MULTIPLEX GENOME EDITING OF IMMUNE CELLS TO ENHANCE FUNCTIONALITY AND RESISTANCE TO SUPPRESSIVE ENVIRONMENT

BACKGROUND

[0001] This application claims priority to U.S. Provisional Patent Application Serial No. 62/772,406, filed November 28, 2018, which is incorporated by reference herein in its entirety.

1. Technical Field

[0002] The present invention relates generally to the fields of immunology, cell biology, molecular biology, and medicine. More particularly, it concerns multiplex editing of immune cells and methods of use thereof.

2. Description of Related Art

[0003] Cellular immunotherapy holds much promise for the treatment of cancer. However, most immunotherapeutic approaches when applied alone are of limited value against the majority of malignancies, especially solid tumors. Reasons for this limited success include reduced expression of tumor antigens on the surface of tumor cells, which reduces their detection by the immune system, the expression of ligands for inhibitory receptors such as PD1, NKG2A, TIGIT or CISH that induce immune cell inactivation; and the induction of cells (*e.g.,* regulatory T cells or myeloid-derived suppressor cells) in the microenvironment that release substances such as transforming growth factor-β (TGFβ) and adenosine that suppress the immune response and promote tumor cell proliferation and survival. Thus, there is an unmet need for improved methods of cellular immunotherapy.

SUMMARY

[0004] The disclosure provides compositions and methods related to cancer immunotherapy particularly including engineered immune cells. Specific examples concern certain immune cells that have been modified by the hand of man to lack expression of or have reduced expression of one, two, or more genes, and in specific cases the cells with such modification(s) also express one or more heterologous proteins, including non-natural proteins such antigen receptors. Also included are methods of producing the non-natural immune cells. In certain cases, the introduction of the heterologous antigen receptor is at the genomic locus of a gene being reduced or eliminated in expression.

[0005] In one example, the present disclosure provides an *in vitro* method for the disruption of at least two genes in an immune cell, wherein the at least two genes are selected from the group consisting of NKG2A, SIGLEC-7, LAG3, TIM3, CISH, FOXO1, TGFBR2, TIGIT, CD96, ADORA2, NR3C1, PD1, PDL-1, PDL-2, CD47, SIRPA, SHIP1, ADAM17, RPS6, 4EBP1, CD25, CD40, IL21R, ICAM1, CD95, CD80, CD86, IL10R, CD5, CD7, and a combination thereof. In one aspect, the present disclosure refers to an *in vitro* method for the disruption of at least two genes in an immune cell, wherein the at least two genes are selected from the group consisting of NKG2A, SIGLEC-7, LAG3, TIM3, CISH, FOXO1, TGFBR2, TIGIT, CD96, ADORA2, NR3C1, PD1, PDL-1, PDL-2, CD47, SIRPA, SHIP1, ADAM17, RPS6, 4EBP1, CD25, CD40, IL21R, ICAM1, CD95, CD80, CD86, IL10R, CD5, and CD7. In particular examples, three, four, five, or six or more genes are disrupted. In specific examples, the disruption of two or more genes is simultaneous, such as in the same method step. The method may comprise introducing a guide RNA (gRNA) for each gene to the immune cell.

