha beta T cells, gamma delta T cells, viral-specific T (VST) cells, cytotoxic T lymphocytes (CTLs), regulatory T cells (Tregs), and tumor infiltrating lymphocytes.
- [0246] Embodiment 37. The cell of any one of embodiments 34 to 36, wherein the population of cells comprise total T cells.
- [0247] Embodiment 38. The cell of any one of embodiments 34 to 36, wherein the population of cells comprise CD8+ T cells.
- [0248] Embodiment 39. The cell of any one of embodiments 34 to 36, wherein the population of cells comprise CD4+ T cells.
- [0249] Embodiment 40. The cell of any one of embodiments 34 to 39, wherein the TRAILR2 agonist is expressed on the cell surface.
- [0250] Embodiment 41. The cell of any one of embodiments 34 to 40, wherein the cell exhibits an anti-tumor activity.
- [0251] Embodiment 42. A method of treating cancer, comprising administering an effective amount of the cell of any one of embodiments 34 to 41 to a patient in need thereof.
- [0252] Embodiment 43. A method of treating cancer, comprising: administering to a subject in need thereof an effective amount of a cell comprising a TRAILR2 agonist, wherein the TRAILR2 agonist comprises one or more TRAILR2 binding domains and a transmembrane domain.
- [0253] Embodiment 44. The method of embodiment 43, wherein the TRAILR2 agonist further comprises a linker, a hinge domain, one or more multimerization domains, and/or one or more intracellular domains.
- [0254] Embodiment 45. The method of either embodiment 43 or embodiment 44, wherein the one or more TRAILR2 binding domains comprises one or more sequences

selected from SEQ ID NO: 1, 2, 23, 28, or 31-39, a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 1, 2, 23, 28, or 31-39, or any combination thereof.

[0255] Embodiment 46. The method of any one of embodiments 43 to 45, wherein the transmembrane domain comprises a CD8 α transmembrane domain, a CD4 transmembrane domain, or a CD28 transmembrane domain.

[0256] Embodiment 47. The method of embodiment 46, wherein the transmembrane domain comprises a CD8 α transmembrane domain.

[0257] Embodiment 48. The method of any one of embodiments 43 to 47, wherein the one or more multimerization domains comprises one or more of a coiled-coil motif, a zinc finger motif, a leucine zipper motif, a helix-turn-helix, a helix-loop-helix, and/or combinations thereof.

[0258] Embodiment 49. The method of any one of embodiments 43 to 48, wherein the one or more intracellular domains comprises a 4-1BB domain, CD3-zeta domain, or a CD28 intracellular domain.

[0259] Embodiment 50. The method of any one of embodiments 43 to 49, wherein the linker comprises (GGSGG) $_x$ (SEQ ID NO:9) or (GGGGS) $_x$ (SEQ ID NO:10), wherein x can be 1, 2, 3, 4, or 5.

[0260] Embodiment 51. The method of any one of embodiments 43 to 50, wherein the hinge domain comprises a CD8 α hinge domain, a CD28 hinge domain, an IgG1 hinge domain, an IgG2 hinge domain, an IgG3 hinge domain, or an IgG4 hinge domain..

[0261] Embodiment 52. The method of any one of embodiments 43 to 51, wherein the linker is located between the TRAILR2 binding domain and the transmembrane domain.

[0262] Embodiment 53. The method of any one of embodiments 43 to 52, wherein the cell further comprises a chimeric antigen receptor (CAR).

[0263] Embodiment 54. The method of any one of embodiments 43 to 53, wherein the TRAILR2 agonist comprises an amino acid sequence having at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence of SEQ ID NO:3, 12, 19, 22, 25, 27, or 30.

[0264] Embodiment 55. The method of any one of embodiments 43 to 54, wherein the cell is an autologous cell or an allogenic cell.

[0265] Embodiment 56. The method of any one of embodiments 43 to 55, wherein the cell is selected from a T cells, Natural Killer (NK) cells, Natural Killer T (NKT) cells, Invariant Natural Killer T (iNKT) cells, alpha beta T cells, gamma delta T cells, viral-specific

T (VST) cells, cytotoxic T lymphocytes (CTLs), regulatory T cells (Tregs), and tumor infiltrating lymphocytes.

[0266] Embodiment 57. The method of any one of embodiments 43 to 56, wherein the population of cells comprise total T cells.

[0267] Embodiment 58. The method of any one of embodiments 43 to 56, wherein the population of cells comprise CD8⁺ T cells.

[0268] Embodiment 59. The method of any one of embodiments 43 to 56, wherein the population of cells comprise CD4⁺ T cells.

[0269] Embodiment 60. The method of any one of embodiments 43 to 59, wherein the TRAILR2 agonist is expressed on the cell surface.

[0270] Embodiment 61. The method of any one of embodiments 43 to 60, wherein the cell exhibits an anti-tumor activity.

[0271] Embodiment 62. The method any one of embodiments 43 to 61 further comprising inhibiting tumor growth, inducing tumor regression, and/or prolonging survival of the subject.

[0272] Embodiment 63. The method of any one of embodiments 43 to 62, wherein the cancer is a solid tumor.

[0273] Embodiment 64. The method of embodiment 63, wherein the solid tumor is bladder cancer, bone cancer, brain cancer, breast cancer, bronchial cancer, cervical cancer, colon cancer, colorectal cancer, endometrial cancer, esophageal cancer, gallbladder cancer, head and neck cancer, kidney cancer, liver cancer, lung cancer, melanoma, mesothelioma, nasal cavity cancer, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, small cell lung cancer, small intestine cancer, soft tissue sarcoma, stomach cancer, testicular cancer, throat cancer, thyroid cancer, uterine cancer, and metastatic forms thereof.

[0274] Embodiment 65. A pharmaceutical composition comprising the TRAILR2 agonist of any one of embodiments 1 to 11, the isolated nucleic acid of any one of embodiments 12 to 30, the vector of any one of embodiments 31 to 33, or the cell of any one of embodiments 34 to 41, and a pharmaceutically acceptable excipient.

[0275] Embodiment 66. A method of treating a disease or condition in a subject in need thereof, comprising administering to the subject the TRAILR2 agonist of any one of embodiments 1 to 11, the isolated nucleic acid of any one of embodiments 12 to 30, the

vector of any one of embodiments 31 to 33, or the cell of any one of embodiments 34 to 41, or the pharmaceutical composition of embodiment 65.

