Claims

1. A modified cell or a population thereof, wherein:
(1) the modified cell is, relative to an unmodified counterpart cell:
deficient in an endogenous inhibitory checkpoint molecule; and
engineered to express a ligand of the deficient endogenous inhibitory checkpoint molecule; preferably the ligand is a variant of cognate ligand and is characterized in having reduced capability to induce immunoinhibitory signaling relative to the cognate ligand; or
(2) the modified cell is engineered to express a ligand of an inhibitory checkpoint molecule, wherein the ligand is a variant of cognate ligand and is characterized in having reduced capability to induce immunoinhibitory signaling relative to the cognate ligand; preferably the cell is deficient in the inhibitory checkpoint molecule corresponding to the ligand.

2. The modified cell of claim 1, wherein the cell is:
a) an animal cell or human cell;
b) an immune cell,
c) a stem cell or a cell differentiated from the stem cell;
d) suitable for transplantation;
e) allogeneic with reference to an intended recipient for the cell; or
f) any combination thereof;

preferably:
a) the cell is an immune cell and the immune cell is a T cell, Natural Killer (NK) cell, NKT cell, B cell, macrophage cell, tumor infiltrating lymphocyte, monocyte, dendritic cell (DC cell), neutrophil, or γδT cell; or
b) the cell is stem cell or a cell differentiated from the stem cell, and the stem cell is a hematopoietic progenitor cell (e.g. T cell progenitor cell, NK cell progenitor cell, macrophage progenitor cell), hematopoietic stem cell (HSCs), CD34+ cell, a cell line of embryonic stem cell, a mesenchymal stem cell or iPSC cell;

more preferably, the T cell is selected from the group consisting of CD4+ T cell, CD8+ T cell, cytotoxic T cell, terminal effector T cell, memory T cell, naïve T cell, regulatory T cell, natural killer T cell, gamma-delta T cell, cytokine-induced killer (CIK) T cell, and tumor infiltrating lymphocyte, and optionally is an activated T cell; or a) the inhibitory checkpoint molecule is selected from a group consisting of PD-1, TIM3, TIGIT, LAG3, A2AR, BTLA (CD272), CTLA-4 (CD152), IDO1, IDO2, TDO, KIR, NOX2, VISTA, SIGLEC7 (CD328), PVR(CD155), and SIGLEC9 (CD329); and/or b) the ligand is selected from a group consisting of PD-L1, PD-L2, HMGB1, Ceacam-1, phosphatidyl serine (PS), LSECtin, a-synuclein, FGL1, adenosine, HVEM (Herpesvirus Entry Mediator), CD28, B7-H3 (CD276), B7-H4 (VTCN1), PVR(CD155), MHC class I, sialoglycoprotein, CD112, CD113, Galectin9, CD24, and CD47;

preferably:
a) the inhibitory checkpoint molecule is PD-1, and the ligand comprises at least one of PD-L1 or a variant thereof, or PD-L2 or a functional equivalent thereof;
b) the inhibitory checkpoint molecule is TIM3, and the ligand comprises at least

one of galectin9, HMGB1, Ceacam-1 or phosphatidyl serine (PS), or a functional equivalent thereof;

c) the inhibitory checkpoint molecule is LAG-3, and the ligand comprises at least one of galectin-3, LSECtin, a-synuclein, or FGL1 or a functional equivalent thereof;
d) the inhibitory checkpoint molecule is TIGIT, and the ligand comprises at least one of CD155, CD113 or CD112 or a functional equivalent thereof;
e) the inhibitory checkpoint molecule is Adenosine A2A receptor (A2AR), and the ligand comprises adenosine;
f) the inhibitory checkpoint molecule is BTLA, and the ligand comprises HVEM (Herpesvirus Entry Mediator) or a functional equivalent thereof;
g) the inhibitory checkpoint molecule is CTLA-4, and the ligand comprises CD28 or a functional equivalent thereof;
h) the inhibitory checkpoint molecule is Killer-cell Immunoglobulin-like Receptor (KIR), and the ligand comprises MHC class I molecule (e.g. other than HLA-A, HLA-B) or a functional equivalent thereof;
i) the inhibitory checkpoint molecule is VISTA (V-domain Ig suppressor of T cell activation), and the ligand comprises at least one of PD-L1 or PD-L2 or a functional equivalent thereof;
j) the inhibitory checkpoint molecule is SIGLEC-7 (Sialic acid-binding immunoglobulin-type lectin 7, CD328), and the ligand comprises sialoglycoprotein or a functional equivalent thereof, or
k) the inhibitory checkpoint molecule is SIGLEC-9 (CD329), and the ligand comprises sialoglycoprotein or a functional equivalent thereof;