[0006] The method can comprise the knockdown of particular combinations of genes, such as the following, for example: (a) NKG2A and CISH, (b) NKG2A and TGFBRII, (c) CISH and TGFBRII, (d) TIGIT and FOXO1, (e) TIGIT and TGFBRII, (f) CD96 and FOXO1, (g) CD96 and TGFBRII, (h) FOXO1 and TGFBRII, (i) CD96 and TIGIT, (j) CISH and TIGIT, (k) TIM3 and CISH, (l) TIM3 and TGFBRII, (m) FOXO1 and TGFBRII, (n) TIM3 and TIGIT, (o) SIGLEC7 and CISH, (p) SIGLEC7 and TGFBRII, (q) CD47 and CISH, (r) CD47 and TGFBRII, (s) SIRPA and CISH, (t) SIRPA and TGFBRII, (u) CD47 and TIGIT, (v) CD47 and SIRPA, (w) A2AR and CISH, (x) A2AR and TGFBRII, (y) ADAM17 and CISH, (z) TGFBRII and ADAM17, (a) A2AR and TIGIT, (b) SHP1 and CISH, (c) CISH and TGFBRII, (d) SHP1 and TGFBRII, (e) SHP1 and TIGIT, or (f) SHP1 and TIM3. The method can comprise the knockdown of (1) NKG2A, CISH, and TGFBRII, (2) TIGIT, FOXO1, and TGFBRII, (3) TGFBRII, CD96, and TIGIT, (4) TGFBR2, CISH, and TIGIT, (5) TIM3, CISH, and TGFBRII, (6) CD96, FOXO1, and TGFBRII, (7) TGFBRII, TIM3, and TIGIT, (8) SIGLEC7, CISH, and TGFBRII, (9) CD47, CISH, and TGFBRII, (10) SIRPA, CISH, and TGFBRII, (11) TGFBRII, CD47, and TIGIT, (12) TGFBRII, CD47, and SIRPA, (13) A2AR, CISH, and TGFBRII, (14) TGFBRII, CISH, and ADAM17, (15) TGFBRII, TIM3, and TIGIT, (16) TGFBRII, A2AR, and TIGIT, (17) SHP1, CISH, and TGFBRII, (18) TGFBRII, CISH, and SHP1, (19) TGFBRII, SHP1, and TIGIT, or (20) TGFBRII, SHP1, and TIM3. Any of the above subgroups may be combined with a second subgroup as disclosed above. For example, any one of subgroups a-j1 may be combined with any one or more of the other subgroups a-j1, any one or more of subgroups a-j1 may be combined with any one or more of the other subgroups 1-23, or any one or more of subgroups 1-23 may be combined with any one or more of the other subgroups 1-23.

[0007] In some examples, the method further comprises introducing to the cell an RNA-guide endonuclease, such as Cas9. Introducing the RNA-guided endonuclease may comprise introducing a nucleic acid, such as mRNA, encoding the RNA-guided endonuclease into the immune cell.

[0008] In certain examples, the immune cell is a T cell, NK cell, B cell, macrophage, NK T cell, or stem cell. In alternative cases, the immune cell is not a T cell, including not a CAR T cell. In some examples, the immune cell is engineered to express one or more chimeric antigen receptors (CAR) and/or one or more T cell receptors (TCR). The immune cell may be virus-specific, such as a virus-specific T cell. The T cell may be a regulatory T cell. The B cell may be a regulatory B cell. In some examples, the stem cell is a mesenchymal stem cell (MSC) or an induced pluripotent stem (iPS) cell. In particular examples, the T cell is a $CD8^+$ T cell, $CD4^+$ T cell, or gamma-delta T cell. The immune cell may be isolated from peripheral blood, cord blood, bone marrow, or a mixture thereof. In some examples, the cord blood is pooled from 2 or more individual cord blood units.

[0009] In some examples, an introducing step comprises transfecting or transducing. For example, introducing comprises electroporation that may performed more than once, such as two or three rounds of electroporation. In some examples, a first group of CRISPR gRNAs are introduced in a first electroporation and a second group of CRISPR gRNAs are introduced in a second round of electroporation. In specific cases, the first group of CRISPR gRNAs are different than the second group of CRISPR gRNAs. In particular examples, the first group and/or second group of CRISPR gRNAs comprise 1, 2, 3, or 4 or more CRISPR gRNAs. In some examples, two CRISPR gRNAs are introduced in a first electroporation and two different CRISPR gRNAs are introduced in a second round of electroporation. In specific examples, a group of CRISPR gRNAs comprises a group of gRNAs at least two of which target different genes; in particular examples, the group of gRNAs each target different genes.

[0010] In particular examples, the method comprises disrupting NKG2A, CD47, TGFβR2, and CISH; NKG2A, CISH, TGFβR2 and ADORA2; NKG2A, TGFβR2 and CISH; TIGIT, CD96, CISH, and ADORA2; or ADAM17, TGFβR2, NKG2A, and SHP1.

[0011] In some examples, the disruption results in enhanced antitumor cytotoxicity, *in vivo* proliferation, *in vivo* persistence, and/or improved function of the immune cell. In particular examples, the immune cell has increased secretion of IFN-γ, CD107, and/or TNFα,

compared to in the absence of the modification(s). In some examples, the immune cell has increased production of perforin and/or granzyme B, compared to in the absence of the modification(s).