[0276] Embodiment 67. The method of embodiment 61, wherein the disease or condition comprises a cancer.

[0277] Embodiment 68. A method of treating a cancer in a subject in need thereof, comprising administering to the subject the TRAILR2 agonist of any one of embodiments 1 to 11, the isolated nucleic acid of any one of embodiments 12 to 30, the vector of any one of embodiments 31 to 33, or the cell of any one of embodiments 34 to 41, or the pharmaceutical composition of embodiment 65.

[0278] Embodiment 69. The method of either embodiment 62 or 63, wherein the cancer is bladder cancer, bone cancer, brain cancer, breast cancer, bronchial cancer, cervical cancer, colon cancer, colorectal cancer, endometrial cancer, esophageal cancer, gallbladder cancer, head and neck cancer, kidney cancer, liver cancer, lung cancer, melanoma, mesothelioma, nasal cavity cancer, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, small cell lung cancer, small intestine cancer, soft tissue sarcoma, stomach cancer, testicular cancer, throat cancer, thyroid cancer, uterine cancer, and metastatic forms thereof.

[0279] Embodiment 70. Use of the TRAILR2 agonist of any one of embodiments 1 to 11, the isolated nucleic acid of any one of embodiments 12 to 30, the vector of any one of embodiments 31 to 33, or the cell of any one of embodiments 34 to 41, or the pharmaceutical composition of embodiment 65 for the manufacture of a medicament for treating a disease or condition in a subject in need thereof.

[0280] Embodiment 71. The use of embodiment 70, wherein the disease or condition comprises a cancer.

[0281] Embodiment 72. Use of the TRAILR2 agonist of any one of embodiments 1 to 11, the isolated nucleic acid of any one of embodiments 12 to 30, the vector of any one of embodiments 31 to 33, or the cell of any one of embodiments 34 to 41, or the pharmaceutical composition of embodiment 65 for the manufacture of a medicament for treating cancer in a subject in need thereof.

[0282] Embodiment 73. The use of either embodiment 71 or 72, wherein the cancer is bladder cancer, bone cancer, brain cancer, breast cancer, bronchial cancer, cervical cancer, colon cancer, colorectal cancer, endometrial cancer, esophageal cancer, gallbladder cancer, head and neck cancer, kidney cancer, liver cancer, lung cancer, melanoma, mesothelioma,

nasal cavity cancer, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, small cell lung cancer, small intestine cancer, soft tissue sarcoma, stomach cancer, testicular cancer, throat cancer, thyroid cancer, uterine cancer, and metastatic forms thereof.

[0283] Embodiment 74. The use of a TNF-related apoptosis-inducing ligand receptor 2 (TRAILR2) agonist for the manufacture of a medicament for treating cancer in a patient, wherein the TRAILR2 agonist comprises one or more TRAILR2 binding domains and a transmembrane domain.

[0284] Embodiment 75. The use of embodiment 74, wherein the TRAILR2 agonist further comprises a linker, a hinge domain, one or more multimerization domains, and/or one or more intracellular domains.

[0285] Embodiment 76. The use of either embodiment 74 or embodiment 75, wherein the one or more TRAILR2 binding domains comprises one or more sequences selected from SEQ ID NO: 1, 2, 23, 28, or 31-39, a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 1, 2, 23, 28, or 31-39, or any combination thereof.

[0286] Embodiment 77. The use of any one of embodiments 74 to 76, wherein the transmembrane domain comprises a CD8 α transmembrane domain, a CD4 transmembrane domain, or a CD28 transmembrane domain.

[0287] Embodiment 78. The use of embodiment 77, wherein the transmembrane domain comprises a CD8 α transmembrane domain.

[0288] Embodiment 79. The use of any one of embodiments 75 to 78, wherein the one or more multimerization domains comprises one or more of a coiled-coil motif, a zinc finger motif, a leucine zipper motif, a helix-turn-helix, a helix-loop-helix, and/or combinations thereof.

[0289] Embodiment 80. The method of any one of embodiments 75 to 79, wherein the one or more intracellular domains comprises a 4-1BB domain, CD3-zeta domain, or a CD28 intracellular domain.

[0290] Embodiment 81. The use of any one of embodiments 75 to 80, wherein the linker comprises (GGSGG) $_x$ (SEQ ID NO:9) or (GGGGS) $_x$ (SEQ ID NO:10), wherein x can be 1, 2, 3, 4, or 5.

[0291] Embodiment 82. The use of any one of embodiments 75 to 81, wherein the hinge domain comprises a CD8 α hinge domain, a CD28 hinge domain, an IgG1 hinge domain, an IgG2 hinge domain, an IgG3 hinge domain, or an IgG4 hinge domain.

[0292] Embodiment 83. The use of any one of embodiments 75 to 82, wherein the linker is located between the TRAILR2 binding domain and the transmembrane domain.

[0293] Embodiment 84. The use of any one of embodiments 74 to 83, wherein the TRAILR2 agonist comprises an amino acid sequence having at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence of SEQ ID NO:3, 12, 19, 22, 25, 27, or 30.

[0294] Embodiment 85. The use of any one of embodiments 74 to 84, wherein the medicament further comprises a chimeric antigen receptor (CAR).

[0295] Embodiment 86. The use of any one of embodiments 74 to 84, wherein the cancer is bladder cancer, bone cancer, brain cancer, breast cancer, bronchial cancer, cervical cancer, colon cancer, colorectal cancer, endometrial cancer, esophageal cancer, gallbladder cancer, head and neck cancer, kidney cancer, liver cancer, lung cancer, melanoma, mesothelioma, nasal cavity cancer, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, small cell lung cancer, small intestine cancer, soft tissue sarcoma, stomach cancer, testicular cancer, throat cancer, thyroid cancer, uterine cancer, and metastatic forms thereof.

[0296] Embodiment 87. A population of cells for the treatment of cancer in a patient, wherein the population of cells comprises TRAILR2 agonist comprising one or more TRAILR2 binding domains and a transmembrane domain.

[0297] Embodiment 88. The population of cells of embodiment 87, wherein the TRAILR2 agonist further comprises a linker, a hinge domain, one or more multimerization domains, and/or one or more intracellular domains.

[0298] Embodiment 89. The population of cells of either embodiment 87 or embodiment 88, wherein the one or more TRAILR2 binding domains comprises one or more sequences selected from SEQ ID NO: 1, 2, 23, 28, or 31-39, a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 1, 2, 23, 28, or 31-39, or any combination thereof.

