

NON-HLA RESTRICTED T CELL RECEPTORS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application Serial No.
5 62/629,072, filed February 11, 2018, the content of which is incorporated by reference in
its entirety, and to which priority is claimed.

INTRODUCTION

The presently disclosed subject matter provides methods and compositions for
enhancing the immune response toward cancers and pathogens. It relates to novel designs
10 of T cell receptors (TCRs) and engineered immunoresponsive cells comprising the same.
The engineered immunoresponsive cells comprising the novel TCRs are antigen-directed.

BACKGROUND OF THE INVENTION

Adoptive immunotherapy using antigen recognizing receptors (e.g., chimeric
15 antigen receptors (CARs)) has shown remarkable clinical results in the treatment of
leukemia and is one of the most promising new strategies to treat cancer. To generate
CAR therapies, current clinical protocols employ autologous T cells and randomly
integrating vectors, including gamma-retroviral, lentiviral and transposons, which all
result in semi-random integration and variable expression of the CAR owing to transgene
20 variegation. Altogether, the conjunction of autologous cell sourcing and random vector
integration is prone to generating cell products with variable potency. Thus, there is a
need of novel designs of antigen recognizing receptors having consistent potency and
increased ability to detect low levels of target antigen.

25 SUMMARY

The presently disclosed subject matter generally provides HLA-independent (or
non-HLA restricted) T cell receptors (referred to as “HI-TCRs”) that bind to an antigen of
interest in an HLA-independent manner and immunoresponsive cells comprising thereof.
The presently disclosed subject matter also provides methods of using such cells for
30 inducing and/or enhancing an immune response to a target antigen, and/or treating and/or
preventing neoplasia or other diseases/disorders where an increase in an antigen-specific
immune response is desired.

The presently disclosed subject matter provides recombinant T cell receptor (TCR) comprising an antigen binding chain that comprises an extracellular antigen-binding domain and a constant domain, wherein the recombinant TCR binds to an antigen in an HLA-independent manner.

5 In one aspect, provided herein is a recombinant T cell receptor (TCR) comprising an antigen binding chain that comprises:

a) an antigen-binding fragment of an immunoglobulin variable region that binds to an antigen in an HLA-independent manner; and

b) a constant domain that is capable of associating with a CD3 ζ polypeptide.

10 In certain examples, the constant domain comprises a native or modified TRAC peptide, and/or a native or modified TRBC peptide. In certain examples, the constant domain is capable of forming a homodimer or a heterodimer with another constant domain.

In certain examples, the recombinant TCR is expressed from an expression
15 cassette placed in an endogenous *TRAC* locus and/or a *TRBC* locus of an immunoresponsive cell. In certain examples, the placement of the recombinant TCR expression cassette disrupts or abolishes the endogenous expression of a TCR comprising a native TCR α chain and/or a native TCR β chain in the immunoresponsive cell. In certain examples, the placement of the recombinant TCR expression cassette prevents or
20 eliminates mispairing between the recombinant TCR and a native TCR α chain and/or a native TCR β chain in the immunoresponsive cell. In certain examples, the antigen binding chain is capable of associating with a CD3 ζ polypeptide. The antigen binding chain, upon binding to an antigen, is capable of activating the CD3 ζ polypeptide associated to the antigen binding chain. The activation of the CD3 ζ polypeptide is
25 capable of activating an immunoresponsive cell. The CD3 ζ polypeptide can be endogenous or exogenous. In certain examples, the CD3 ζ polypeptide is endogenous and is endogenous and integrated in the native CD3 complex. In certain examples, the CD3 ζ polypeptide is exogenous and optionally integrated with a co-stimulatory molecule selected from the group consisting of a CD28 polypeptide, a 4-1BB polypeptide, an
30 OX40 polypeptide, an ICOS polypeptide, a DAP-10 polypeptide and any combination thereof. In certain examples, the antigen binding chain further comprises a co-stimulatory region, wherein the recombinant TCR, upon binding to an antigen, is capable of stimulating an immunoresponsive cell. The co-stimulatory region can include

a co-stimulatory molecule selected from the group consisting of a CD28 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, an ICOS polypeptide, a DAP-10 polypeptide and any combination thereof. In certain examples, the co-stimulatory region comprises a CD28 polypeptide.