or

a) the cell is T cell and the inhibitory checkpoint molecule is selected from the group consisting of: PD-1, TIM3, TIGIT, LAG3, A2AR, BTLA (CD272), CTLA-4 (CD152), IDO1, IDO2, TDO, NOX2, VISTA, SIGLEC7 (CD328), PVR(CD155) and SIGLEC9 (CD329);
b) the cell is Natural Killer (NK) cell and the inhibitory checkpoint molecule is selected from the group consisting of: PD-1, TIM3, TIGIT, LAG3, A2AR, BTLA (CD272), IDO1, IDO2, KIR, TDO, NOX2, VISTA, SIGLEC7 (CD328), PVR(CD155) and SIGLEC9 (CD329);
c) the cell is dendritic cell (DC cell) and the inhibitory checkpoint molecule is selected from the group consisting of: A2AR, CD47, IDO1, IDO2, and TDO; or
d) the cell is macrophage and the inhibitory checkpoint molecule is selected from the group consisting of: A2AR, CD47, IDO1, IDO2, and CD24.

3. The modified cell of claim 1 or 2, wherein the variant lacks a functional immunoinhibitory signaling domain of the cognate ligand and/or the cognate ligand comprises an extracellular domain, a transmembrane domain and an intracellular domain; the at least one mutation is preferably within the intracellular domain of the cognate ligand, or within the transmembrane domain of cognate ligand, or within the extracellular domain of cognate ligand, or any combination thereof;
preferably, the variant comprises at least one mutation that reduces capability of the cognate ligand to induce immunoinhibitory signaling; the at least one mutation

preferably comprises a substitution, deletion, insertion, or any combination thereof; for example, the at least one mutation comprises deletion of at least 1 amino acid residue.

4. The modified cell of claim 2 or 3, wherein the variant is PD-L1 variant or PD-L2 variant; preferably the PD-L1 variant or the PD-L2 variant meets one or more of the following conditions:
(i) the PD-L1 variant comprises at least one mutation within the intracellular domain (e.g. SEQ ID NO: 1), or within the transmembrane domain (e.g. SEQ ID NO: 2), or within the extracellular domain (e.g. SEQ ID NO: 3), or within the domain spanning the extracellular domain and the intracellular domain (e.g. SEQ ID NO: 5), or any combination thereof, wherein the at least one mutation reduces capability of the PD-L1 to induce immunoinhibitory signaling;
(ii) the PD-L1 variant comprises deletion of at least part of the intracellular domain of PD-L1 (e.g. SEQ ID NO: 1), or deletion of at least part of the transmembrane domain of PD-L1 (e.g. SEQ ID NO: 2), or deletion of at least part of the extracellular domain of PD-L1 (e.g. SEQ ID NO: 3), or deletion of at least part of the domain spanning the extracellular domain and the intracellular domain (e.g. SEQ ID NO: 5), or any combination thereof; the PD-L1 variant preferably comprises an amino acid sequence of SEQ ID NO: 7;
(iii) the PD-L1 variant comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, 100% identical to SEQ ID NO: 11 or to an equal length portion of SEQ ID NO: 11, and the PD-L1 variant does not comprise an amino acid sequence of SEQ ID NO: 11.