[0299] Embodiment 90. The population of cells of any one of embodiments 87 to 89, wherein the transmembrane domain comprises a CD8 α transmembrane domain, a CD4 transmembrane domain, or a CD28 transmembrane domain.

[0300] Embodiment 91. The population of cells of embodiment 87, wherein the transmembrane domain comprises a CD8 α transmembrane domain.

[0301] Embodiment 92. The method of any one of embodiments 88 to 91, wherein the one or more multimerization domains comprises one or more of a coiled-coil motif, a zinc finger motif, a leucine zipper motif, a helix-turn-helix, a helix-loop-helix, and/or combinations thereof.

[0302] Embodiment 93. The method of any one of embodiments 88 to 92, wherein the one or more intracellular domains comprises a 4-1BB domain, CD3-zeta domain, or a CD28 intracellular domain.

[0303] Embodiment 94. The population of cells of any one of embodiments 87 to 93, wherein the linker comprises (GGSGG)_x (SEQ ID NO:9) or (GGGGS)_x (SEQ ID NO:10), wherein x can be 1, 2, 3, 4, or 5.

[0304] Embodiment 95. The population of cells of any one of embodiments 87 to 94, wherein the hinge domain comprises a CD8 α hinge domain, a CD28 hinge domain, an IgG1 hinge domain, an IgG2 hinge domain, an IgG3 hinge domain, or an IgG4 hinge domain.

[0305] Embodiment 96. The population of cells of any one of embodiments 87 to 95, wherein the linker is located between the TRAILR2 binding domain and the transmembrane domain.

[0306] Embodiment 97. The population of cells of any one of embodiments 87 to 96, wherein the TRAILR2 agonist comprises an amino acid sequence having at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence of SEQ ID NO:3, 12, 19, 22, 25, 27, or 30.

[0307] Embodiment 98. The population of cells of any one of embodiments 87 to 97, wherein the cancer is bladder cancer, bone cancer, brain cancer, breast cancer, bronchial cancer, cervical cancer, colon cancer, colorectal cancer, endometrial cancer, esophageal cancer, gallbladder cancer, head and neck cancer, kidney cancer, liver cancer, lung cancer, melanoma, mesothelioma, nasal cavity cancer, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, small cell lung cancer, small intestine cancer, soft tissue sarcoma, stomach cancer, testicular cancer, throat cancer, thyroid cancer, uterine cancer, and metastatic forms thereof.

[0308] Embodiment 99. The population of cells of any one of embodiments 87 to 98, wherein the population of cells is an autologous cell population or an allogenic cell population.

[0309] Embodiment 100. The population of cells of any one of embodiments 87 to 99, wherein the population of cells is selected from T cells, Natural Killer (NK) cells, Natural Killer T (NKT) cells, Invariant Natural Killer T (iNKT) cells, alpha beta T cells, gamma delta T cells, viral-specific T (VST) cells, cytotoxic T lymphocytes (CTLs), regulatory T cells (Tregs), and tumor infiltrating lymphocytes.

[0310] Embodiment 101. The population of cells of any one of embodiments 87 to 100, wherein the population of cells comprise total T cells.

[0311] Embodiment 102. The population of cells of any one of embodiments 87 to 100, wherein the population of cells comprise CD8+ T cells.

[0312] Embodiment 103. The population of cells of any one of embodiments 87 to 100, wherein the population of cells comprise CD4+ T cells.

[0313] Embodiment 104. The population of cells of any one of embodiments 87 to 103, wherein the TRAILR2 agonist is expressed on the cell surface.

[0314] Embodiment 105. The population of cells of any one of embodiments 87 to 104, wherein the population of cells exhibits an anti-tumor activity.

[0315] Embodiment 106. The population of cells of any one of embodiments 87 to 105, wherein the population of cells exhibits an enhanced anti-tumor activity.

Embodiment 107. The population of cells of any one of embodiments 87 to 106, wherein the cancer is bladder cancer, bone cancer, brain cancer, breast cancer, bronchial cancer, cervical cancer, colon cancer, colorectal cancer, endometrial cancer, esophageal cancer, gallbladder cancer, head and neck cancer, kidney cancer, liver cancer, lung cancer, melanoma, mesothelioma, nasal cavity cancer, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, small cell lung cancer, small intestine cancer, soft tissue sarcoma, stomach cancer, testicular cancer, throat cancer, thyroid cancer, uterine cancer, and metastatic forms thereof.

[0316] It is to be understood that the particular aspects of the specification are described herein are not limited to specific embodiments presented, and can vary. It also will be understood that the terminology used herein is for the purpose of describing particular aspects only and, unless specifically defined herein, is not intended to be limiting. Moreover, particular embodiments disclosed herein can be combined with other embodiments disclosed herein, as would be recognized by a skilled person, without limitation.

EXAMPLES

[0317] The Examples that follow are illustrative of specific embodiments of the disclosure, and various uses thereof. They are set forth for explanatory purposes only and should not be construed as limiting the scope of the disclosure in any way.

Materials and Methods:

Example 1. Development and characterization of DR5 agonists.

[0318] TNF-related apoptosis inducing ligand receptor 2 (TRAILR2, also called DR5) is a member of the TNF receptor superfamily that, when activated, induces apoptosis in a broad range of cancer cells and immunosuppressive myeloid cells, but not normal cells. For this reason, it was decided to express on the membrane of CAR-T cells an agonistic-anti DR5 molecule as an armoring strategy to enhance their efficacy. The anti-DR5 binding domain (G6) derives from a monobody protein scaffold based upon the third fibronectin type III domain of tenascin C (Swers *et al.*, “Multivalent scaffold proteins as superagonists of TRAIL receptor 2-induced apoptosis.” *Mol Cancer Ther.* 2013 Jul;12(7):1235-44). To characterize CAR-T cells armored with membrane bound anti-DR5, a construct encoding for G6 alone (FIG.1A) was tested or coexpressed with a GPC3 CAR (FIG.1B). Unarmored CAR-T cells were used as a control (FIG.1C).

[0319] Upon lentiviral transduction, the membrane-bound anti-DR5 was expressed on the surface on T cells (FIG. 2A). The anti-DR5 binding domain G6 was detected by flow cytometry, incubating the cells with recombinant DR5-Fc, followed by staining with BV421-anti-Fc antibody. T cell expansion in vitro was not affected by expression of G6 (FIG 2B). In addition, T cells expressing membrane-bound G6 had a similar CD4/8 ratio compared to untransduced (UT) T cells (FIG.2C). This demonstrates that membrane-bound G6 can be successfully expressed and detected on the surface of CAR-T cells, and that its expression does not affect T cell in vitro expansion or CD4/CD8 ratio.