- 5 In certain examples, the recombinant TCR is capable of associating with a CD3 complex. In certain examples, the recombinant TCR is capable of integrating

with a CD3 complex and providing HLA-independent antigen recognition. In certain examples, the CD3 complex is endogenous. In certain examples, the recombinant TCR replaces an endogenous TCR in a CD3/TCR complex.

In certain examples, the extracellular antigen-binding domain is capable of dimerizing with another extracellular antigen-binding domain. The extracellular antigen-binding domain can include a ligand for a cell-surface receptor, a receptor for a cell surface ligand, an antigen binding portion of an antibody or a fragment thereof or an antigen binding portion of a TCR. In certain examples, the extracellular antigen-binding domain comprises a heavy chain variable region (V_H) of an antibody, or a VHH from a camelid V_H -only antibody and/or a light chain variable region (V_L) of an antibody. In certain examples, the extracellular antigen-binding domain is capable of dimerizing with another extracellular antigen-binding domain. In certain examples, the extracellular antigen-binding domain comprises a V_H of an antibody, wherein the human, murine, or camelid V_H is capable of dimerizing with another extracellular antigen-binding domain comprising a V_L of the antibody and form a fragment variable (Fv). In certain examples, the extracellular antigen-binding domain comprises a V_L of an antibody, wherein the V_L is capable of dimerizing with another extracellular antigen-binding domain comprising a V_H of the antibody and form a fragment variable (Fv).

In certain examples, the recombinant TCR binds to a tumor antigen. The tumor antigen can be selected from the group consisting of CD19, MUC16, MUC1, CAIX, CEA, CD8, CD7, CD10, CD20, CD22, CD30, CLL1, CD33, CD34, CD38, CD41, CD44, CD49f, CD56, CD74, CD133, CD138, EGP-2, EGP-40, EpCAM, erb-B2,3,4, FBP, Fetal acetylcholine receptor, folate receptor-a, GD2, GD3, HER-2, hTERT, IL-13R-a2, K-light chain, KDR, LeY, L1 cell adhesion molecule, MAGE-A1, Mesothelin, ERBB2, MAGEA3, p53, MART1, GP100, Proteinase3 (PR1), Tyrosinase, Survivin, hTERT, EphA2, NKG2D ligands, NY-ES0-1, oncofetal antigen (h5T4), PSCA, PSMA, ROR1, TAG-72, VEGF-R2, WT-1, BCMA, CD123, CD44V6, NKCS1, EGF1R, EGFR-VIII, and CD99, CD70, ADGRE2, CCR1, LILRB2, LILRB4, PRAME, and ERBB. In certain examples, the tumor antigen is CD19.

In certain examples, the recombinant TCR exhibits greater antigen sensitivity than a CAR targeting the same antigen. In certain examples, the recombinant TCR is capable of inducing an immune response when binds to an antigen that has a low density

on the surface of a tumor cell. In certain examples, the antigen that has a low density on the cell surface has below about 2,000 molecules per cell.