5. The modified cell of any of claims 1-4, wherein the modified cell meets one or more of the following conditions:
(I) the modified cell comprise: a) a first mutation within a coding sequence or a regulatory sequence within a genomic region of the inhibitory checkpoint molecule, wherein the first mutation reduces expression or activity of the inhibitory checkpoint molecule; or b) a first interfering oligonucleotide targeting mRNA of the inhibitory checkpoint molecule, wherein the interfering oligonucleotide reduces expression of the inhibitory checkpoint molecule, c) an introduced protein, polypeptide or small molecule that inhibits the expression and/or activity of the inhibitory checkpoint molecule;
(II) the modified cell comprises a first exogenous polynucleotide comprising a coding sequence for the ligand; preferably, the first exogenous polynucleotide further comprises a coding sequence for a signal peptide operably linked to the coding sequence for the ligand; and/or the first exogenous polynucleotide further comprises a promoter operably linked to the coding sequence for the ligand; and/or the first exogenous polynucleotide is mRNA or an expression vector, optionally a viral expression vector.

6. The modified cell of any of the preceding claims, wherein one or more of the following conditions is met:

(A) the modified cell has introduced expression of the ligand;

(B) the cell is further deficient in a) MHC class I protein, or b) MHC class II protein or MHC class II transactivator, or c) both a) and b); preferably, the MHC class I

protein comprises HLA-A, or HLA-B, HLA-C, B2M, or any combination thereof, optionally, the cell is further deficient in both HLA-A and HLA-B; and/or the MHC class II protein comprises HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, HLA-DR, or any combination thereof, optionally, the cell is further deficient in HLA-DR; and/or the MHC class II transactivator is CIITA, optionally, the cell is deficient in CIITA;

(C) the cell is further deficient in endogenous TCR, optionally, the cell is further deficient in T cell receptor alpha chain constant region (TRAC), T cell receptor beta constant 1 (TRBC1), T cell receptor beta constant 2 (TRBC2), or any combination thereof;
preferably, in (B), the modified cell comprises: a) a second mutation within a coding sequence or a regulatory sequence within a genomic region of the MHC class I protein, wherein the second mutation reduces expression or activity of the MHC class I protein; or b) a second interfering oligonucleotide targeting mRNA of the MHC class I protein, thereby causing the MHC class I protein to have reduced expression therein; and/or the modified cell comprise: a) a third mutation within a coding sequence or a regulatory sequence within a genomic region of the MHC class II protein or MHC class II transactivator (e.g. CIITA), wherein the third mutation reduces expression or activity of the MHC class II protein or MHC class II transactivator (e.g. CIITA); or b) a third interfering oligonucleotide targeting mRNA of the MHC class II protein or MHC class II transactivator (e.g. CIITA), thereby causing the MHC class II protein or MHC class II transactivator (e.g. CIITA) to have reduced expression therein; and/or the modified cell comprise: a) a fourth mutation within a coding sequence or a regulatory sequence within a genomic region of the endogenous TCR or TRAC or TRBC1 or TRBC2, wherein the fourth mutation reduces expression or activity of the endogenous TCR or TRAC or TRBC1 or TRBC2; or b) a fourth interfering oligonucleotide targeting mRNA of the endogenous TCR or TRAC or TRBC1 or TRBC2, thereby causing the endogenous TCR or TRAC or TRBC1 or TRBC2 to have reduced expression therein;
more preferably, in (B), the second mutation, or the third mutation, or the fourth mutation or any combination thereof is introduced by means of gene editing;

(D) the modified cell has been modified, or is further modified, to introduce a second exogenous polynucleotide comprising a coding sequence for a polypeptide of interest, and optionally the modified cell expresses the polypeptide of interest; preferably, the polypeptide of interest comprises a chimeric antigen receptor (CAR) or an engineered TCR or other cell surface receptor or ligand; and/or the modified cell is a modified T cell, and optionally the modified T cell is further deficient in endogenous T cell receptor; more preferably, the CAR comprises an antigen binding domain, a transmembrane domain, and an intracellular signaling domain, further optionally the CAR further comprises a costimulatory signaling region; and/or the engineered TCR comprises an engineered antigen binding domain; and/or the antigen binding domain of the CAR or of the engineered TCR is capable of binding to a target antigen, optionally a target cell antigen; the target cell antigen comprises a tumor antigen, an inflammation-associated antigen, an autoimmune-associated antigen, or an infectious agent associated antigen; even more preferably, the intracellular signaling domain comprises a TCR signaling domain; and/or the target cell

