activation properties of the TNF receptor family member 4-1BB have been shown to involve the specific ability of its cytoplasmic domain to associate with the tyrosine kinase p56lck. The sequence of the cytoplasmic domain of CD30 shows little sequence similarity to any of these receptors; CD30 lacks an obvious death domain or a p56lck-binding site.

SUMMARY

[0005] The present ~~invention~~disclosure provides, among other things, chimeric stimulating receptors (CSRs) that use a costimulatory domain from CD30 (also referred to herein as a CD30 costimulatory domain). As described in detail herein, T cells with CSRs containing a costimulatory domain from CD30 express far less PD-1, an inhibitor of T cell activation, than T cells with CSRs containing a costimulatory domain from, *e.g.,* CD28 or 4-1BB, and at the same time demonstrate equal cytotoxic potential. In some ~~embodiment~~examples, T cells with CSRs containing a costimulatory domain from CD30 express far less PD-1 than T cells with CSRs containing a costimulatory domain from Dap10. The examples suggest that the costimulatory domain from CD30 ameliorates the functional unresponsiveness that leads to T cell exhaustion, also called anergy, and subsequently, provides superior persistence of tumor cell killing and increased tumor infiltration as compared to the commonly used costimulatory domains such as CD28. It is unexpected since CD30 lacks a p56lck-binding site that is thought to be crucial for CSR costimulation.

[0006] In one aspect, the disclosure features an immune cell comprising: (a) a chimeric antigen receptor (CAR) comprising: (i) an extracellular target-binding domain comprising an antibody moiety (a CAR antibody moiety); (ii) a transmembrane domain (a CAR transmembrane domain); and (iii) a primary signaling domain, and (b) a chimeric stimulating receptor (CSR) comprising: (i) a ligand-binding module that is capable of binding or interacting with a target ligand; (ii) a transmembrane domain (a CSR transmembrane domain); and (iii) a CD30 costimulatory domain, wherein the CSR lacks a functional primary signaling domain.

[0007] In some ~~embodiment~~examples, the CD30 costimulatory domain comprises a sequence that can bind to an intracellular TRAF signaling protein. In some ~~embodiment~~examples, the sequence that can bind to an intracellular TRAF signaling protein corresponds to residues 561–573 or 578–586 of a full-length CD30 having the sequence of SEQ ID NO:65. In some ~~embodiment~~examples, the CD30 costimulatory domain comprises a sequence that is at least 80%, 85%, 90%, 95%, or 100% (*e.g.,* 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%,

94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to residues 561–573 or 578–586 of SEQ ID NO:65. In some ~~embodiment~~examples, the CD30 costimulatory domain comprises a sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, or 100% (*e.g.,* 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the sequence of SEQ ID NO:75.

[0008] In some ~~embodiment~~examples ~~of this aspect~~, the CSR comprises more than one CD30 costimulatory domain. In some ~~embodiment~~examples, the CSR further comprises at least one costimulatory domain which comprises the intracellular sequence of a costimulatory molecule that is different from CD30. The costimulatory molecule that is different from CD30 can be selected from the group consisting of CD27, CD28, 4-1BB (CD137), OX40, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and Dap10.

[0009] In some ~~embodiment~~examples, the CAR further comprises a costimulatory domain (a CAR costimulatory domain). The CAR costimulatory domain can be derived from the intracellular domain of a costimulatory receptor. The costimulatory receptor can be selected from the group consisting of CD30, CD27, CD28, 4-1BB (CD137), OX40, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and Dap10.

[0010] In some ~~embodiment~~examples, the ligand-binding module of the CSR is derived from the extracellular domain of a receptor. In some ~~embodiment~~examples, the ligand-binding module of the CSR comprises an antibody moiety (a CSR antibody moiety). The CSR antibody moiety can be a single chain antibody fragment. The CAR antibody moiety can be a single chain antibody fragment. In some ~~embodiment~~examples, the CAR antibody moiety and/or the CSR antibody moiety is a single chain Fv (scFv), a single chain Fab, a single chain Fab', a single domain antibody fragment, a single domain multispecific antibody, an intrabody, a nanobody, or a single chain immunokine. In some ~~embodiment~~examples, the CAR antibody moiety and/or the CSR antibody moiety is a single domain multispecific antibody. In some ~~embodiment~~examples, the single domain multispecific antibody is a single domain bispecific antibody. In some ~~embodiment~~examples, the CAR antibody moiety and/or the CSR antibody moiety is a single chain Fv (scFv). In some ~~embodiment~~examples, the scFv is a tandem scFv.