In one aspect, the presently disclosed subject matter further provides immunoresponsive cells comprising a recombinant TCR described herein. In certain examples, the expression cassette of at least one antigen binding chain of the recombinant TCR is placed at an endogenous gene locus of the immunoresponsive cell. In certain examples, the expression cassettes of two antigen binding chains of the recombinant TCR are placed at an endogenous gene locus of the immunoresponsive cell, wherein the two antigen binding chains are capable of dimerization. The placement of the expression cassette of the recombinant TCR can disrupt or abolish the endogenous expression of a TCR comprising a native TCR α chain and/or a native TCR β chain in the immunoresponsive cell, whereby preventing or eliminating mispairing between the recombinant TCR and a native TCR α chain and/or a native TCR β chain in the immunoresponsive cell. The endogenous gene locus can be a *CD3 δ* locus, a *CD3 ϵ* locus, a *CD247* locus, a *B2M* locus, a *TRAC* locus, a *TRBC* locus or a *TRGC* locus or a *TRDC* locus. In certain examples, the endogenous gene locus is a *TRAC* locus and/or a *TRBC* locus. The endogenous gene locus can include a modified transcription terminator region. In certain examples, the modified transcription terminator region comprises a genomic element selected from the group consisting of a TK transcription terminator, a GCSF transcription terminator, a TCRA transcription terminator, an HBB transcription terminator, a bovine growth hormone transcription terminator, a SV40 transcription terminator and a P2A element; the P2A element allows the use of the endogenous transcription terminator of the targeted gene. In certain examples, when one endogenous T cell receptor locus in a cell is modified to express the at least one antigen binding chain of the recombinant TCR, one or more other endogenous T cell receptor locus in the cell is modified to eliminate the expression of an endogenous TCR chain. In certain examples, the one or more other endogenous T cell receptor loci are further modified to express a gene of interest. The gene of interest can be an anti-tumor cytokine, a co-stimulatory molecule ligand, a tracking gene or a suicide gene. In certain examples, one or more endogenous TCR loci are further modified to incorporate a sequence encoding co-stimulatory signaling domain(s) to generate a TCR chain containing such signaling domain(s) at the carboxy terminus.

In certain examples, the immunoresponsive cell is selected from the group consisting of a T cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, a human embryonic stem cell, and a pluripotent stem cell from

which lymphoid cells may be differentiated. In certain examples, the immunoresponsive cell is autologous.

In certain examples, the immunoresponsive cell further comprises at least one exogenous co-stimulatory ligand. In certain examples, the co-stimulatory ligand is
5 selected from the group consisting of CD80, CD86, 41BBL, CD275, CD40L, OX40L and any combination thereof. In certain examples, the cell further comprises or consists of one exogenous co-stimulatory ligand. In certain examples, the one exogenous co-stimulatory ligand is CD80 or 4-1BBL. In certain examples, the cell further comprises or consists of two exogenous co-stimulatory ligands. In certain examples, In certain
10 examples, the two exogenous co-stimulatory ligands are CD80 and 4-1BBL.

In certain examples, the immunoresponsive cell further comprises at least one chimeric costimulatory receptor (CCR). In certain examples, the CCR comprising a co-stimulatory molecule selected from the group consisting of a CD28 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, an ICOS polypeptide, a DAP-10 polypeptide and any
15 combination thereof.

In another aspect, the presently disclosed subject matter also provides pharmaceutical compositions comprising the immunoresponsive cell(s) disclosed herein and a pharmaceutically acceptable excipient. The pharmaceutical composition can be used for treating a neoplasia.

20 Further provided are methods of reducing tumor burden in a subject. In addition, the presently disclosed subject matter provides methods of lengthening survival of a subject having a neoplasm (e.g., cancer). In certain examples, the method comprises administering to the subject an effective amount of the immunoresponsive cells described herein or the pharmaceutical composition described herein.

25 The presently disclosed subject matter also provides methods of treating or preventing a neoplasm. In certain examples, the method comprises administering to the subject an effective amount of the immunoresponsive cells described herein or the pharmaceutical composition described herein. The neoplasm can be selected from the group consisting of blood cancer, B cell leukemia, multiple myeloma, acute
30 lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, non-Hodgkin's lymphoma and adenocarcinoma. In certain examples, the neoplasm is B cell leukemia, multiple myeloma, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, or non-Hodgkin's lymphoma, and the recombinant TCR binds to CD19. In