[0012] In additional examples, the method further comprises introducing a CAR and/or TCR to an immune cell, such as introducing a nucleic acid encoding the CAR and/or TCR into the immune cell. In some examples, the nucleic acid is in an expression vector, such as a retroviral vector. In certain examples, the vector is an adenovirus-associated vector, such as AAV6. In some examples, the vector further comprises an inhibitory gene sequence, such as an inhibitory gene sequence selected from the group consisting of NKG2A, SIGLEC-7, LAG3, TIM3, CISH, FOXO1, TGFBR2, TIGIT, CD96, ADORA2, NR3C1, PD1, PDL-1, PDL-2, CD47, SIRPA, SHIP1, ADAM17, RPS6, 4EBP1, CD25, CD40, IL21R, ICAM1, CD95, CD80, CD86, IL10R, CD5, CD7, and a combination thereof. In particular examples, the vector further comprises a guide RNA for the inhibitory gene. The CAR may be flanked by homology arms for the inhibitory gene. In some examples, introducing the vector comprising the CAR sequence results in insertion of the CAR at an inhibitory gene locus, such as an exon of an inhibitory gene, in the immune cell, such that the CAR is under the control of the endogenous promoter of the inhibitory gene. In particular examples, introducing the vector further disrupts expression of the inhibitory gene.

[0013] In another example, there is provided an immune cell, such as an immune cell of the disclosed examples, with disrupted expression of at least two genes in the immune cell, produced at least by the step comprising introducing a CRISPR guide RNA (gRNA) for each gene to said immune cell, wherein at least two genes are selected from the group consisting of NKG2A, SIGLEC-7, LAG3, TIM3, CISH, FOXO1, TGFBR2, TIGIT, CD96, ADORA2, NR3C1, PD1, PDL-1, PDL-2, CD47, SIRPA, SHIP1, ADAM17, RPS6, 4EBP1, CD25, CD40, IL21R, ICAM1, CD95, CD80, CD86, IL10R, CD5, CD7, and a combination thereof. In some examples, three, four, five, or six or more genes are disrupted.

[0013a] In another aspect, the present disclosure refers to an immune cell with disrupted expression of at least two genes in the immune cell, wherein the at least two genes are selected from the group consisting of NKG2A, SIGLEC-7, LAG3, TIM3, CISH, FOXO1, TGFBR2, TIGIT, CD96, ADORA2, NR3C1, PD1, PDL-1, PDL-2, CD47, SIRPA, SHIP1, ADAM17, RPS6, 4EBP1, CD25, CD40, IL21R, ICAM1, CD95, CD80, CD86, IL10R, CD5, and CD7.

[0014] In certain examples, the immune cell is a T cell, NK cell, B cell, or stem cell. In some examples, the immune cell is engineered to express a chimeric antigen receptor (CAR) and/or T cell receptor (TCR). The immune cell may be virus-specific, such as a virus-specific T cell. The T cell may be a regulatory T cell. The B cell may be a regulatory B cell. In some examples, the stem cell is a mesenchymal stem cell (MSC) or an induced pluripotent stem (iPS) cell. In particular examples, the T cell is a $CD8^+$ T cell, $CD4^+$ T cell, or gamma-delta T cell. The

immune cell may be isolated from peripheral blood, cord blood, or bone marrow. In some examples, the cord blood is pooled from 2 or more individual cord blood units.

[0015] In particular examples, the method comprises disrupting particular groups of genes, such as NKG2A, CD47, TGFβR2, and CISH; NKG2A, CISH, TGFβR2 and ADORA2; NKG2A, TGFβR2 and CISH; TIGIT, CD96, CISH, and ADORA2; or ADAM17, TGFβR2 NKG2A and SHP1.

[0016] In some examples, the disruption results in enhanced antitumor cytotoxicity, *in vivo* proliferation, *in vivo* persistence, and/or improved function of the immune cell. In particular examples, the immune cell has increased secretion of IFN-γ, CD107, and/or TNFα. In some examples, the immune cell has increased production of perforin and/or granzyme B.