[0011] In some ~~embodiment~~examples, the CAR antibody moiety and/or the CSR antibody moiety specifically binds to a disease-related antigen. The disease-related antigen is a cancer-related antigen. The disease-related antigen is a virus-related antigen. In some ~~embodiment~~examples, the CAR antibody moiety and/or the CSR antibody moiety specifically binds to a cell surface antigen. The cell surface antigen can be selected from the group consisting of protein, carbohydrate, and lipid. The cell surface antigen can be CD19, CD20, CD22, CD47, CD158e, GPC3, ROR1, ROR2, BCMA, GPRC5D, FcRL5, MUC16, MCT4, PSMA, or a variant or mutant thereof.

[0012] In some ~~embodiment~~examples, the CAR antibody moiety and the CSR antibody moiety specifically bind to the same antigen. In particular ~~embodiment~~examples, the CAR antibody moiety and the CSR antibody moiety specifically bind to different epitopes on the same antigen.

[0013] In some ~~embodiment~~examples, the CAR antibody moiety and/or the CSR antibody moiety specifically binds to a MHC-restricted antigen. In some ~~embodiment~~examples, the MHC-restricted antigen is a complex comprising a peptide and an MHC protein, and the peptide is derived from a protein selected from the group consisting of WT-1, AFP, HPV16-E7, NY-ESO-1, PRAME, EBV-LMP2A, HIV-1, KRAS, FoxP3, Histone H3.3, PSA, and a variant or mutant thereof.

[0014] In some ~~embodiment~~examples ~~of this aspect~~, the CAR antibody moiety binds to CD19, and the ligand-binding module of the CSR binds to CD19. In some ~~embodiment~~examples, the CAR antibody moiety binds to CD22, and the ligand-binding module of the CSR binds to CD22. In some ~~embodiment~~examples, the CAR antibody moiety binds to CD20, and the ligand-binding module of the CSR binds to CD20. In some ~~embodiment~~examples, the CAR antibody moiety binds to CD19, and the ligand-binding module of the CSR binds to CD22. In some ~~embodiment~~examples, the CAR antibody moiety binds to CD19, and the ligand-binding module of the CSR binds to CD20. In some ~~embodiment~~examples, the CAR antibody moiety binds to CD22, and the ligand-binding module of the CSR binds to CD20. In some ~~embodiment~~examples, the CAR antibody moiety binds to CD22, and the ligand-binding module of the CSR binds to CD19. In some ~~embodiment~~examples, the CAR antibody moiety binds to CD20, and the ligand-binding module of the CSR binds to CD19. In some ~~embodiment~~examples, the CAR antibody moiety binds to CD20, and the ligand-binding module of the CSR binds to CD22. In some ~~embodiment~~examples, the CAR antibody moiety and/or the ligand-binding module of the CSR binds to both CD19 and CD22. In some ~~embodiment~~examples, the CAR antibody moiety and/or the ligand-binding module of the CSR binds to both CD19 and CD20. In some ~~embodiment~~examples, the CAR antibody moiety and/or the ligand-binding module of the CSR

binds to both CD20 and CD22. In some ~~embodiment~~examples, the CAR antibody moiety and/or the ligand-binding module of the CSR binds to CD19, CD20, and CD22.

[0015] In some ~~embodiment~~examples ~~of this aspect~~, the CAR antibody moiety specifically binds to a complex comprising an alpha-fetoprotein (AFP) peptide and an MHC class I protein. In some ~~embodiment~~examples, the ligand-binding module of the CSR specifically binds to glypican 3 (GPC3). In some ~~embodiment~~examples, the CAR antibody moiety binds to a complex comprising an AFP peptide and an MHC class I protein, and the ligand-binding module of the CSR binds to GPC3.

[0016] In some ~~embodiment~~examples, both the CAR antibody moiety and the ligand-binding module of the CSR bind to GPC3. In particular ~~embodiment~~examples, the CAR antibody moiety and the ligand-binding module of the CSR specifically bind to different epitopes on GPC3.

[0017] In some ~~embodiment~~examples, the CAR transmembrane domain is the transmembrane domain of CD30. In some ~~embodiment~~examples, the CAR transmembrane domain is the transmembrane domain of CD8. In some ~~embodiment~~examples, the CAR transmembrane domain and/or the CSR transmembrane domain is derived from the transmembrane domain of a TCR co-receptor or a T cell co-stimulatory molecule. The TCR co-receptor or T cell co-stimulatory molecule can be selected from the group consisting of CD8, 4-1BB, CD27, CD28, CD30, OX40, CD3ε, CD3ζ, CD45, CD4, CD5, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, and Dap10. In certain ~~embodiment~~examples, the TCR co-receptor or T cell co-stimulatory molecule is CD30 or CD8. In some ~~embodiment~~examples, the T cell co-stimulatory molecule can be CD30. In some ~~embodiment~~examples, the TCR co-receptor is CD8.