certain examples, the neoplasm is B cell leukemia, multiple myeloma, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, an adenocarcinoma, or non-Hodgkin's lymphoma, and the recombinant TCR binds to CD19, MUC16, MUC1, CAIX, CEA, CD8, CD7, CD10, CD20, CD22, CD30, CLL1, CD33, CD34, CD38, CD41, CD44, CD49f, CD56, CD74, CD133, CD138, EGP-2, EGP-40, EpCAM, erb-B2,3,4, FBP, Fetal acetylcholine receptor, folate receptor-a, GD2, GD3, HER-2, hTERT, IL-13R-a2, K-light chain, KDR, LeY, L1 cell adhesion molecule, MAGE-A1, Mesothelin (MSLN), ERBB2, MAGEA3, p53, MART1, GP100, Proteinase3 (PR1), Tyrosinase, Survivin, hTERT, EphA2, NKG2D ligands, NY-ES0-1, oncofetal antigen (h5T4), PSCA, PSMA, ROR1, TAG-72, VEGF-R2, WT-1, BCMA, CD123, CD44V6, NKCS1, EGF1R, EGFR-VIII, CD99, CD70, ADGRE2, CCR1, LILRB2, LILRB4, PRAME, and ERBB. In certain examples, the neoplasm is CD19+ ALL.

In one aspect, the presently disclosed subject matter further provides methods for producing an antigen-specific immunoresponsive cell. In certain examples, the method comprises introducing into an immunoresponsive cell a nucleic acid sequence encoding a recombinant TCR described herein. The nucleic acid sequence can be comprised in a vector. In certain examples, the expression cassette of at least one antigen binding chain of the recombinant TCR is placed at an endogenous gene locus of the immunoresponsive cell. In certain examples, the expression cassettes of two antigen binding chains of the recombinant TCR are placed at an endogenous gene locus of the immunoresponsive cell, wherein the two antigen binding chains are capable of dimerization. The endogenous gene locus can be a *CD3δ* locus, a *CD3ε* locus, a *CD247* locus, a *B2M* locus, a *TRAC* locus, a *TRBC* locus, a *TRDC* locus and/or a *TRGC* locus. In certain examples, the endogenous gene locus is a *TRAC* locus or a *TRBC* locus. In certain examples, the placement of the expression cassette of the recombinant TCR disrupts or abolishes the endogenous expression of a TCR comprising a native TCR α chain and/or a native TCR β chain in the immunoresponsive cell, whereby preventing or eliminating mispairing between the recombinant TCR and a native TCR α chain and/or a native TCR β chain in the immunoresponsive cell. In certain examples, the endogenous gene locus comprises a modified transcription terminator region. In certain examples, the modified transcription terminator region comprises a genomic element selected from the group consisting of a TK transcription terminator, a GCSF transcription terminator, a TCRA transcription terminator, an HBB transcription terminator, a bovine growth hormone transcription terminator, an SV40 transcription

terminator and a P2A element. In certain examples, when one endogenous T cell receptor locus in a cell is modified to express the at least one antigen binding chain of the recombinant TCR, one or more other endogenous T cell receptor locus in the cell is modified to eliminate the expression of an endogenous TCR chain. In certain examples, the one or more other
5 endogenous T cell receptor locus are further modified to express a gene of interest. In certain examples, the gene of interest is an anti-tumor cytokine, a co-stimulatory molecule ligand, a tracking gene or a suicide gene.

In one aspect, the presently disclosed subject matter further provides nucleotide acids encoding a recombinant TCR described herein, and nucleic acid compositions comprising a
10 recombinant TCR described herein. In certain examples, the nucleic acid sequences are comprised in a vector. In another aspect, the presently disclosed subject matter also provides vectors comprising the nucleic acid composition described herein. In another aspect, further provided are kits comprising a recombinant TCR described herein, an immunoresponsive cell described herein, a pharmaceutical composition described herein, a nucleic acid composition
15 described herein, or a vector described herein. In certain examples, the kit further comprises written instructions for treating and/or preventing a neoplasm, a pathogen infection, an autoimmune disorder, or an allogeneic transplant.

Illustrative neoplasms for which the presently disclosed subject matter can be used include, but are not limited to leukemias (e.g., acute leukemia, acute lymphocytic leukemia,
20 acute myelocytic leukemia a, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute monocytic leukemia, acute erythroleukemia, chronic leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia), polycythemia vera, lymphoma (Hodgkin's disease, non-Hodgkin's disease), Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors such as sarcomas and carcinomas (e.g., fibrosarcoma,
25 myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangi endotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma,
30 papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, uterine cancer, testicular cancer, lung carcinoma, small cell lung carcinoma, bladder

carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, schwannoma, meningioma, melanoma, neuroblastoma, and retinoblastoma).