[0017] In some examples, the cell is engineered to express a CAR and/or TCR. The CAR may be inserted at an endogenous inhibitory gene locus of the cell, such as and inhibitory gene locus is selected from the group consisting of NKG2A, SIGLEC-7, LAG3, TIM3, CISH, FOXO1, TGFBR2, TIGIT, CD96, ADORA2, NR3C1, PD1, PDL-1, PDL-2, CD47, SIRPA, SHIP1, ADAM17, RPS6, 4EBP1, CD25, CD40, IL21R, ICAM1, CD95, CD80, CD86, IL10R, CD5, CD7, and a combination thereof. In some examples, the CAR is under the control of the endogenous promoter of the inhibitory gene. In certain examples, the CAR is inserted at the inhibitory gene locus by CRISPR-mediated gene editing.

[0018] In some examples, the CAR comprises an antigen-binding domain selected from the group consisting of F(ab')2, Fab', Fab, Fv, and scFv. In certain examples, the CAR targets one or more tumor associated antigens selected from the group consisting of CD19, CD319 (CS1), ROR1, CD20, carcinoembryonic antigen, alphafetoprotein, CA-125, MUC-1, epithelial tumor antigen, melanoma-associated antigen, mutated p53, mutated ras, HER2/Neu, ERBB2, folate binding protein, HIV-1 envelope glycoprotein gp120, HIV-1 envelope glycoprotein gp41, GD2, CD5, CD123, CD23, CD30, CD56, c-Met, mesothelin, GD3, HERV-K, IL-11Ralpha, kappa chain, lambda chain, CSPG4, ERBB2, WT-1, TRAIL/DR4, VEGFR2, CD33, CD47, CLL-1, U5snRNP200, CD200, BAFF-R, BCMA, CD99, and a combination thereof. In particular examples, the CAR comprises at least one signaling domain selected from the group consisting of CD3ξ, CD28, OX40/CD134, 4-1BB/CD137, FcεRIγ, ICOS/CD278, ILRB/CD122, IL-2RG/CD132, DAP12, CD70, CD40 and a combination thereof. In some examples, the immune cell comprises one or more heterologous cytokines, such as one or more

of IL-7, IL-2, IL-15, IL-12, IL-18, and IL-21. In certain examples, the CAR further comprises a suicide gene, such as a membrane bound nonsecretable TNF-alpha mutant or inducible caspase 9.

[0019] Further provided herein is an expression vector encoding at least one CAR and/or TCR, at least one inhibitory gene sequence, and at least one gRNA. In some examples, the inhibitory gene sequence is from an inhibitory gene selected from the group consisting of NKG2A, SIGLEC-7, LAG3, TIM3, CISH, FOXO1, TGFBR2, TIGIT, CD96, ADORA2, NR3C1, PD1, PDL-1, PDL-2, CD47, SIRPA, SHIP1, ADAM17, RPS6, 4EBP1, CD25, CD40, IL21R, ICAM1, CD95, CD80, CD86, IL10R, CD5, and CD7. In particular examples, the gRNA is specific to the inhibitory gene. In some examples, the vector is a viral vector, such as an AAV vector. The CAR may be flanked by homology arms for the inhibitory gene. Also provided herein is a host cell, such as a cell of the examples, engineered to express the vector of the examples. In examples, the cell is a T cell, NK cell, B cell, or stem cell.

[0019a] In another aspect, the present disclosure refers to a host cell engineered to express an expression vector encoding a CAR, inhibitory gene sequence, and gRNA.

[0020] Also provided herein is a pharmaceutical composition comprising a population of immune cells of the disclosed examples. In another aspect, the present disclosure refers to a pharmaceutical composition comprising a population of cells as disclosed herein. In another aspect, the present disclosure refers to a composition comprising a population of cells as disclosed herein for the treatment of an immune-related disorder, infectious disease, and/or cancer.

[0021] In a further example, there is provided a method of treating a disease or disorder in a subject comprising administering an effective amount of immune cells of the disclosed examples to the subject. In some examples, the disease or disorder is an infectious disease, cancer, such as a solid cancer or a hematologic malignancy, or an immune-related disorder. In another aspect, the present disclosure refers to use of immune cells as disclosed herein in the manufacture of a medicament for treating a disease or disorder in a subject. The immune-related disorder may be an autoimmune disorder, graft versus host disease, allograft rejection, or inflammatory condition, for example. In some examples, the immune-related disorder is an inflammatory condition and the immune cells have essentially no expression of glucocorticoid receptor. In certain examples, the immune cells are autologous or allogeneic with respect to a recipient individual.

