USE OF ANTI-HA-1 AND ANTI-HA-2 BINDING PROTEINS FOR TREATMENT OF AML, ALL, AND MDS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. Provisional Application Serial No. 63/631,379, filed on 08 April 2024, U.S. Provisional Application Serial No. 63/716,079, filed on 04 November 2024, and U.S. Provisional Application Serial No. 63/729,220, filed on 06 December 2024; the entire contents of each of said applications are incorporated herein in their entirety by this reference.

BACKGROUND

[0002] Hematopoietic cell transplantation (HCT) is a standard treatment for acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), and myelodysplastic disorder (MDS). However, relapse after HCT is common, occurring in approximately 30-40% of patients. Relapsed patients have a one-year mortality rate of about 90%, and few options for treatment.

SUMMARY

[0003] The present disclosure encompasses, among other things, methods and compositions for treatment of acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), and myelodysplastic disorder (MDS) in subjects that received HCT, e.g., where the subjects were not cured by and/or relapsed after reduced intensity conditioning (RIC) HCT (RIC-HCT) by administration of T cells engineered to express particular T Cell Receptors (TCRs). TCRs can recognize portions of antigens that are presented via major histocompatibility complex (MHC) molecules, resulting in T cell activation. The human MHC, known as the HLA (human leukocyte antigen), includes three class I genes that present endogenous peptides: HLA-A, HLA-B, and HLA-C. Individuals can have different alleles, of each HLA class I gene, though some alleles are more common than others. TCRs of the present disclosure recognize peptide-MHC (pMHC) complexes of HA-1 (VLHDDLLEA; SEQ ID NO: 1) or HA-2 (YIGEVLVSV; SEQ ID NO: 2), which are HLA-A*02:01- and hematopoietically-restricted minor histocompatibility antigens.

[0004] In at least one aspect, the present disclosure encompasses a method of treating a malignancy selected from the group consisting of acute myeloid leukemia (AML), acute

lymphocytic leukemia (ALL), and myelodysplastic disorder (MDS) in an adult human subject that previously received reduced intensity conditioning (RIC) hematopoietic cell therapy (HCT), the treatment including infusing a first dose of haploidentical T cells engineered to express a TCR that binds HA-1 (VLHDDLLEA; SEQ ID NO: 1) when presented by HLA-A*02:01, where the TCR includes: a TCR alpha chain variable domain including a CDR1 including the amino acid sequence of SEQ ID NO: 5, a CDR2 including the amino acid sequence of SEQ ID NO: 6, and a CDR3 including the amino acid sequence of SEQ ID NO: 7, and a TCR beta chain variable domain including a CDR1 including the amino acid sequence of SEQ ID NO: 11, a CDR2 including the amino acid sequence of SEQ ID NO: 12, and a CDR3 including the amino acid sequence of SEQ ID NO: 13, where cells of the malignancy encode and/or express each of HA-1 and HLA-A*02:01, where the HCT includes administration of hematopoietic cells from a donor that: (i) does not encode and/or express HA-1; and/or (ii) does not encode and/or express an HLA-A*02 allele, and wherein the first dose is selected from the group consisting of about 5x10^6 cells/kg of the haploidentical T cells, about 0.8 billion of the haploidentical T cells, and about 1.2 billion of the haploidentical T cells, and the first dose is infused at a time that is between about 14 and about 42 days after the subject received HCT, optionally at a time that is about 21 days after the subject received HCT, optionally where at least 80% of cells of the first dose of haploidentical T cells express a marker, optionally where the marker is Qtag-CD34. In some embodiments, the TCR alpha chain variable domain sequence includes an amino acid sequence having at least 90% identity with SEQ ID NO: 8 and/or the TCR beta chain variable domain sequence includes an amino acid sequence having at least 90% identity with SEQ ID NO: 14. In some embodiments, the TCR alpha chain variable domain sequence includes the amino acid sequence of SEQ ID NO: 8 and/or the TCR beta chain variable domain sequence includes the amino acid sequence of SEQ ID NO: 14. In some embodiments, the TCR includes a TCR alpha chain including an amino acid sequence having at least 90% identity with SEQ ID NO: 10, and/or a TCR beta chain including an amino acid sequence having at least 90% identity with SEQ ID NO: 16. In some embodiments, the TCR alpha chain includes the amino acid sequence of SEQ ID NO: 10, and/or the TCR beta chain includes the amino acid sequence of SEQ ID NO: 16.