[0320] To investigate if expression of membrane-bound anti-DR5 was sufficient to induce apoptosis of sensitive target cells, untransduced (UT) T cells or G6-expressing T cells were incubated with PLC/PRF/5, a DR5-sensitive HCC cell line (FIG.3A). An equal number of T cells was added to the tumor cells (E:T = 1:1) and cell death was analyzed by real-time impedance-based assay. Soluble trimeric anti-DR5 binding domain (sG6T3, 10 nM) was used as a positive control. Addition of UT T cells did not affect cell index compared to tumor alone control, as expected, indicating lack of PLC/PRF/5 death. Addition of G6-expressing T cells caused a rapid decrease in cell index, indicating target cell death. The effect of membrane-bound G6 was similar to that of sG6T3. To characterize the effect of membrane-

bound G6 on additional target cells, UT or G6-expressing T cells were co-incubated with a TRAIL-sensitive cell line (Colo 205) and a TRAIL-resistant cell line (SNU-398) at an 1:1 E:T ratio (FIG. 3B). Co-incubation of UT or G6-expressing T cells with the TRAIL-resistant cell line SNU-398 for three days did not affect the viability of tumor cells, as expected. In contrast, G6-expressing, but not UT, T cells induced death of Colo 205 similarly to the sG6T3 control. In summary, membrane-bound anti-DR5 induces death of sensitive targets in a specific way and to a similar extent to the soluble agonist.

Example 2. DR5 agonist synergizes with chimeric antigen receptors (CAR) to induce cell death *in vitro*.

[0321] To investigate if CAR-dependent killing would synergize with DR-mediated death, UT T cells, unarmored GPC3 CAR-T cells or CAR-T cells expressing membrane-bound G6 were incubated with PLC/PRF/5 and analyzed target cell killing by xCELLigence assays. The PLC/PRF/5 cell line is sensitive to DR5 and also expresses the CAR target antigen, GPC3 (FIG. 4A). Suboptimal dose of T cells expressing either the CAR or the membrane bound DR5 (E:T 1:3) resulted in a partial killing of target cells, that was greatly improved by co-expression of the CAR and membrane bound DR5, demonstrating *in vitro* synergy between the two modalities against target cell lines. Target cells knocked out for DR5 were insensitive to mDR5-induced cell death while retaining sensitivity to CAR-induced death (FIG.4B), demonstrating the specificity of the mDR5 moiety. In summary, anti-DR5 synergizes with a CAR to induce death of cells expressing the CAR target and sensitive to DR5.

[0322] Engagement of CAR with its target antigen results in CAR-T cell activation and release of the effector cytokine IFN γ . In contrast, engagement of membrane-bound G6 with DR5 does not result in T cell activation and therefore it should not lead to IFN γ release. To verify this hypothesis, IFN γ levels in supernatants collected from samples treated as in FIG.4 were analyzed. Cell culture supernatant was collected after 24 hours and the concentration of IFN- γ analyzed by MSD (FIG.5). UT T cells did not release IFN γ , as expected (level comparable to tumor alone control) while IFN- γ was produced by T cells upon CAR-induced activation and killing of GPC3 expressing tumor cells, irrespective of DR5 expression on target cells. In contrast, the DR5 agonist (G6) mediated tumor killing did not result in production of IFN- γ , indicating that expression of the surface agonist is sufficient to induce apoptosis of target cells and does not require T cell activation (FIG.5).

Example 3. DR5 agonist synergizes with chimeric antigen receptors (CAR) to induce cell death *in vivo*.

[0323] To investigate the *in vivo* anti-tumor efficacy of membrane-bound G6 armored CAR-T cells, UT, G6-expressing, unarmored CAR-T or G6-armored CAR-T cells were infused in PLC/PRF/5 tumor bearing mice. PLC/PRF/5 cells were implanted s.c. in the flank of NSG mice, and when the tumor size reached approximately 200 mm³, 0.8E6 CAR-T cells were dosed i.v. and tumor size was measured biweekly. At this suboptimal dose, GPC3 CAR-T cells partially delayed tumor growth compared to the UT control and did not substantially improve survival. DR5 agonist (G6) expressing T cells were not able to control tumor growth, in line with the fact that the DR5 agonist (G6) does not have an intracellular domain resulting in T cell activation, proliferation, and persistence. In contrast, cells co-expressing the GPC3 CAR and the membrane-bound G6 were able to efficiently control tumor growth (FIG.6A) and induced complete regression in 6 out of 10 mice (FIG.6B). These data demonstrate *in vivo* synergy between a CAR and a DR5-agonist.

Example 4. *In vivo* efficacy of DR5 agonist requires DR5 expression on target cells.

[0324] To confirm if the enhanced *in vivo* activity of DR5-armored CAR-T was dependant on DR5 expression on target cells, the *in vivo* efficacy against a DR5 KO target cell line (FIG. 7) was evaluated. UT, unarmored CAR-T or DR5-armored CAR-T cells were infused in mice bearing parental (FIG. 7) or DR5 KO (FIG. 7B) PLC/PRF/5 DR5 KO tumors. For both models, cells were implanted s.c. in the flank of NSG mice, and when the tumor size reached approximately 200 mm³, 1E6 CAR-T cells were dosed i.v. and tumor size was measured biweekly. Unarmored CAR-T cells achieved a transient control of parental or DR5 KO PLC/PRF/5 tumors, while expression of membrane-bound DR5 enhanced CAR-T efficacy against parental but not DR5 KO tumors. This result demonstrates that expression of membrane-bound DR5 induces death of sensitive cells *in vivo* in a DR5-specific manner.

Example 5. Expression of membrane-bound DR5 agonist enhances CART *in vivo* efficacy in a TGF β positive xenograft model

[0325] DR5-induced cell death does not require T cell activation. Therefore, it was hypothesized that CAR-T cells armored with a membrane-bound DR5 agonist would be more efficacious than unarmored CART when exposed to immunosuppressive stimuli. To answer this question, CART *in vivo* efficacy was evaluated in a model of hepatocellular carcinoma overexpressing TGF- β (HUH7-TGF- β). UT, unarmored CAR-T or DR-armored CAR-T cells were infused in tumor-bearing mice. Tumor cells were implanted s.c. in the flank of NSG

mice, and when the tumor size reached approximately 100 mm³, 5E6 CAR-T cells were dosed i.v. and tumor size was measured biweekly. While unarmored CART cells had a modest anti-tumor efficacy and mice had to be euthanized in the first 10 days after infusion, DR5-armored cells induced tumor regression and controlled tumor relapse for over 20 days after infusion. These data demonstrate that DR5-armored CART can kill tumor cells in a T cell activation-independent manner.