[0022] In additional examples, the method further comprises administering at least a second therapeutic agent to the individual receiving the immune cells. In some examples, the at least a second therapeutic agent comprises chemotherapy, immunotherapy, surgery, radiotherapy, hormone therapy, or biotherapy. In certain examples, the immune cells and/or the at least a second therapeutic agent are administered intravenously, intraperitoneally,

intratracheally, intratumorally, intramuscularly, endoscopically, intralesionally, percutaneously, subcutaneously, regionally, or by direct injection or perfusion.

[0023] In another aspect, the present disclosure refers to a method for engineering an immune cell to express a CAR, said method comprising using a CRISPR gRNA to insert the CAR at an inhibitory gene locus of said immune cell. In some examples, the CAR is encoded by an expression vector, such as a retroviral vector, plasmid, lentiviral vector, adenoviral vector, adenovirus-associated viral vector, and so forth. In certain examples, the viral vector is an adenovirus-associated vector, such as AAV6.

[0024] In some examples, the vector further comprises an inhibitory gene sequence, such as an inhibitory gene sequence is selected from the group consisting of NKG2A, SIGLEC-7, LAG3, TIM3, CISH, FOXO1, TGFBR2, TIGIT, CD96, ADORA2, NR3C1, PD1, PDL-1, PDL-2, CD47, SIRPA, SHIP1, ADAM17, RPS6, 4EBP1, CD25, CD40, IL21R, ICAM1, CD95, CD80, CD86, IL10R, CD5, CD7 and a combination thereof. In some examples, the CRISPR gRNA is to the inhibitory gene. In certain examples, the CAR is flanked by homology arms for the inhibitory gene. In particular examples, the CAR is inserted at an inhibitory gene locus at any part of the gene, such as an exon of the inhibitory gene. The CAR may be under the control of the endogenous promoter of the inhibitory gene. In specific examples, the CAR disrupts the expression of the inhibitory gene.

[0025] In some examples, the CAR targets one or more tumor associated antigens selected from the group consisting of CD19, CD319 (CS1), ROR1, CD20, carcinoembryonic antigen, alphafetoprotein, CA-125, MUC-1, epithelial tumor antigen, melanoma-associated antigen, mutated p53, mutated ras, HER2/Neu, ERBB2, folate binding protein, HIV-1 envelope glycoprotein gp120, HIV-1 envelope glycoprotein gp41, GD2, CD5, CD123, CD23, CD30, CD56, c-Met, mesothelin, GD3, HERV-K, IL-11Ralpha, kappa chain, lambda chain, CSPG4, ERBB2, WT-1, TRAIL/DR4, VEGFR2, CD33, CD47, CLL-1, U5snRNP200, CD200, BAFF-R, BCMA, CD99, and a combination thereof. In particular examples, the CAR comprises at least one signaling domain selected from the group consisting of CD3ξ, CD28, OX40/CD134, 4-1BB/CD137, FcεRIγ, ICOS/CD278, ILRB/CD122, IL-2RG/CD132, DAP12, CD70, and CD40. In some examples, a vector that encodes the CAR also encodes a cytokine, such as IL-7, IL-2, IL-15, IL-12, IL-18, IL-21, or a combination thereof. In alternative cases, the cytokine is on a vector separate from the vector that encodes the CAR. In certain examples, an expression

construct that encodes the CAR further comprises a suicide gene, such as inducible caspase 9 or a membrane bound, nonsecretable TNF-alpha mutant.

[0026] Further provided herein is an immune cell with at least one CAR inserted at an inhibitory gene of the immune cell, such as an immune cell produced by the present methods. In another aspect, the present disclosure refers to an immune cell with a CAR inserted at an inhibitory gene of said immune cell. Also provided herein is a composition comprising a population of immune cells of examples of the disclosure, such as a population of T cells, B cells, NK cells, NK T cells, macrophages, stem cells, mixture thereof, and so forth. In another aspect, the present disclosure refers to a composition comprising a population of immune cells as disclosed herein.

[0027] Another example provides a composition comprising a population of cells of the examples, and in certain examples the population is utilized for treatment of a medical condition of any kind, including at least for the treatment of an immune-related disorder, infectious disease, and/or cancer. In another aspect, the present disclosure refers to a composition comprising a population of cells as disclosed herein for the treatment of an immune-related disorder, infectious disease, or cancer.