antigen is associated with a disease selected from the group consisting of a cancer, an autoimmune disease, an infectious disease, a metabolic disease and a genetic disease; and/or the tumor antigen is BCMA, CD7, CD10, CD19, CD20, CD22, CD24, CD30, CD33, CD34, CD38, CD44, CD79a, CD79b, CD123, CD138, CD 179b, CEA, CLEC12A, Claudin18.2, CS-l, DLL3, EGFR, EGFRvIII, EPCAM, FLT-3, FOLR1, FOLR3, GD2, gpA33, GPC3, HER2, HM1.24, LGR5, Mesothelin, MSLN, MCSP, MICA/B, PSMA, PAMA, P-cadherin, or ROR1; and/or the cell is expanded *ex vivo*; and/or the population of the modified cells has one or more characteristics of the following:

a) reduced self-suppression,
b) improved effector cell function, and/or
c) improved cell activation and/or expansion,

relative to a comparative population of counterpart cells expressing both the endogenous inhibitory checkpoint molecule and the ligand to the endogenous inhibitory checkpoint molecule, or counterpart cells that are native cells (e.g. native immune cells).

7. A method of producing the modified cell of any of the preceding claims, comprising:

(1):

a) providing a starting cell;
b) modifying the starting cell to reduce expression or activity of the endogenous inhibitory checkpoint molecule and to increase expression or activity of the ligand of the endogenous inhibitory checkpoint molecule, thereby obtaining the modified cell; or

(2):

a) providing a starting cell expressing the endogenous inhibitory checkpoint and the ligand to the endogenous inhibitory checkpoint molecule; and
b) modifying the starting cell to reduce expression or activity of the endogenous inhibitory checkpoint molecule, thereby obtaining the modified cell; or

(3):

a) providing a starting cell deficient in the endogenous inhibitory checkpoint molecule; and
b) modifying the starting cell to increase expression or activity of the ligand to the endogenous inhibitory checkpoint molecule, thereby obtaining the modified cell;

preferably the step of modifying the starting cell to reduce expression or activity of the endogenous inhibitory checkpoint molecule comprises:

a) introducing to the starting cell a first mutation to a coding sequence or a regulatory sequence within a genomic region of the inhibitory checkpoint molecule, preferably the first mutation is introduced by means of gene editing, thereby causing the inhibitory checkpoint molecule to have reduced expression or reduced activity therein; or
b) introducing to the starting cell a first interfering oligonucleotide targeting mRNA of the inhibitory checkpoint molecule, thereby causing the inhibitory checkpoint molecule to have reduced expression therein;

more preferably, the gene editing comprises:

a) introducing to the cell i) a sequence-targeting protein, or a polynucleotide encoding the same, and ii) an oligonucleotide that is complementary to a target sequence in the coding sequence or a regulatory sequence within a genomic

region of the inhibitory checkpoint molecule; the sequence-targeting protein preferably comprises an RNA-guided DNA endonuclease, optionally Cas9; and/or the oligonucleotide preferably comprises a guide RNA sequence that is complementary to the target sequence.

8. The method of claim 7, wherein the step of modifying the starting cell to increase expression or activity of the ligand of the inhibitory checkpoint molecule comprises:
   a) introducing to the starting cell a first exogenous polynucleotide comprising a coding sequence for the ligand, thereby causing expression of the ligand therefrom; the first exogenous polynucleotide preferably further comprises a promoter operably linked to the coding sequence for the ligand;
   b) knocking out or knocking down one or more suppressive transcription factors;
   c) editing one or more regulatory sequences for expression of the gene encoding the ligand; or
   d) knocking in the gene encoding the ligand;

   preferably the first exogenous polynucleotide is an mRNA, an expression vector, optionally a retroviral vector, a lentiviral vector, an adenoviral vector, an adeno-associated viral (AAV) vector, a virus-like particle (VLP), a herpes simplex virus (HSV) vector, a plasmid, a minicircle, a nanoplasmid, a DNA vector, or an RNA vector; the starting cell is preferably a native cell or a differentiated cell, or an engineered cell genetically engineered from a native cell or a differentiated cell; more preferably, the expression vector is a viral expression vector, optionally a retroviral vector, a lentiviral vector, an adenoviral vector, an adeno-associated viral (AAV) vector, a virus-like particle (VLP), or a herpes simplex virus (HSV) vector; and/or the differentiated cell is differentiated from a stem cell; the stem cell is preferably a hematopoietic progenitor cell (e.g. T cell progenitor cell, NK cell progenitor cell, macrophage progenitor cell), hematopoietic stem cell (HSCs), CD34+ cell, a cell line of embryonic stem cell, a mesenchymal stem cell or iPSC cell.