[0005] In at least one aspect, the present disclosure encompasses a method of treating a malignancy selected from the group consisting of acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), and myelodysplastic disorder (MDS) in an adult human subject that previously received reduced intensity conditioning (RIC) hematopoietic cell therapy (HCT), the treatment including infusing a first dose of haploidentical T cells engineered to

express a TCR that binds HA-2 (YIGEVLVSV; SEQ ID NO: 2) when presented by HLA-A*02:01, where the TCR includes: a TCR alpha chain variable domain including a CDR1 including the amino acid sequence of SEQ ID NO: 17, a CDR2 including the amino acid sequence of SEQ ID NO: 18, and a CDR3 including the amino acid sequence of SEQ ID NO: 19, and a TCR beta chain variable domain including a CDR1 including the amino acid sequence of SEQ ID NO: 23, a CDR2 including the amino acid sequence of SEQ ID NO: 24, and a CDR3 including the amino acid sequence of SEQ ID NO: 25, where cells of the malignancy encode and/or express each of HA-2 and HLA-A*02:01, where the HCT includes administration of hematopoietic cells from a donor that: (i) does not encode and/or express HA-1; and/or (ii) does not encode and/or express an HLA-A*02 allele, and optionally where the HCT includes administration of hematopoietic cells from a donor that does not encode and/or express an HLA-A*02 allele, and where the first dose is selected from the group consisting of about 5x10^6 cells/kg of the haploidentical T cells, about 0.8 billion of the haploidentical T cells, and about 1.2 billion of the haploidentical T cells, and the first dose is infused at a time that is between about 14 and about 42 days after the subject received HCT, optionally at a time that is about 21 days after the subject received HCT, optionally where at least 80% of cells of the first dose of haploidentical T cells express a marker, optionally where the marker is Qtag-CD34. In some embodiments, the TCR alpha chain variable domain sequence includes an amino acid sequence having at least 90% identity with SEQ ID NO: 20 and/or the TCR beta chain variable domain sequence includes an amino acid sequence having at least 90% identity with SEQ ID NO: 26. In some embodiments, the TCR alpha chain variable domain sequence includes the amino acid sequence of SEQ ID NO: 20 and/or the TCR beta chain variable domain sequence includes the amino acid sequence of SEQ ID NO: 26. In some embodiments, the TCR includes a TCR alpha chain including an amino acid sequence having at least 90% identity with SEQ ID NO: 22, and/or a TCR beta chain including an amino acid sequence having at least 90% identity with SEQ ID NO: 28. In some embodiments, the TCR alpha chain includes the amino acid sequence of SEQ ID NO: 22, and/or the TCR beta chain includes the amino acid sequence of SEQ ID NO: 28.

[0006] In some embodiments, a cell expresses CD8α, CD8β, and/or a selectable protein marker, optionally where the selectable protein marker is DHFR and further optionally where the CD8α, CD8β, and/or the selectable protein marker is fused to a CD34 enrichment tag. In some embodiments, cells are enriched using the CD34 enrichment tag. In some embodiments, the cell includes a vector encoding the TCR, optionally where i) the

vector is a cloning vector, expression vector, or viral vector. In some embodiments, the vector further includes a nucleic acid sequence encoding CD8α, CD8β, and/or a selectable protein marker, optionally where the selectable protein marker is dihydrofolate reductase (DHFR). In some embodiments, the nucleic acid sequence encoding CD8α, CD8β, and/or the selectable protein marker is operably linked to a nucleic acid encoding a tag. In some embodiments, the nucleic acid encoding a tag is at the 5' upstream of the nucleic acid sequence encoding CD8 α, CD8β, and/or the selectable protein marker such that the tag is fused to the N-terminus of CD8α, CD8β, and/or the selectable protein marker. In some embodiments, the tag is a CD34 enrichment tag. In some embodiments, the nucleic acid encoding the binding protein, the nucleic acid sequence encoding TCRα, TCRβ, CD8α, CD8β, and/or the selectable protein marker are interconnected with an internal ribosome entry site or a nucleic acid sequence encoding a self-cleaving peptide.