[0028] A further example provides a method of treating a disease or disorder in a subject comprising administering an effective amount of immune cells of the examples to the subject. In another aspect, the present disclosure refers to use of cells as disclosed herein in the manufacture of a medicament for treating a disease or disorder in a subject. In some examples, the disease or disorder is an infectious disease; cancer, such as a solid cancer or a hematologic malignancy; and/or an immune-related disorder. The immune-related disorder may be an autoimmune disorder, graft versus host disease, allograft rejection, and/or inflammatory condition, in some cases. In some examples, the immune-related disorder is an inflammatory condition and the immune cells have essentially no expression of glucocorticoid receptor. In certain examples, the immune cells are autologous or allogeneic with respect to a recipient individual.

[0029] In additional examples, the method further comprises administering at least a second therapeutic agent to an individual. In some examples, the at least a second therapeutic agent comprises chemotherapy, immunotherapy, surgery, radiotherapy, hormone therapy, or biotherapy. In certain examples, the immune cells and/or the at least a second therapeutic agent are administered intravenously, intraperitoneally, intratracheally, intratumorally, intramuscularly, endoscopically, intralesionally, percutaneously, subcutaneously, regionally, or by direct injection or perfusion. The immune cells and the at least a second therapeutic agent may be administered at the same time or at different times, and when they are administered at different times or at the same time but not in the same formulation, they may or may not be administered by the same route.

[0030] Other objects, features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating particular examples of the disclosure, are given by way of illustration only, since various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0032] FIG. 1: CRISPR/Cas9 mediates efficient multiple genes (NKG2A, CD47, TGFBR2, and CISH) disruption in NK cells. In this set of genes, NKG2A and CD47 were knocked out in the first round of electroporation and in the second round of electroporation CISH and TGFBR2 were targeted. Knockout efficiency was successfully validated using PCR and flow-cytometry for both rounds of electroporation. The red (peaks on the right) and blue (peaks on the left) histograms in the flow panels represent expression of the protein before and after CRISPR KO, respectively.

[0033] FIG. 2: Validation of multiplex gene editing in NK cells using another set of genes (TIGIT (T), CD96 (C), CISH (CH), Adenosine (A)). In this set of genes, TIGIT and CD96 were knocked out in the first round of electroporation and in the second round of electroporation CISH and Adenosine were targeted. Knockout efficiency was successfully validated using PCR and flow-cytometry for both rounds of electroporation. The red (peaks on the right) and blue (peaks on the left) histograms in the flow panels represent expression of the protein before and after CRISPR KO, respectively.

[0034] FIG. 3: Disruption of multiple genes (NKG2A, CD47, TGFBR2 and CISH) in NK cells leads to enhanced functionality against target tumor cells. There was enhanced IFN-γ, TNFα and CD107 secretion following stimulation with target cell lines. Flow cytometric analysis of IFN-γ, TNFα and CD107 production was performed with varying NK cells (Edited vs Cas9 alone) co-stimulated with target cell lines for 5 hr in the presence of Brefeldin A.

WHAT IS CLAIMED IS:

1. An *in vitro* method for the disruption of at least two genes in an immune cell, wherein the at least two genes are selected from the group consisting of NKG2A, SIGLEC-7, LAG3, TIM3, CISH, FOXO1, TGFBR2, TIGIT, CD96, ADORA2, NR3C1, PD1, PDL-1, PDL-2, CD47, SIRPA, SHIP1, ADAM17, RPS6, 4EBP1, CD25, CD40, IL21R, ICAM1, CD95, CD80, CD86, IL10R, CD5, and CD7.