Example 6. Expression of membrane-bound DR5 agonist results in killing of tumor associated macrophages

[0326] To investigate whether the expression of membrane-bound DR5-agonist would result in killing of myeloid suppressive cells, T cell expressing or not membrane-bound DR5-agonist were co-incubated with tumor-derived macrophages (TAMs) generated *in vitro*. Approximately 50% of *in vitro* generated TAMs express DR5 upon polarization with MDA-MB-231 supernatant. In line with this, the number of live TAMs recovered after 3 days of culture was 50% lower upon incubation with DR5-agonist expressing T cells compared to non-DR5 expressing control, suggesting that expression of membrane-bound DR5 results in death of TAMs.

[0327] The embodiments described herein can be practiced in the absence of any element or elements, limitation or limitations that are not specifically disclosed herein. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the embodiments claimed. Thus, it should be understood that although the present description has been specifically disclosed by embodiments, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of these embodiments as defined by the description and the appended claims. Although some aspects of the present disclosure can be identified herein as particularly advantageous, it is contemplated that the present disclosure is not limited to these particular aspects of the disclosure.

[0328] Claims or descriptions that include “or” between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The disclosure includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a

given product or process. The disclosure includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process.

[0329] Furthermore, the disclosure encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, and descriptive terms from one or more of the listed claims is introduced into another claim. For example, any claim that is dependent on another claim can be modified to include one or more limitations found in any other claim that is dependent on the same base claim. Where elements are presented as lists, e.g., in Markush group format, each subgroup of the elements is also disclosed, and any element(s) can be removed from the group.

[0330] It should be understood that, in general, where the disclosure, or aspects of the disclosure, is/are referred to as comprising particular elements and/or features, certain embodiments of the disclosure or aspects of the disclosure consist, or consist essentially of, such elements and/or features. For purposes of simplicity, those embodiments have not been specifically set forth in haec verba herein.

[0331] All patents and publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent patent and publication was specifically and individually indicated to be incorporated by reference. Citation or identification of any reference in any section of this application shall not be construed as an admission that such reference is available as prior art to the present disclosure.

WHAT IS CLAIMED IS:

Claim 1. A TNF-related apoptosis-inducing ligand receptor 2 (TRAILR2) agonist comprising one or more TRAILR2 binding domains and a transmembrane domain.

Claim 2. The TRAILR2 agonist of claim 1, wherein the TRAILR2 agonist further comprises a linker, a hinge domain, one or more multimerization domains, and/or one or more intracellular domains.

Claim 3. The TRAILR2 agonist of either claim 1 or claim 2, wherein the one or more TRAILR2 binding domains comprises one or more sequences selected from SEQ ID NO:1, 2, 23, 28, or 31-39 a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 1, 2, 23, 28, or 31-39, or any combination thereof.

Claim 4. The TRAILR2 agonist of any one of claims 1 to 3, wherein the transmembrane domain comprises a CD8 α transmembrane domain, a CD4 transmembrane domain, or a CD28 transmembrane domain.

Claim 5. The TRAILR2 agonist of claim 4, wherein the transmembrane domain comprises a CD8 α transmembrane domain.

Claim 6. The TRAILR2 agonist of any one of claims 2 to 5, wherein the one or more multimerization domains comprises one or more of a coiled-coil motif, a zinc finger motif, a leucine zipper motif, a helix-turn-helix, a helix-loop-helix, and/or combinations thereof.

Claim 7. The TRAILR2 agonist of any one of claims 2 to 6, wherein the one or more intracellular domains comprises a 4-1BB domain, CD3-zeta domain, or a CD28 intracellular domain.

Claim 8. The TRAILR2 agonist of any one of claims 2 to 7, wherein the linker comprises (GGSGG)_x (SEQ ID NO:9) or (GGGGS)_x (SEQ ID NO:10), wherein x can be 1, 2, 3, 4, or 5.

Claim 9. The TRAILR2 agonist of any one of claims 2 to 8, wherein the hinge domain comprises a CD8 α hinge domain, a CD28 hinge domain, an IgG1 hinge domain, an IgG2 hinge domain, an IgG3 hinge domain, or an IgG4 hinge domain.

Claim 10. The TRAILR2 agonist of any one of claims 2 to 9, wherein the linker is located between the TRAILR2 binding domain and the transmembrane domain.

Claim 11. The TRAILR2 agonist of any one of claims 1 to 10, wherein the TRAILR2 agonist comprises an amino acid sequence having at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence of SEQ ID NO:3, 12, 19, 22, 25, 27, or 30.

Claim 12. An isolated nucleic acid sequence encoding a TNF-related apoptosis-inducing ligand receptor 2 (TRAILR2) agonist, wherein the TRAILR2 agonist comprises one or more TRAILR2 binding domains and a transmembrane domain.

Claim 13. The isolated nucleic acid sequence of claim 12, wherein the TRAILR2 agonist further comprises a linker, a hinge domain, one or more multimerization domains, and/or one or more intracellular domains.

Claim 14. The isolated nucleic acid sequence of either claim 12 or claim 13, wherein the one or more TRAILR2 binding domains comprises one or more sequences selected from SEQ ID NO: 1, 2, 23, 28, or 31-39, a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 1, 2, 23, 28, or 31-39, or any combination thereof.

Claim 15. The isolated nucleic acid sequence of any one of claims 12 to 14, wherein the transmembrane domain comprises a CD8 α transmembrane domain, a CD4 transmembrane domain, or a CD28 transmembrane domain.

Claim 16. The isolated nucleic acid sequence of claim 15, wherein the transmembrane domain comprises a CD8 α transmembrane domain.

Claim 17. The isolated nucleic acid sequence of any one of claims 12 to 16, wherein the one or more multimerization domains comprises one or more of a coiled-coil motif, a zinc finger

motif, a leucine zipper motif, a helix-turn-helix, a helix-loop-helix, and/or combinations thereof.