9. A pharmaceutical composition or a kit comprising the modified cells of any one of claims 1-6, and a pharmaceutically acceptable medium.

10. The modified cells of any one of claims 1-6 for use in medicine.

11. Use of the modified cells of any one of claims 1-6 in the manufacture of a medicament for treating a condition or a disease, wherein the disease is selected from a cancer, an autoimmune disease, an infectious disease, aging, metabolic disease, and cardiovascular disease.

12. A protein comprising a PD-L1 variant, wherein the variant is characterized in having reduced capability to induce immunoinhibitory signaling relative to native PD-L1; preferably the PD-L1 variant lacks a functional immunoinhibitory signaling domain of native PD-L1; and/or the PD-L1 variant comprises at least one mutation that reduces capability to induce immunoinhibitory signaling; the at least one mutation preferably comprises a substitution, deletion, insertion, or any combination thereof; the at least one mutation is more preferably within the intracellular domain of PD-L1 (e.g. SEQ ID NO: 1), or within the transmembrane

domain of PD-L1(e.g. SEQ ID NO: 2), or within the extracellular domain of PD-L1 (e.g. SEQ ID NO: 3), or within the domain spanning the extracellular domain and the intracellular domain (e.g. SEQ ID NO: 5), or any combination thereof; and/or the at least one mutation comprises deletion of at least 1 amino acid residue; the deletion preferably comprises deletion of at least part of the intracellular domain of PD-L1 (e.g. SEQ ID NO: 1), or deletion of at least part of the transmembrane domain of PD-L1 (e.g. SEQ ID NO: 2), or deletion of at least part of the extracellular domain of PD-L1 (e.g. SEQ ID NO: 3), or deletion of at least part of the domain spanning the extracellular domain and the intracellular domain (e.g. SEQ ID NO: 5), or any combination thereof; the PD-L1 variant more preferably comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, 100% identical to SEQ ID NO: 11 or to an equal length portion of SEQ ID NO: 11 and does not comprise an amino acid sequence of SEQ ID NO: 11; the PD-L1 variant even more preferably comprises an amino acid sequence of SEQ ID NO: 7; for example, the PD-L1 variant is linked to a polypeptide of interest, optionally via a linker; the linker is preferably cleavable; the polypeptide of interest more preferably comprises a chimeric antigen receptor (CAR), an engineered TCR or other cell surface receptor or ligand; and/or the protein further comprises a signal peptide.

13. A polynucleotide encoding the protein of claim 12; an expression vector comprising the polynucleotide; or a recombinant cell comprising the expression vector.

14. A method of producing the recombinant cell, comprising:
(1) the recombinant cell is the recombinant cell of claim 13, and introduce to a starting cell the expression vector under conditions suitable for expression of the polynucleotide in the cell; or
(2) the recombinant cell is the recombinant cell with increased immunotolerance, and introduce to the cell the expression vector under conditions suitable for expression of the polynucleotide in the cell, thereby increasing immunotolerance of the recombinant cell;
wherein the polynucleotide and the expression vector are as defined in claim 13.

15. A population of cells produced ex vivo by the method of claim 14, wherein the recombinant cell is the recombinant cell of claim 13, and introduce to a starting cell the expression vector under conditions suitable for expression of the polynucleotide in the cell; preferably at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of the population of cells express a detectable level of the protein of claim 12.

16. Use of the recombinant cell of claim 13, or modified cells of any one of claims 1-6 in the manufacture of a medicament for transplanting cells to a subject with reduced risk of immune rejection, wherein the recombinant cell or modified cells are allogeneic to the subject.