[0018] In some ~~embodiment~~examples, the CAR transmembrane domain and/or the CSR transmembrane domain is the transmembrane domain of CD8, 4-1BB, CD27, CD28, CD30, OX40, CD3ε, CD3ζ, CD45, CD4, CD5, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, or Dap10. In certain ~~embodiment~~examples, the CAR transmembrane domain and/or the CSR transmembrane domain is the transmembrane domain of CD30 or CD8. In certain ~~embodiment~~examples, the CAR transmembrane domain and/or the CSR transmembrane domain is the transmembrane domain of CD30. In certain ~~embodiment~~examples, the CSR transmembrane domain is the transmembrane domain of CD30. In certain ~~embodiment~~examples, the CAR transmembrane domain and/or the CSR transmembrane domain is the transmembrane domain of CD8. In certain ~~embodiment~~examples, the CAR transmembrane domain and/or the CSR

transmembrane domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:66-71.

[0019] In some ~~embodiment~~examples~~ of this aspect~~, the primary signaling domain comprises a sequence derived from the intracellular signaling sequence of a molecule selected from the group consisting of CD3ζ, TCRζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD5, CD22, CD79a, CD79b, and CD66d. In some ~~embodiment~~examples, the primary signaling domain comprises a sequence derived from the intracellular signaling sequence of CD3ζ. In some ~~embodiment~~examples, the primary signaling domain comprises the intracellular signaling sequence of CD3ζ. In cetain ~~embodiment~~examples, the primary signaling domain comprises a sequence that is at least 80%, 85%, 90%, 95%, or 100% (*e.g.,* 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the sequence of SEQ ID NO:77.

[0020] In some ~~embodiment~~examples~~ of this aspect~~, the CAR in the immune cell further comprises a peptide linker between the extracellular target-binding domain and the transmembrane domain of the CAR. In some ~~embodiment~~examples, the CAR in the immune cell further comprises a peptide linker between the transmembrane domain and the costimulatory domain of the CAR. In some ~~embodiment~~examples, the CAR in the immune cell further comprises a peptide linker between the costimulatory domain and the primary signaling domain of the CAR. In some ~~embodiment~~examples, the CSR in the immune cell further comprises a peptide linker between the ligand-binding module and the transmembrane domain of the CSR. In some ~~embodiment~~examples, the CSR in the immune cell further comprises a peptide linker between the transmembrane domain and the CD30 costimulatory domain of the CSR.

[0021] In some ~~embodiment~~examples~~ of this aspect~~, the expression of the CSR is inducible. In some ~~embodiment~~examples, the expression of the CSR is inducible upon activation of the immune cell. In some ~~embodiment~~examples, the immune cell is selected from the group consisting of a cytotoxic T cell, a helper T cell, a natural killer T cell, and a suppressor T cell.

[0022] In another aspect, the disclosure features one or more nucleic acids encoding the CAR and CSR comprised by the immune cell described herein, wherein the CAR and CSR each consist of one or more polypeptide chains encoded by the one or more nucleic acids.

[0023] In another aspect, the disclosure features one or more vectors comprising the one or more nucleic acids described above.

[0024] In another aspect, the disclosure features a pharmaceutical composition comprising: (a) the immune cell described herein, the nucleic acid(s) described herein, or the vector(s) described herein, and (b) a pharmaceutically acceptable carrier or diluent.