Claim 18. The isolated nucleic acid sequence of any one of claims 12 to 17, wherein the one or more intracellular domains comprises a 4-1BB domain, CD3-zeta domain, or a CD28 intracellular domain.

Claim 19. The isolated nucleic acid sequence of claim 18, wherein the one or more transmembrane domains comprises a 4-1BB domain.

Claim 20. The isolated nucleic acid sequence of any one of claims 13 to 19, wherein the linker comprises (GGSGG)_x (SEQ ID NO:9) or (GGGS)_x (SEQ ID NO:10), wherein x can be 1, 2, 3, 4, or 5.

Claim 21. The isolated nucleic acid sequence of any one of claims 13 to 20, wherein the hinge domain comprises a CD8 α hinge domain, a CD28 hinge domain, an IgG1 hinge domain, an IgG2 hinge domain, an IgG3 hinge domain, or an IgG4 hinge domain.

Claim 22. The isolated nucleic acid sequence of any one of claims 13 to 21, wherein the linker is located between the TRAILR2 binding domain and the transmembrane domain.

Claim 23. The isolated nucleic acid sequence of any one of claims 12 to 22, wherein the nucleic acid sequence encodes a TRAILR2 agonist with an amino acid sequence as set forth in SEQ ID NO: 3, 12, 19, 22, 25, 27, or 30, optionally wherein the nucleic acid sequence is as set forth in SEQ ID NO: 8, 11, 18, 21, 24, 26, or 29.

Claim 24. The isolated nucleic acid sequence of any one of claims 12 to 23 further comprising a CD33 signal peptide, and/or an inducible promoter, and/or a constitutive promoter.

Claim 25. The isolated nucleic acid sequence of any one of claims 12 to 24 further comprising a chimeric antigen receptor (CAR).

Claim 26. The isolated nucleic acid sequence of claim 25, wherein the TRAILR2 agonist and the CAR are operably linked under the control of a single promoter.

Claim 27. The isolated nucleic acid sequence of claim 25, wherein the TRAILR2 agonist and the CAR are operably linked by an internal ribosome entry site (IRES).

Claim 28. The isolated nucleic acid sequence of claim 25, wherein the TRAILR2 agonist and the CAR are linked by a nucleotide sequence encoding a cleavable peptide.

Claim 29. The isolated nucleic acid sequence of claim 28, wherein the cleavable peptide is a self-cleaving peptide (for example, T2A).

Claim 30. The isolated nucleic acid sequence of any one of claims 25 to 29, wherein the CAR comprises an antigen binding domain, a transmembrane domain, and one or more intracellular domains.

Claim 31. A vector comprising the isolated nucleic acid sequence of any one of claims 12 to 30.

Claim 32. The vector of claim 31, wherein the vector is a virus, a lentivirus, an adenovirus, a retrovirus, an adeno-associated virus (AAV), a transposon, a DNA vector, a mRNA, a lipid nanoparticle (LNP), or a CRISPR-Cas System.

Claim 33. The vector of either claim 31 or claim 32, wherein the vector is a lentivirus.

Claim 34. A cell comprising the TRAILR2 agonist of any one of claims 1 to 11, the isolated nucleic acid sequence of any one of claims 12 to 30, or the vector of any one of claims 31 to 33.

Claim 35. The cell of claim 34, wherein the cell is an autologous cell or an allogenic cell.

Claim 36. The cell of either claim 34 or claim 35, wherein the cell is selected from T cells, Natural Killer (NK) cells, Natural Killer T (NKT) cells, Invariant Natural Killer T (iNKT) cells, alpha beta T cells, gamma delta T cells, viral-specific T (VST) cells, cytotoxic T lymphocytes (CTLs), regulatory T cells (Tregs), and tumor infiltrating lymphocytes.

Claim 37. The cell of any one of claims 34 to 36, wherein the population of cells comprise total T cells.

Claim 38. The cell of any one of claims 34 to 36, wherein the population of cells comprise CD8⁺ T cells.

Claim 39. The cell of any one of claims 34 to 36, wherein the population of cells comprise CD4⁺ T cells.

Claim 40. The cell of any one of claims 34 to 39, wherein the TRAILR2 agonist is expressed on the cell surface.

Claim 41. The cell of any one of claims 34 to 40, wherein the cell exhibits an anti-tumor activity.

Claim 42. A method of treating cancer, comprising administering an effective amount of the cell of any one of claims 34 to 41 to a patient in need thereof.

Claim 43. A method of treating cancer, comprising:

administering to a subject in need thereof an effective amount of a cell comprising a TRAILR2 agonist, wherein the TRAILR2 agonist comprises one or more TRAILR2 binding domains and a transmembrane domain.

Claim 44. The method of claim 43, wherein the TRAILR2 agonist further comprises a linker, a hinge domain, one or more multimerization domains, and/or one or more intracellular domains.

Claim 45. The method of either claim 43 or claim 44, wherein the one or more TRAILR2 binding domains comprises one or more sequences selected from SEQ ID NO: 1, 2, 23, 28, or 31-39, a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 1, 2, 23, 28, or 31-39, or any combination thereof.

Claim 46. The method of any one of claims 43 to 45, wherein the transmembrane domain comprises a CD8 α transmembrane domain, a CD4 transmembrane domain, or a CD28 transmembrane domain.

Claim 47. The method of claim 46, wherein the transmembrane domain comprises a CD8 α transmembrane domain.

Claim 48. The method of any one of claims 43 to 47, wherein the one or more multimerization domains comprises one or more of a coiled-coil motif, a zinc finger motif, a leucine zipper motif, a helix-turn-helix, a helix-loop-helix, and/or combinations thereof.

Claim 49. The method of any one of claims 43 to 48, wherein the one or more intracellular domains comprises a 4-1BB domain, CD3-zeta domain, or a CD28 intracellular domain.

Claim 50. The method of any one of claims 43 to 49, wherein the linker comprises (GGSGG)_x (SEQ ID NO:9) or (GGGGS)_x (SEQ ID NO:10), wherein x can be 1, 2, 3, 4, or 5.

Claim 51. The method of claim any one of claims 43 to 50, wherein the hinge domain comprises a CD8 α hinge domain, a CD28 hinge domain, an IgG1 hinge domain, an IgG2 hinge domain, an IgG3 hinge domain, or an IgG4 hinge domain.

Claim 52. The method of any one of claims 43 to 51, wherein the linker is located between the TRAILR2 binding domain and the transmembrane domain.