2. The method of claim 1, wherein the at least two genes are selected from the group consisting of (a) NKG2A and CISH, (b) NKG2A and TGFBRII, (c) CISH and TGFBRII, (d) TIGIT and FOXO1, (e) TIGIT and TGFBRII, (f) CD96 and FOXO1, (g) CD96 and TGFBRII, (h) FOXO1 and TGFBRII, (i) CD96 and TIGIT, (j) CISH and TIGIT, (k) TIM3 and CISH, (l) TIM3 and TGFBRII, (m) FOXO1 and TGFBRII, (n) TIM3 and TIGIT, (o) SIGLEC7 and CISH, (p) SIGLEC7 and TGFBRII, (q) CD47 and CISH, (r) CD47 and TGFBRII, (s) SIRPA and CISH, (t) SIRPA and TGFBRII, (u) CD47 and TIGIT, (v) CD47 and SIRPA, (w) A2AR and CISH, (x) A2AR and TGFBRII, (y) ADAM17 and CISH, (z) TGFBRII and ADAM17, (a1) A2AR and TIGIT, (b1) SHP1 and CISH, (c1) CISH and TGFBRII, (d1) SHP1 and TGFBRII, (e1) SHP1 and TIGIT, and (f1) SHP1 and TIM3.

3. The method of claim 1 or 2, wherein at least 3 genes are selected from the group consisting of (1) NKG2A, CISH, and TGFBRII, (2) TIGIT, FOXO1, and TGFBRII, (3) TGFBRII, CD96, and TIGIT, (4) TGFBR2, CISH, and TIGIT, (5) TIM3, CISH, and TGFBRII, (6) CD96, FOXO1, and TGFBRII, (7) TGFBRII, TIM3, and TIGIT, (8) SIGLEC7, CISH, and TGFBRII, (9) CD47, CISH, and TGFBRII, (10) SIRPA, CISH, and TGFBRII, (11) TGFBRII, CD47, and TIGIT, (12) TGFBRII, CD47, and SIRPA, (13) A2AR, CISH, and TGFBRII, (14) TGFBRII, CISH, and ADAM17, (15) TGFBRII, TIM3, and TIGIT, (16) TGFBRII, A2AR, and TIGIT, (17) SHP1, CISH, and TGFBRII,

(18) TGFBRII, CISH, and SHP1, (19) TGFBRII, SHP1, and TIGIT, and (20) TGFBRII, SHP1, and TIM3.

4. The method of claim 1, 2 or 3, wherein the immune cell is a T cell, NK cell, NK T cell, B cell, or stem cell.

5. The method of claim 4, wherein the immune cell is engineered to express a chimeric antigen receptor (CAR) and/or T cell receptor (TCR).

6. The method of claim 4, wherein the immune cell is virus-specific.

7. The method of any one of claims 1-6, wherein the immune cell is isolated from cord blood and the cord blood is pooled from 2 or more individual cord blood units.

8. The method of any one of claims 1-7, further comprising introducing a CAR or TCR to said immune cell.

9. The method of claim 8, further comprising introducing a CAR or TCR into said immune cell, wherein said introducing comprises introducing a nucleic acid encoding said CAR or TCR into said immune cells, wherein the nucleic acid is in an expression vector, wherein the vector further comprises an inhibitory gene sequence.

10. The method of claim 9, wherein the inhibitory gene sequence is selected from the group consisting of NKG2A, SIGLEC-7, LAG3, TIM3, CISH, FOXO1, TGFBR2, TIGIT, CD96, ADORA2, NR3C1, PD1, PDL-1, PDL-2, CD47, SIRPA, SHIP1, ADAM17, RPS6, 4EBP1, CD25, CD40, IL21R, ICAM1, CD95, CD80, CD86, IL10R, CD5, and CD7.

11. The method of claim 9, wherein the vector further comprises a guide RNA for said inhibitory gene.

12. The method of claim 9, wherein the vector further comprises a guide RNA for said inhibitory gene, wherein the CAR is flanked by homology arms for said inhibitory

gene, wherein introducing the vector comprising the CAR sequence results in insertion of the CAR at the inhibitory gene locus in said immune cell.

13. The method of claim 12, wherein the CAR is inserted at an exon of said inhibitory gene.

14. The method of claim 12, wherein the CAR is under the control of the endogenous promoter of the inhibitory gene.

15. The method of claim 12, wherein introducing the vector further disrupts expression of said inhibitory gene.

16. An immune cell with disrupted expression of at least two genes in the immune cell, wherein the at least two genes are selected from the group consisting of NKG2A, SIGLEC-7, LAG3, TIM3, CISH, FOXO1, TGFBR2, TIGIT, CD96, ADORA2, NR3C1, PD1, PDL-1, PDL-2, CD47, SIRPA, SHIP1, ADAM17, RPS6, 4EBP1, CD25, CD40, IL21R, ICAM1, CD95, CD80, CD86, IL10R, CD5, and CD7.