[0025] In another aspect, the disclosure features a method of killing target cells, comprising: contacting one or more target cells with the immune cell described herein under conditions and for a time sufficient so that the immune cells mediate killing of the target cells, wherein the target cells express an antigen specific to the immune cell, and wherein the immune cell expresses a low cell exhaustion level upon contacting the target cells. In some ~~embodiment~~examples, the immune cell expresses a low cell exhaustion level of an exhaustion marker selected from the group consisting of PD-1, TIM-3, TIGIT, and LAG-3. In certain ~~embodiment~~examples, the immune cell is a T cell. In certain ~~embodiment~~examples, the immune cell expresses a low cell exhaustion level of PD-1. In some ~~embodiment~~examples, the ratio of PD-1 from immune cells (*e.g.,* $CD8^+$ T cells, $CD4^+$ T cells) expressing a 1^{st} generation CAR (*e.g.,* αAFP-CD8T-z-CAR) and CD30-CSR to PD-1 from immune cells expressing the 1^{st} generation CAR alone is between 0.05 and 0.5 (*e.g.,* between 0.05 and 0.45, between 0.05 and 0.4, between 0.05 and 0.35, between 0.05 and 0.3, between 0.05 and 0.25, between 0.05 and 0.2, between 0.05 and 0.15, between 0.05 and 0.1, between 0.1 and 0.45, between 0.15 and 0.45, between 0.2 and 0.45, between 0.25 and 0.45, between 0.3 and 0.45, between 0.35 and 0.45, or between 0.4 and 0.45). In some ~~embodiment~~examples, the ratio of PD-1 from immune cells (*e.g.,* $CD8^+$ T cells, $CD4^+$ T cells) expressing a 2nd generation CAR (*e.g.,* αAFP-CD28z-CAR) and CD30-CSR to PD-1 from immune cells expressing the 2nd generation CAR alone is between 0.05 and 0.5 (*e.g.,* between 0.05 and 0.45, between 0.05 and 0.4, between 0.05 and 0.35, between 0.05 and 0.3, between 0.05 and 0.25, between 0.05 and 0.2, between 0.05 and 0.15, between 0.05 and 0.1, between 0.1 and 0.45, between 0.15 and 0.45, between 0.2 and 0.45, between 0.25 and 0.45, between 0.3 and 0.45, between 0.35 and 0.45, or between 0.4 and 0.45). In certain ~~embodiment~~examples, the immune cell expresses a low cell exhaustion level of TIM-3. In certain ~~embodiment~~examples, the immune cell expresses a low cell exhaustion level of TIGIT. In certain ~~embodiment~~examples, the immune cell expresses a low cell exhaustion level of LAG-3. In some ~~embodiment~~examples, the ratio of LAG-3 from immune cells (*e.g.,* $CD8^+$ T cells, $CD4^+$ T cells) expressing a 2nd generation CAR (*e.g.,* αAFP-CD28z-CAR) and CD30-CSR to LAG-3 from immune cells expressing the 2nd

generation CAR alone is between 0.1 and 0.9 (*e.g.,* between 0.1 and 0.8, between 0.1 and 0.7, between 0.1 and 0.6, between 0.1 and 0.5, between 0.1 and 0.4, between 0.1 and 0.3, between 0.1 and 0.2, between 0.2 and 0.9, between 0.3 and 0.9, between 0.4 and 0.9, between 0.5 and 0.9, between 0.6 and 0.9, between 0.7 and 0.9, or between 0.8 and 0.9).

[0026] In some ~~embodiment~~examples, the immune cell expresses a lower level of PD-1, TIM-3, TIGIT, or LAG-3 than corresponding immune cell expressing a CSR comprising a CD28 costimulatory domain. In some ~~embodiment~~examples, the immune cell expresses a lower level of PD-1 than the corresponding CD28 CSR immune cell, and wherein the ratio of PD-1 expression level of the immune cell to the corresponding CD28 CSR immune cell is 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 or lower. In some ~~embodiment~~examples, the immune cell expresses a lower level of TIM-3 than the corresponding CD28 CSR immune cell, and wherein the ratio of TIM-3 expression level of the immune cell to the corresponding CD28 CSR immune cell is 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 or lower. In some ~~embodiment~~examples, the immune cell expresses a lower level of LAG-3 than the corresponding CD28 CSR immune cell, and wherein the ratio of LAG-3 expression level of the immune cell to the corresponding CD28 CSR immune cell is 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 or lower. In some ~~embodiment~~examples, the immune cell expresses a lower level of TIGIT than the corresponding CD28 CSR immune cell, and wherein the ratio of TIGIT expression level of the immune cell to the corresponding CD28 CSR immune cell is 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 or lower.

[0027] In some ~~embodiment~~examples, the immune cell expresses a lower level of PD-1, TIM-3, TIGIT, or LAG-3 than corresponding immune cell expressing a CSR comprising a 4-1BB costimulatory domain. In some ~~embodiment~~examples, the immune cell expresses a lower level of PD-1 than the corresponding 4-1BB CSR immune cell, and wherein the ratio of PD-1 expression level of the immune cell to the corresponding 4-1BB CSR immune cell is 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 or lower. In some ~~embodiment~~examples, the immune cell expresses a lower level of TIM-3 than the corresponding 4-1BB CSR immune cell, and wherein the ratio of TIM-3 expression level of the immune cell to the corresponding 4-1BB CSR immune cell is 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 or lower. In some ~~embodiment~~examples, the immune cell expresses a lower level of LAG-3 than the corresponding 4-1BB CSR immune cell, and wherein the ratio of LAG-3 expression level of the immune cell to the corresponding 4-1BB CSR immune cell is 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 or lower. In some ~~embodiment~~examples, the immune cell expresses a lower level of TIGIT than the corresponding 4-1BB CSR immune cell, and wherein

the ratio of TIGIT expression level of the immune cell to the corresponding 4-1BB CSR immune cell is 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 or lower.