Claim 53. The method of any one of claims 43 to 52, wherein the cell further comprises a chimeric antigen receptor (CAR).

Claim 54. The method of any one of claims 43 to 53, wherein the TRAILR2 agonist comprises an amino acid sequence having at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence of SEQ ID NO:3, 12, 19, 22, 25, 27, or 30.

Claim 55. The method of any one of claims 43 to 54, wherein the cell is an autologous cell or an allogenic cell.

Claim 56. The method of any one of claims 43 to 55, wherein the cell is selected from a T cells, Natural Killer (NK) cells, Natural Killer T (NKT) cells, Invariant Natural Killer T (iNKT) cells, alpha beta T cells, gamma delta T cells, viral-specific T (VST) cells, cytotoxic T lymphocytes (CTLs), regulatory T cells (Tregs), and tumor infiltrating lymphocytes.

Claim 57. The method of any one of claims 43 to 56, wherein the population of cells comprise total T cells.

Claim 58. The method of any one of claims 43 to 56, wherein the population of cells comprise CD8⁺ T cells.

Claim 59. The method of any one of claims 43 to 56, wherein the population of cells comprise CD4⁺ T cells.

Claim 60. The method of any one of claims 43 to 59, wherein the TRAILR2 agonist is expressed on the cell surface.

Claim 61. The method of any one of claims 43 to 60, wherein the cell exhibits an anti-tumor activity.

Claim 62. The method any one of claims 43 to 61 further comprising inhibiting tumor growth, inducing tumor regression, and/or prolonging survival of the subject.

Claim 63. The method of any one of claims 43 to 62, wherein the cancer is a solid tumor.

Claim 64. The method of claim 63, wherein the solid tumor is bladder cancer, bone cancer, brain cancer, breast cancer, bronchial cancer, cervical cancer, colon cancer, colorectal cancer, endometrial cancer, esophageal cancer, gallbladder cancer, head and neck cancer, kidney cancer, liver cancer, lung cancer, melanoma, mesothelioma, nasal cavity cancer, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, small cell lung cancer, small intestine cancer, soft tissue sarcoma, stomach cancer, testicular cancer, throat cancer, thyroid cancer, uterine cancer, and metastatic forms thereof.

Claim 65. A pharmaceutical composition comprising the TRAILR2 agonist of any one of claims 1 to 11, the isolated nucleic acid of any one of claims 12 to 30, the vector of any one of claims 31 to 33, or the cell of any one of claims 34 to 41, and a pharmaceutically acceptable excipient.

Claim 66. A method of treating a disease or condition in a subject in need thereof, comprising administering to the subject the TRAILR2 agonist of any one of claims 1 to 11, the isolated nucleic acid of any one of claims 12 to 30, the vector of any one of claims 31 to 33, or the cell of any one of claims 34 to 41, or the pharmaceutical composition of claim 65.

Claim 67. The method of claim 61, wherein the disease or condition comprises a cancer.

Claim 68. A method of treating a cancer in a subject in need thereof, comprising administering to the subject the TRAILR2 agonist of any one of claims 1 to 11, the isolated nucleic acid of any one of claims 12 to 30, the vector of any one of claims 31 to 33, or the cell of any one of claims 34 to 41, or the pharmaceutical composition of claim 65.

Claim 69. The method of any one of claims 62, 63 or 67-68, wherein the cancer is bladder cancer, bone cancer, brain cancer, breast cancer, bronchial cancer, cervical cancer, colon cancer, colorectal cancer, endometrial cancer, esophageal cancer, gallbladder cancer, head and neck cancer, kidney cancer, liver cancer, lung cancer, melanoma, mesothelioma, nasal cavity cancer, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, small cell lung cancer, small intestine cancer, soft tissue sarcoma, stomach cancer, testicular cancer, throat cancer, thyroid cancer, uterine cancer, and metastatic forms thereof.

Claim 70. Use of the TRAILR2 agonist of any one of claims 1 to 11, the isolated nucleic acid of any one of claims 12 to 30, the vector of any one of claims 31 to 33, or the cell of any one of claims 34 to 41, or the pharmaceutical composition of claim 65 for the manufacture of a medicament for treating a disease or condition in a subject in need thereof.

Claim 71. The use of claim 70, wherein the disease or condition comprises a cancer.

Claim 72. Use of the TRAILR2 agonist of any one of claims 1 to 11, the isolated nucleic acid of any one of claims 12 to 30, the vector of any one of claims 31 to 33, or the cell of any one of claims 34 to 41, or the pharmaceutical composition of claim 65 for the manufacture of a medicament for treating cancer in a subject in need thereof.

Claim 73. The use of either claim 71 or 72, wherein the cancer is bladder cancer, bone cancer, brain cancer, breast cancer, bronchial cancer, cervical cancer, colon cancer, colorectal cancer, endometrial cancer, esophageal cancer, gallbladder cancer, head and neck cancer, kidney cancer, liver cancer, lung cancer, melanoma, mesothelioma, nasal cavity cancer, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, small cell lung cancer, small intestine cancer, soft tissue sarcoma, stomach cancer, testicular cancer, throat cancer, thyroid cancer, uterine cancer, and metastatic forms thereof.

Claim 74. The use of a TNF-related apoptosis-inducing ligand receptor 2 (TRAILR2) agonist for the manufacture of a medicament for treating cancer in a patient, wherein the TRAILR2 agonist comprises one or more TRAILR2 binding domains and a transmembrane domain.

Claim 75. The use of claim 74, wherein the TRAILR2 agonist further comprises a linker, a hinge domain, one or more multimerization domains, and/or one or more intracellular domains.

Claim 76. The use of either claim 74 or claim 75, wherein the one or more TRAILR2 binding domains comprises one or more sequences selected from SEQ ID NO: 1, 2, 23, 28, or 31-39, a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 1, 2, 23, 28, or 31-39, or any combination thereof.

Claim 77. The use of any one of claims 74 to 76, wherein the transmembrane domain comprises a CD8 α transmembrane domain, a CD4 transmembrane domain, or a CD28 transmembrane domain.

Claim 78. The use of claim 77, wherein the transmembrane domain comprises a CD8 α transmembrane domain.

Claim 79. The use of any one of claims 75 to 78, wherein the one or more multimerization domains comprises one or more of a coiled-coil motif, a zinc finger motif, a leucine zipper motif, a helix-turn-helix, a helix-loop-helix, and/or combinations thereof.