17. The cell of claim 16, wherein the cell is produced according to any one of claims 1-15.

18. The cell of claim 16, wherein the immune cell is a T cell, NK cell, NK T cell, B cell, or stem cell.

19. The cell of claim 16, wherein the immune cell is engineered to express a chimeric antigen receptor (CAR) and/or T cell receptor (TCR).

20. The cell of claim 16, wherein the immune cell is virus-specific.

21. The cell of claim 16, wherein the cell is engineered to express a CAR and/or TCR.

22. The cell of claim 21, wherein the CAR is inserted at an endogenous inhibitory gene locus of said cell.

23. The cell of claim 22, wherein the inhibitory gene locus is selected from the group consisting of NKG2A, SIGLEC-7, LAG3, TIM3, CISH, FOXO1, TGFBR2, TIGIT, CD96, ADORA2, NR3C1, PD1, PDL-1, PDL-2, CD47, SIRPA, SHIP1, ADAM17,

RPS6, 4EBP1, CD25, CD40, IL21R, ICAM1, CD95, CD80, CD86, IL10R, CD5, and CD7.

24. The cell of claim 22, wherein the CAR is under the control of the endogenous promoter of said inhibitory gene.

25. The cell of claim 22, wherein the CAR was inserted at the inhibitory gene locus by CRISPR-mediated gene editing.

26. The cell of any one of claims 16-25, wherein the cell is engineered to express a heterologous cytokine selected from the group consisting of IL-7, IL-2, IL-15, IL-12, IL-18, IL-21, and a combination thereof.

27. A host cell engineered to express an expression vector encoding a CAR, inhibitory gene sequence, and gRNA.

28. The cell of claim 27, wherein the cell is a T cell, NK cell, B cell, or stem cell.

29. A pharmaceutical composition comprising a population of cells of any one of claims 16-28.

30. A composition comprising a population of cells of any one of claims 16-28 for the treatment of an immune-related disorder, infectious disease, or cancer.

31. Use of immune cells of any one of claims 16-26 in the manufacture of a medicament for treating a disease or disorder in a subject.

32. The use of claim 31, wherein the disease or disorder is an infectious disease, cancer, or immune-related disorder.

33. A method for engineering an immune cell to express a CAR, said method comprising using a CRISPR gRNA to insert the CAR at an inhibitory gene locus of said immune cell.

34. The method of claim 33, wherein the CAR is encoded by a vector that further comprises an inhibitory gene sequence.

35. The method of claim 34, wherein the inhibitory gene sequence is from an inhibitory gene selected from the group consisting of NKG2A, SIGLEC-7, LAG3, TIM3, CISH, FOXO1, TGFBR2, TIGIT, CD96, ADORA2, NR3C1, PD1, PDL-1, PDL-2, CD47, SIRPA, SHIP1, ADAM17, RPS6, 4EBP1, CD25, CD40, IL21R, ICAM1, CD95, CD80, CD86, IL10R, CD5, and CD7.

36. The method of any one of claims 33-35, wherein the CAR is flanked by homology arms for said inhibitory gene.

37. The method of any one of claims 33-36, wherein the CAR is inserted at an exon of said inhibitory gene.

38. The method of any one of claims 33-38, wherein the CAR is under the control of the endogenous promoter of the inhibitory gene.

39. The method of any one of claims 33-38, wherein the CAR disrupts the expression of said inhibitory gene.

40. The method of any one of claims 33-39, wherein the cell is engineered to express at least one heterologous cytokine selected from the group consisting of IL-7, IL-2, IL-15, IL-12, IL-18, IL-21, and a combination thereof.

41. An immune cell with a CAR inserted at an inhibitory gene of said immune cell.

42. The immune cell of claim 41, wherein the cell is produced by the method of any one of claims 33-40.

43. A composition comprising a population of immune cells of claim 41 or 42.

44. A composition comprising a population of cells of any one of claims 41-43 for the treatment of an immune-related disorder, infectious disease, or cancer.

45. Use of cells of any one of claims 16-26, 27, or 41-42 in the manufacture of a medicament for treating a disease or disorder in a subject.

46. The use of claim 45, wherein the disease or disorder is an infectious disease, cancer or immune-related disorder.