5 The presently disclosed subject matter further provides use of any recombinant TCR, any pharmaceutical composition or any immunoresponsive cell disclosed herein for used in a therapy.

 In another aspect, the presently disclosed subject matter provides an immunoresponsive cell as described herein or the pharmaceutical composition as
10 described herein for use in therapy.

 In another aspect, the presently disclosed subject matter provides a use of the immunoresponsive cell as described herein or the pharmaceutical composition as described herein in the manufacture of a medicament for reducing tumor burden in a subject, treating and/or preventing a neoplasm, a pathogen infection, and/or an
15 autoimmune disorder, and/or lengthening survival of a subject having neoplasm.

BRIEF DESCRIPTION OF THE FIGURES

 The following Detailed Description, given by way of example, but not intended to limit the presently disclosed subject matter to specific examples described, may be understood in conjunction with the accompanying drawings.

20 Figures 1A-1E depict HLA-Independent TCR-based Chimeric Antigen Receptor HIT (HIT-CAR, i.e., HI-TCR or HIT) and gene targeting strategy at the TRAC locus in human T cells. (A) Schematic representation of the T Cell Receptor (TCR), the B Cell Receptor (BCR), a Chimeric Antigen Receptor (CAR) and the HLA-Independent TCR-based Chimeric Antigen Receptor (HIT-CAR, i.e., HI-TCR or HIT). (B) CRISPR/Cas9-
25 targeted integration of the 3 receptors into the TRAC locus. Top: TRAC locus; middle: rAAV6 containing the different receptor cassette flanked by homology arms. (C) Representative TCR/Mouse F(ab')₂ flow cytometry plots 4 days after TRAC targeting. The TCR antibody epitope recognize the constant chain of the TCR alpha and beta. (D) Cytotoxic activity using an 18-hour bioluminescence assay, using firefly luciferase
30 (FFL)-expressing NALM-6 as targets cells (n=3). (E) Relative CAR MFI (1=MFI at 0h) of CAR T cells after 1, 2 or 4 (arrows) stimulations on CD19 positive target cells.

 Figures 2A-2B depict expression and therapeutic efficacy of HI-TCR. (A) Representative TCR/Mouse F(ab')₂ flow cytometry plots 4 days after TRAC targeting.

(B) Kaplan-Meier analysis of the mice survival where NALM-6- bearing mice were treated with 5×10^5 CAR T cells.

Figures 3A-3E depict gene targeting strategy and expression of NYESO TCR.

- (A) Schematic representation of the NYESO TCR genes integrated into the TCR alpha or
5 beta chain. (B) Representative TCR-V-beta-1 flow cytometry plots 4 days after TRAC or TRBC targeting. (C) Cytotoxic activity using an 18-hour bioluminescence assay, using firefly luciferase (FFL)-expressing PC3 as targets cells (n=3). (D) Schematic representation of a co-targeting into both the TCR alpha and the TCR beta. (E)

What is claimed is:

1. A recombinant T cell receptor (TCR) comprising an antigen binding chain that comprises:
 - a) an antigen-binding fragment of an immunoglobulin variable region that binds to an antigen in an HLA-independent manner; and
 - b) a constant domain that is capable of associating with a CD3 ζ polypeptide.
2. The immunoresponsive cell of claim 1, wherein the constant domain of the TCR comprises a native or modified TRAC polypeptide.
3. The immunoresponsive cell of claim 1 or 2, wherein the constant domain of the TCR comprises a native or modified TRBC polypeptide.
4. The immunoresponsive cell of any one of claims 1-3, wherein the CD3 ζ polypeptide is a native CD3 ζ polypeptide or a modified CD3 ζ polypeptide.
5. The recombinant TCR of any one of claims 1-4, wherein the CD3 ζ polypeptide is fused to an intracellular domain of a co-stimulatory molecule or a fragment thereof.
6. The recombinant TCR of claim 5, wherein the co-stimulatory molecule is CD28, 4-1BB, OX40, ICOS, DAP-10, or a combination thereof.
7. The recombinant TCR of any one of claims 1-6, wherein the antigen binding chain further comprises a co-stimulatory region that is capable of stimulating an immunoresponsive cell upon the binding of the antigen binding chain to the antigen.
8. The recombinant TCR of claim 7, wherein the co-stimulatory region comprises an intracellular domain of a co-stimulatory molecule or a fragment thereof, optionally wherein the co-stimulatory molecule is CD28, 4-1BB, OX40 polypeptide, ICOS polypeptide, DAP-10, or a combination thereof.
9. The recombinant TCR of claim 8, wherein the co-stimulatory molecule comprises CD28.
10. The recombinant TCR of any one of claims 1-9, wherein the antigen-binding fragment comprises:

- (i) a heavy chain variable region (V_H) of an antibody; and/or
 - (ii) a light chain variable region (V_L) of an antibody.
11. The recombinant TCR of any one of claims 1-10, wherein the recombinant TCR binds to a tumor antigen.
 12. The recombinant TCR of claim 11, wherein the tumor antigen is selected from the group consisting of CD19, MUC16, MUC1, CAIX, CEA, CD8, CD7, CD10, CD20, CD22, CD30, CLL1, CD33, CD34, CD38, CD41, CD44, CD49f, CD56, CD74, CD133, CD138, EGP-2, EGP-40, EpCAM, Erb-B2, Erb-B3, Erb-B4, FBP, Fetal acetylcholine receptor, folate receptor- α , GD2, GD3, HER-2, hTERT, IL-13R- α 2, κ -light chain, KDR, LeY, L1 cell adhesion molecule, MAGE-A1, Mesothelin, ERBB2, MAGEA3, p53, MART1, GP100, Proteinase3 (PR1), Tyrosinase, Survivin, hTERT, EphA2, NKG2D ligands, NY-ESO-1, oncofetal antigen (h5T4), PSCA, PSMA, ROR1, TAG-72, VEGF-R2, WT-1, BCMA, CD123, CD44V6, NKCS1, EGF1R, EGFR-VIII, CD99, CD70, ADGRE2, CCR1, LILRB2, LILRB4, PRAME, and ERBB; optionally wherein the tumor antigen is CD19.
 13. The recombinant TCR of any one of claims 1-12, wherein the recombinant TCR is capable of inducing an immune response when binds to an antigen that has a low density on the surface of a tumor cell; optionally wherein the antigen that has a low density on the cell surface has a density of less than about 10,000 molecules per cell.
 14. An immunoresponsive cell comprising a recombinant TCR of any one of claims 1-13.
 15. The immunoresponsive cell of claim 14, wherein the recombinant TCR is expressed by a transgene that is integrated at an endogenous gene locus of the immunoresponsive cell.
 16. The immunoresponsive cell of claim 15, wherein the endogenous gene locus is a *CD3 δ* locus, a *CD3 ϵ* locus, a *CD247* locus, a *B2M* locus, a *TRAC* locus, a *TRBC* locus, a *TRDC* locus, and/or a *TRGC* locus; optionally wherein the endogenous gene locus is a *TRAC* locus and/or a *TRBC* locus.
 17. The immunoresponsive cell of claim 15 or 16, wherein the endogenous expression of a TCR comprising a native TCR α chain and/or a native TCR β chain is disrupted or abolished in

the immunoresponsive cell, thereby preventing or eliminating mispairing between the recombinant TCR and a native TCR α chain and/or a native TCR β chain in the immunoresponsive cell.

18. The immunoresponsive cell of any one of claims 15-17, wherein the endogenous gene locus comprises a modified transcription terminator region; optionally wherein the modified transcription terminator region comprises a genomic element selected from the group consisting of a TK transcription terminator, a GCSF transcription terminator, a TCRA transcription terminator, an HBB transcription terminator, a bovine growth hormone transcription terminator, an SV40 transcription terminator, and a P2A element.