[0028] In some ~~embodiment~~examples, the immune cell expresses a lower level of PD-1, TIM-3, TIGIT, or LAG-3 than corresponding immune cell expressing a CSR comprising a Dap10 costimulatory domain. In some ~~embodiment~~examples, the immune cell expresses a lower level of PD-1 than the corresponding Dap10 CSR immune cell, and wherein the ratio of PD-1 expression level of the immune cell to the corresponding Dap10 CSR immune cell is 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 or lower. In some ~~embodiment~~examples, the immune cell expresses a lower level of TIM-3 than the corresponding Dap10 CSR immune cell, and wherein the ratio of TIM-3 expression level of the immune cell to the corresponding Dap10 CSR immune cell is 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 or lower. In some ~~embodiment~~examples, the immune cell expresses a lower level of LAG-3 than the corresponding Dap10 CSR immune cell, and wherein the ratio of LAG-3 expression level of the immune cell to the corresponding Dap10 CSR immune cell is 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 or lower. In some ~~embodiment~~examples, the immune cell expresses a lower level of TIGIT than the corresponding Dap10 CSR immune cell, and wherein the ratio of TIGIT expression level of the immune cell to the corresponding Dap10 CSR immune cell is 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 or lower.

[0029] In some ~~embodiment~~examples of this ~~aspect~~example, the target cells are cancer cells. The cancer cells can be from a cancer selected from the group consisting of adrenocortical carcinoma, bladder cancer, breast cancer, cervical cancer, cholangiocarcinoma, colorectal cancers, esophageal cancer, glioblastoma, glioma, hepatocellular carcinoma, head and neck cancer, kidney cancer, leukemia, lymphoma, lung cancer, melanoma, mesothelioma, multiple myeloma, pancreatic cancer, pheochromocytoma, plasmacytoma, neuroblastoma, ovarian cancer, prostate cancer, sarcoma, stomach cancer, uterine cancer, and thyroid cancer. The cancer cells can be hematological cancer cells. The cancer cells can be solid tumor cells.

[0030] In some ~~embodiment~~examples, the target cells are virus-infected cells. The virus-infected cells can be from a viral infection caused by a virus selected from the group consisting of Cytomegalovirus (CMV), Epstein-Barr Virus (EBV), Hepatitis B Virus (HBV), Kaposi's Sarcoma associated herpesvirus (KSHV), Human papillomavirus (HPV), Molluscum contagiosum virus (MCV), Human T cell leukemia virus 1 (HTLV-1), HIV (Human immunodeficiency virus), and Hepatitis C Virus (HCV).

[0031] In another ~~aspect~~example, the disclosure features a method of treating a disease, the method comprising a step of administering to a subject the immune cell described herein, the nucleic acid(s) described herein, or the vector(s) described herein, or the pharmaceutical composition described herein to the subject. In some ~~embodiment~~examples, the disease is a viral infection. In some ~~embodiment~~examples, the disease is cancer. The cancer can be a hematological cancer. The cancer can be a solid tumor cancer.

[0032] In some ~~embodiment~~examples, the subject has a higher density of the immune cell described herein in the solid tumor cancer than in the rest of the subject's body.

[0033] In some ~~embodiment~~examples, the cancer is selected from the group consisting of adrenocortical carcinoma, bladder cancer, breast cancer, cervical cancer, cholangiocarcinoma, colorectal cancers, esophageal cancer, glioblastoma, glioma, hepatocellular carcinoma, head and neck cancer, kidney cancer, leukemia, lymphoma, lung cancer, melanoma, mesothelioma, multiple myeloma, pancreatic cancer, pheochromocytoma, plasmacytoma, neuroblastoma, ovarian cancer, prostate cancer, sarcoma, stomach cancer, uterine cancer, and thyroid cancer.

[0034] In another ~~aspect~~example, the disclosure features a method for preventing and/or reversing T cell exhaustion in a subject, comprising administering to the subject the nucleic acid(s) described herein, the vector(s) described herein, or the pharmaceutical composition described herein comprising the nucleic acid(s) or the vector(s) to the subject. In some ~~embodiment~~examples, the method decreases the expression of an exhaustion marker in a T cell. The exhaustion marker can be selected from the group consisting of PD-1, TIM-3, TIGIT, and LAG-3.