Claim 80. The method of any one of claims 75 to 79, wherein the one or more intracellular domains comprises a 4-1BB domain, CD3-zeta domain, or a CD28 intracellular domain.

Claim 81. The use of any one of claims 75 to 80, wherein the linker comprises (GGSGG)_x (SEQ ID NO:9) or (GGGGS)_x (SEQ ID NO:10), wherein x can be 1, 2, 3, 4, or 5.

Claim 82. The use of any one of claims 75 to 81, wherein the hinge domain comprises a CD8 α hinge domain, a CD28 hinge domain, an IgG1 hinge domain, an IgG2 hinge domain, an IgG3 hinge domain, or an IgG4 hinge domain.

Claim 83. The use of any one of claims 75 to 82, wherein the linker is located between the TRAILR2 binding domain and the transmembrane domain.

Claim 84. The use of any one of claims 74 to 83, wherein the TRAILR2 agonist comprises an amino acid sequence having at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence of SEQ ID NO:3, 12, 19, 22, 25, 27, or 30.

Claim 85. The use of any one of claims 74 to 84, wherein the medicament further comprises a chimeric antigen receptor (CAR).

Claim 86. The use of any one of claims 74 to 85, wherein the cancer is bladder cancer, bone cancer, brain cancer, breast cancer, bronchial cancer, cervical cancer, colon cancer, colorectal cancer, endometrial cancer, esophageal cancer, gallbladder cancer, head and neck cancer, kidney cancer, liver cancer, lung cancer, melanoma, mesothelioma, nasal cavity cancer, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, small cell lung cancer, small intestine cancer, soft tissue sarcoma, stomach cancer, testicular cancer, throat cancer, thyroid cancer, uterine cancer, and metastatic forms thereof.

Claim 87. A population of cells for the treatment of cancer in a patient, wherein the population of cells comprises TRAILR2 agonist comprising one or more TRAILR2 binding domains and a transmembrane domain.

Claim 88. The population of cells of claim 87, wherein the TRAILR2 agonist further comprises a linker, a hinge domain, one or more multimerization domains, and/or one or more intracellular domains.

Claim 89. The population of cells of either claim 87 or claim 88, wherein the one or more TRAILR2 binding domains comprises one or more sequences selected from SEQ ID NO: 1, 2, 23, 28, or 31-39, a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 1, 2, 23, 28, or 31-39, or any combination thereof.

Claim 90. The population of cells of any one of claims 87 to 89, wherein the transmembrane domain comprises a CD8 α transmembrane domain, a CD4 transmembrane domain, or a CD28 transmembrane domain.

Claim 91. The population of cells of claim 87, wherein the transmembrane domain comprises a CD8 α transmembrane domain.

Claim 92. The method of any one of claims 88 to 91, wherein the one or more multimerization domains comprises one or more of a coiled-coil motif, a zinc finger motif, a leucine zipper motif, a helix-turn-helix, a helix-loop-helix, and/or combinations thereof.

Claim 93. The method of any one of claims 88 to 92, wherein the one or more intracellular domains comprises a 4-1BB domain, CD3-zeta domain, or a CD28 intracellular domain.

Claim 94. The population of cells of any one of claims 87 to 93, wherein the linker comprises (GGSGG)_x (SEQ ID NO:9) or (GGGGS)_x (SEQ ID NO:10), wherein x can be 1, 2, 3, 4, or 5.

Claim 95. The population of cells of any one of claims 87 to 94, wherein the hinge domain comprises a CD8 α hinge domain, a CD28 hinge domain, an IgG1 hinge domain, an IgG2 hinge domain, an IgG3 hinge domain, or an IgG4 hinge domain.

Claim 96. The population of cells of any one of claims 87 to 95, wherein the linker is located between the TRAILR2 binding domain and the transmembrane domain.

Claim 97. The population of cells of any one of claims 87 to 96, wherein the TRAILR2 agonist comprises an amino acid sequence having at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence of SEQ ID NO:3, 12, 19, 22, 25, 27, or 30.

Claim 98. The population of cells of any one of claims 87 to 97, wherein the cancer is bladder cancer, bone cancer, brain cancer, breast cancer, bronchial cancer, cervical cancer, colon cancer, colorectal cancer, endometrial cancer, esophageal cancer, gallbladder cancer, head and neck cancer, kidney cancer, liver cancer, lung cancer, melanoma, mesothelioma, nasal cavity cancer, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, small cell lung cancer, small intestine cancer, soft tissue sarcoma, stomach cancer, testicular cancer, throat cancer, thyroid cancer, uterine cancer, and metastatic forms thereof.

Claim 99. The population of cells of any one of claims 87 to 98, wherein the population of cells is an autologous cell population or an allogenic cell population.

Claim 100. The population of cells of any one of claims 87 to 99, wherein the population of cells is selected from T cells, Natural Killer (NK) cells, Natural Killer T (NKT) cells, Invariant Natural Killer T (iNKT) cells, alpha beta T cells, gamma delta T cells, viral-specific T (VST) cells, cytotoxic T lymphocytes (CTLs), regulatory T cells (Tregs), and tumor infiltrating lymphocytes.

Claim 101. The population of cells of any one of claims 87 to 100, wherein the population of cells comprise total T cells.

Claim 102. The population of cells of any one of claims 87 to 100, wherein the population of cells comprise CD8⁺ T cells.

Claim 103. The population of cells of any one of claims 87 to 100, wherein the population of cells comprise CD4⁺ T cells.

Claim 104. The population of cells of any one of claims 87 to 103, wherein the TRAILR2 agonist is expressed on the cell surface.

Claim 105. The population of cells of any one of claims 87 to 104, wherein the population of cells exhibits an anti-tumor activity.

Claim 106. The population of cells of any one of claims 87 to 105, wherein the population of cells exhibits an enhanced anti-tumor activity.

Claim 107. The population of cells of any one of claims 87 to 106, wherein the cancer is bladder cancer, bone cancer, brain cancer, breast cancer, bronchial cancer, cervical cancer, colon cancer, colorectal cancer, endometrial cancer, esophageal cancer, gallbladder cancer, head and neck cancer, kidney cancer, liver cancer, lung cancer, melanoma, mesothelioma, nasal cavity cancer, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, small cell lung cancer, small intestine cancer, soft tissue sarcoma, stomach cancer, testicular cancer, throat cancer, thyroid cancer, uterine cancer, and metastatic forms thereof.