19. The immunoresponsive cell of any one of claims 15-18, wherein the endogenous gene locus is an endogenous TCR locus, and a second endogenous TCR locus is modified to eliminate the expression of an endogenous TCR chain.

20. The immunoresponsive cell of claim 19, wherein the second endogenous TCR locus is further modified to express a gene of interest; optionally wherein the gene of interest is selected from the group consisting of an anti-tumor cytokine, a co-stimulatory molecule ligand, a tracking gene, and a suicide gene.

21. The immunoresponsive cell of any one of claims 14-20, wherein the cell is selected from the group consisting of a T cell, a Natural Killer T (NKT) cell, an embryonic stem cell, and a pluripotent stem cell from which a lymphoid cell may be differentiated.

22. The immunoresponsive cell of claim 21, wherein the cell is a T cell; optionally wherein the T cell is selected from the group consisting of a cytotoxic T lymphocyte (CTL), a regulatory T cell, and a Natural Killer T (NKT) cell; and/or the T cell is a CD4⁺ T cell or a CD8⁺ T cell.

23. The immunoresponsive cell of any one of claims 14-22, further comprising:

a) at least one exogenous co-stimulatory ligand; optionally wherein the exogenous co-stimulatory ligand is selected from the group consisting of CD80, CD86, 41BBL, CD275, CD40L, OX40L and any combination thereof; optionally wherein the exogenous co-stimulatory ligand is selected from CD80, 4-1BBL, and a combination thereof;

b) at least one chimeric costimulatory receptor (CCR); optionally wherein the CCR

comprises an intracellular domain of a co-stimulatory molecule of a fragment thereof; optionally wherein the co-stimulatory molecule is CD28, 4-1BB, OX40, ICOS, DAP-10, or a combination thereof; and/or

c) at least one chimeric antigen receptor (CAR).

24. A pharmaceutical composition comprising an effective amount of an immunoresponsive cell of any one of claims 14-23 and a pharmaceutically acceptable excipient.

25. A method for producing an antigen-specific immunoresponsive cell, the method comprising introducing into an immunoresponsive cell a nucleic acid molecule encoding a recombinant TCR according to any one of claims 1-13; optionally wherein the nucleic acid molecule is comprised in a vector.

26. A nucleic acid molecule encoding a recombinant TCR of any one of claims 1-13.

27. A nucleic acid composition comprising the nucleic acid molecule of claim 26.

28. A vector comprising the nucleic acid composition of claim 27.

29. A kit comprising a recombinant TCR of any one of claims 1-13, an immunoresponsive cell of any one of claims 14-23, a pharmaceutical composition of claim 24, a nucleic acid composition of claim 27, or a vector of claim 28; optionally further comprising written instructions for treating and/or preventing a neoplasm, a pathogen infection, and/or an autoimmune disorder.

30. The kit of claim 29, wherein the kit.

31. The immunoresponsive cell of any one of claims 14-23 or the pharmaceutical composition of claim 24 for use in therapy.

32. Use of the immunoresponsive cell of any one of claims 14-23 or the pharmaceutical composition of claim 24 in the manufacture of a medicament for reducing tumor burden in a subject, treating and/or preventing a neoplasm, a pathogen infection, and/or an autoimmune disorder, and/or lengthening survival of a subject having neoplasm.

33. The use of claim 32, wherein the neoplasm is selected from the group consisting of blood cancer, B cell leukemia, multiple myeloma, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia, non-Hodgkin's lymphoma adenocarcinomas; optionally wherein

i) the neoplasm is B cell leukemia, multiple myeloma, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, or non-Hodgkin's lymphoma, and the recombinant TCR binds to CD19; optionally wherein the neoplasm is CD19⁺ ALL; or

ii) the neoplasm is B cell leukemia, multiple myeloma, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia, or non-Hodgkin's lymphoma, and the recombinant TCR binds to BCMA, ADGRE2, CCR1, CD22, CD70 or combinations thereof.