[0035] In another ~~aspect~~example, the disclosure features a method of treating a solid tumor cancer in a subject with increased tumor infiltration or immune cell expansion as compared to treating the same type of solid tumor cancer with immune cells expressing a CAR and a CSR comprising a CD28 or 4-1BB costimulatory domain, wherein the method comprises administering to the subject corresponding immune cells expressing the same CAR and a corresponding CSR comprising a CD30 costimulatory domain, and wherein the corresponding immune cells comprise the immune cell described herein. In another ~~aspect~~example, the disclosure features a method of treating a solid tumor cancer in a subject with increased tumor infiltration or immune cell expansion as compared to treating the same type of solid tumor cancer with immune cells expressing a CAR and a CSR comprising a Dap10 costimulatory domain, wherein the method comprises administering to the subject corresponding immune cells

expressing the same CAR and a corresponding CSR comprising a CD30 costimulatory domain, and wherein the corresponding immune cells comprise the immune cell described herein. In some ~~embodiment~~examples, experiments can be conducted in animals, *e.g.,* mice, to compare the effects of the immune cells in treating a solid tumor cancer by using one group of immune cells comprising a CAR and a CSR with a CD30 costimulatory domain and another group of immune cells comprising the same CAR and a corresponding CSR with a non-CD30 costimulatory domain, *e.g.,* a 4-1BB costimlaroty domain, a CD28 costimulatory domain, or a Dap10 costimulatory domain.

[0036] In some ~~embodiment~~examples of the methods described herein, the ratio of the number of tumor cells infiltrated by immune cells expressing a 2nd generation CAR (*e.g.,* αAFP-CD28z-CAR, αGPC3-CD28z-CAR) and CD30-CSR to the number of tumor cells infiltrated by immune cells expressing the 2nd generation CAR alone is between 1 and 20 (*e.g.,* between 1 and 18, between 1 and 16, between 1 and 14, between 1 and 12, between 1 and 10, between 1 and 8, between 1 and 6, between 1 and 4, between 1 and 2, between 2 and 20, between 4 and 20, between 6 and 20, between 8 and 20, between 10 and 20, between 12 and 20, between 14 and 20, between 16 and 20, or between 18 and 20).

[0037] In some ~~embodiment~~examples of the methods described herein, the ratio of the blood concentration of immune cells expressing a 2nd generation CAR (*e.g.,* αAFP-CD28z-CAR, αGPC3-CD28z-CAR) and CD30-CSR to the blood concentration of immune cells expressing the 2nd generation CAR alone is between 1 and 5 (*e.g.,* between 1 and 4, between 1 and 3, between 1 and 2, between 2 and 5, between 3 and 5, or between 4 and 5).

[0038] In another ~~aspect~~example, the disclosure features a method of treating a solid tumor cancer in a subject with increased tumor regression as compared to treating the same type of solid tumor cancer with immune cells expressing a CAR and a CSR comprising a CD28 or 4-1BB costimulatory domain, wherein the method comprises administering to the subject corresponding immune cells expressing the same CAR and a corresponding CSR comprising a CD30 costimulatory domain, and wherein the corresponding immune cells comprise the immune cell described herein. In another ~~aspect~~example, the disclosure features a method of treating a solid tumor cancer in a subject with increased tumor regression as compared to treating the same type of solid tumor cancer with immune cells expressing a CAR and a CSR comprising a Dap10 costimulatory domain, wherein the method comprises administering to the subject corresponding immune cells expressing the same CAR and a corresponding CSR comprising

a CD30 costimulatory domain, and wherein the corresponding immune cells comprise the immune cell described herein. In some ~~embodiment~~examples, experiments can be conducted in animals, *e.g.,* mice, to compare the effects of the immune cells on tumor regression by using one group of immune cells comprising a CAR and a CSR with a CD30 costimulatory domain and another group of immune cells comprising the same CAR and a corresponding CSR with a non-CD30 costimulatory domain, *e.g.,* a 4-1BB costimlaroty domain, a CD28 costimulatory domain, or a Dap10 costimulatory domain.

[0039] In another aspect, the disclosure features a method for generating central memory T cells in a subject, comprising administering to the subject the nucleic acid(s) described herein, the vector(s) described herein, or the pharmaceutical composition described herein comprising the nucleic acid(s) or the vector(s) to the subject.

[0040] In some ~~embodiment~~examples, the method increases the number of central memory T cells and/or the percentage of central memory T cells among all T cells in the subject.

[0041] In another aspect, the disclosure provides a method for generating central memory T cells *in vitro* comprising: contacting one or more target cells with the immune cell described herein under conditions and for a time sufficient so that the immune cell develops into central memory T cells, wherein the target cells express an antigen specific to the immune cell.

[0042] In some ~~embodiment~~examples, the method increases the number of central memory T cells and/or the percentage of central memory T cells among all T cells decended from the immune cell.

[0043] In some ~~embodiment~~examples, the method generates higher number of central memory T cells and/or higher percentage of central memory T cells than corresponding immune cell expressing a CSR comprising a CD28 costimulatory domain.

[0044] In some ~~embodiment~~examples, the method generates at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, or 500% higher number of central memory T cells and/or percentage of central memory T cells than corresponding immune cell expressing a CSR comprising a CD28 costimulatory domain.

[0045] In some ~~embodiment~~examples of the methods described herein, immune cells (*e.g.,* $CD8^+$ T cells) expressing a 1^{st} generation CAR (*e.g.,* αAFP-CD8T-z-CAR) and CD30-CSR generates

more central memory T cells than immune cells (*e.g.,* $CD8^+$ T cells) expressing the 1st generation CAR alone. For example, in some ~~embodiment~~examples, the ratio of the number of central memory T cells generated by immune cells (*e.g.,* $CD8^+$ T cells) expressing a 1st generation CAR (*e.g.,* αAFP-CD8T-z-CAR) and CD30-CSR to the number of central memory T cells generated by immune cells (*e.g.,* $CD8^+$ T cells) expressing the 1st generation CAR alone is between 5 and 1000 (*e.g.,* between 5 and 900, between 5 and 800, between 5 and 700, between 5 and 600, between 5 and 500, between 5 and 400, between 5 and 300, between 5 and 200, between 5 and 100, between 5 and 50, between 5 and 10, between 10 and 1000, between 50 and 1000, between 100 and 1000, between 200 and 1000, between 300 and 1000, between 400 and 1000, between 500 and 1000, between 600 and 1000, between 700 and 1000, between 800 and 1000, or between 900 and 1000). For example, in some ~~embodiment~~examples, the ratio of the number of central memory T cells generated by immune cells (*e.g.,* $CD8^+$ T cells) expressing a 1st generation CAR (*e.g.,* αAFP-CD8T-z-CAR) and CD30-CSR to the number of central memory T cells generated by immune cells (*e.g.,* $CD8^+$ T cells) expressing the 1st generation CAR alone is between 1.5 and 8000 (*e.g.,* between 1.5 and 7000, between 1.5 and 6000, between 1.5 and 5000, between 1.5 and 4000, between 1.5 and 3000, between 1.5 and 2000, between 1.5 and 1000, between 1.5 and 500, between 1.5 and 100, between 10 and 8000, between 500 and 8000, between 1000 and 8000, between 2000 and 8000, between 3000 and 8000, between 4000 and 8000, between 5000 and 8000, between 6000 and 8000, or between 7000 and 8000).

[0046] In some ~~embodiment~~examples of the methods described herein, immune cells (*e.g.,* $CD8^+$ T cells) expressing a 2nd generation CAR (*e.g.,* αAFP-CD28z-CAR) and CD30-CSR generates more central memory T cells than immune cells (*e.g.,* $CD8^+$ T cells) expressing the 2nd generation CAR alone. For example, in some ~~embodiment~~examples, the ratio of the number of central memory T cells generated by immune cells (*e.g.,* $CD8^+$ T cells) expressing a 2nd generation CAR (*e.g.,* αAFP-CD28z-CAR) and CD30-CSR to the number of central memory T cells generated by immune cells (*e.g.,* $CD8^+$ T cells) expressing the 2nd generation CAR alone is between 0.5 and 3500 (*e.g.,* between 0.5 and 3000, between 0.5 and 2500, between 0.5 and 2000, between 0.5 and 1500, between 0.5 and 1000, between 0.5 and 500, between 0.5 and 100, between 0.5 and 50, between 50 and 3500, between 100 and 3500, between 500 and 3500, between 1000 and 3500, between 1500 and 3500, between 2000 and 3500, between 2500 and 3500, or between 3000 and 3500). For example, in some ~~embodiment~~examples, the ratio of the number of central memory T cells generated by immune cells (*e.g.,* $CD8^+$ T cells) expressing a 2nd

generation CAR (*e.g.,* αAFP-CD28z-CAR) and CD30-CSR to the number of central memory T cells generated by immune cells (*e.g.,* $CD8^+$ T cells) expressing the 2nd generation CAR alone is between 1.5 and 20,000 (*e.g.,* between 1.5 and 18,000, between 1.5 and 16,000, between 1.5 and 14,000, between 1.5 and 12,000, between 1.5 and 10,000, between 1.5 and 8,000, between 1.5 and 6,000, between 1.5 and 4,000, between 1.5 and 2,000, between 1.5 and 1,800, between 1.5 and 1,600, between 1.5 and 1,400, between 1.5 and 1,200, between 1.5 and 1,000, between 1.5 and 800, between 1.5 and 600, between 1.5 and 400, between 1.5 and 200, between 1.5 and 100, between 100 and 20,000, between 200 and 20,000, between 400 and 20,000, between 600 and 20,000, between 800 and 20,000, between 1000 and 20,000, between 1,200 and 20,000, between 1,400 and 20,000, between 1,600 and 20,000, between 1,800 and 20,000, between 2,000 and 20,000, between 4,000 and 20,000, between 6,000 and 20,000, between 8,000 and 20,000, between 10,000 and 20,000, between 12,000 and 20,000, between 14,000 and 20,000, between 16,000 and 20,000, or between 18,000 and 20,000).

[0047] In some ~~embodiment~~examples, the central memory T cells express high levels of CCR7 and low levels of CD45RA.

[0048] In some ~~embodiment~~examples, the central memory T cells are $CD8^+$ T cells.

[0048a] In another aspect, the disclosure provides the immune cell as described herein, the nucleic acid(s) as described herein, or the vector(s) as described herein, or the pharmaceutical composition as described herein for use in therapy.

[0048b] In another aspect, the disclosure provides a use of the immune cell as described herein, the nucleic acid(s) as described herein, or the vector(s) as described herein, or the pharmaceutical composition as described herein in the manufacture of a medicament for treating a disease in a subject, wherein the disease is a viral infection or cancer, optionally wherein the cancer is a hematological cancer or a solid tumor cancer; and/or optionally wherein the cancer is selected from the group consisting of adrenocortical carcinoma, bladder cancer, breast cancer, cervical cancer, cholangiocarcinoma, colorectal cancers, esophageal cancer, glioblastoma, glioma, hepatocellular carcinoma, head and neck cancer, kidney cancer, leukemia, lymphoma, lung cancer, melanoma, mesothelioma, multiple myeloma, pancreatic cancer, pheochromocytoma, plasmacytoma, neuroblastoma, ovarian cancer, prostate cancer, sarcoma, stomach cancer, uterine cancer, and thyroid cancer.

[0048c] In another aspect, the disclosure provides a use of the nucleic acid(s) as described herein, or the vector(s) as described herein, or the pharmaceutical composition as described herein

comprising the nucleic acid(s) or the vector(s) in the manufacture of a medicament for preventing and/or reversing T cell exhaustion in a subject, optionally wherein the expression of an exhaustion marker in a T cell is decreased, further optionally wherein the exhaustion marker is selected from the group consisting of PD-1, TIM-3, TIGIT, and LAG-3.

[0048d] In another aspect, the disclosure provides a use of corresponding immune cells and a corresponding CSR comprising a CD30 costimulatory domain in the manufacture of a medicament for treating a solid tumor cancer in a subject with increased tumor infiltration as compared to treating the same type of solid tumor cancer with immune cells expressing a CAR and a CSR comprising a CD28 or 4-1BB costimulatory domain, wherein the corresponding immune cells express the same CAR, and wherein the corresponding immune cells comprise the immune cell as described herein.

[0048e] In another aspect, the disclosure provides a use of corresponding immune cells and a corresponding CSR comprising a CD30 costimulatory domain in the manufacture of a medicament for treating a solid tumor cancer in a subject with increased tumor regression as compared to treating the same type of solid tumor cancer with immune cells expressing a CAR and a CSR comprising a CD28 or 4-1BB costimulatory domain, wherein the corresponding immune cells express the same CAR, and wherein the corresponding immune cells comprise the immune cell as described herein.

[0048f] In another aspect, the disclosure provides a use of the nucleic acid(s) as described herein, or the vector(s) as described herein, or the pharmaceutical composition as described herein comprising the nucleic acid(s) or the vector(s) in the manufacture of a medicament for generating central memory T cells in a subject, optionally wherein the number of central memory T cells and/or the percentage of central memory T cells among all T cells in the subject is increased.

[0048g] In another aspect, the disclosure provides corresponding immune cells and a corresponding CSR comprising a CD30 costimulatory domain for use in therapy, wherein the corresponding immune cells express the same CAR as a solid tumor cancer with immune cells expressing a CAR and a CSR comprising a CD28 or 4-1BB costimulatory domain.

DEFINITIONS

[0049] The scope of present invention is defined by the claims appended hereto and is not limited by particular embodiments described herein; those skilled in the art, reading the present disclosure, will be aware of various modifications that may be equivalent to such described embodiments, or otherwise within the scope of the claims.

[0050] In general, terminology used herein is in accordance with its understood meaning in the art, unless clearly indicated otherwise. Explicit definitions of certain terms are provided below; meanings of these and other terms in particular instances throughout this specification will be clear to those skilled in the art from context.

[0051] In order that the present invention may be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms are set forth throughout the specification.

[0052] Administration: As used herein, the term "administration" refers to the administration of a composition to a subject or system (*e.g.,* to a cell, organ, tissue, organism, or relevant