[0007] In some embodiments, the self-cleaving peptide is P2A, E2A, F2A or T2A.

[0008] In some embodiments, the haploidentical T cells engineered to express the TCR are derived from the same donor from which the hematopoietic T cells are derived. In some embodiments, the T cells engineered to express the TCR are produced from T cells derived from the donor by leukapheresis 6 to 13 days prior to HCT. In some embodiments, the HCT includes hematopoietic stem cells derived from a donor one day prior to HCT by leukapheresis, optionally where the donor received a G-CSF mobilization treatment between 6 days before HCT and 2 days before HCT. In some embodiments, the RIC includes: (i) an RIC regimen including fludarabine, cyclophosphamide, and total-body irradiation, where the total-body irradiation includes a dosage of 200 cGy or 400 cGy; (ii) an RIC regimen including fludarabine and total-body irradiation, where the total-body irradiation includes a dosage of 200 cGy, optionally where the RIC regimen includes melphalan; or (iii) an RIC regimen including thiotepa, busulfan and fludarabine. In some embodiments, the RIC is administered between 6 days before and 1 day before HCT. In some embodiments, the subject is infused with a second dose of the engineered haploidentical T cells if infusion of the first dose does not result in excessive toxicity and if persistence of the engineered haploidentical T cells infused in the first dose in the subject is greater than 3% of total T cells, optionally where at least 80% of cells of the second dose of haploidentical T cells express a marker, optionally where the marker is Qtag-CD34. In some embodiments, the subject is infused a second dose of the engineered haploidentical T cells at a time that is around 40 days after the first infusion; between about 54 days and about 82 days after the subject received HCT, optionally where the second dose of the engineered haploidentical T

cells is infused at a time that is about 61 days after the subject received HCT. In some embodiments, the subject receives a second dose of the engineered haploidentical T cells at a time that is between about 40 days (or about 41 days) and about 55 days after the first dose, optionally where the second dose of the engineered haploidentical T cells is infused at a time that is about 40 days after the first dose. In some embodiments, the second dose is selected from the group consisting of about 5x10^6 cells/kg of the haploidentical T cells, about 2x10^7 cells/kg of the haploidentical T cells, about 1.2 billion of the haploidentical T cells, and about 1.6 billion of the haploidentical T cells.

[0009] In some embodiments, a method includes administering to the subject a prophylactic regimen against graft-versus-host disease (GvHD) after HCT, where the prophylactic regimen includes cyclophosphamide (PTCy), mycophenolate mofetil (MMF), and/or tacrolimus, optionally where the prophylactic regimen includes cyclophosphamide. In some embodiments, the prophylactic regimen is administered on the third and fourth days after HCT. In some embodiments, a method includes administering to the subject a maintenance therapy including one or more of FLT3, BCR/Abl, and IDH inhibitors. In some embodiments, the subject is not administered a further anti-leukemia agent, optionally where the further anti-leukemia agent is oral azacytidine.

[0010] In some embodiments, the subject meets the following criteria (inclusion criteria): (i) diagnosed as a candidate for RIC and haploidentical donorHCT; and (ii) Eastern Cooperative Oncology Group performance status (ECOG-PS) score of two or more during at least one screening. In some embodiments, the subject meets the following criteria: (i) does not encode and/or express HLA-A*02:07; (ii) does not have levels of donor-specific HLA antibodies high enough to warrant desensitization protocols; (iii) not suffering a clinically significant infection or uncontrolled viral reactivation of cytomegalovirus (CMV), Epstein-Barr virus (EBV), Adenovirus, BK virus (BKV), or human herpesvirus 6 (HHV-6); and (iv) not a prior recipient of allogeneic HCT.

[0011] In some embodiments, the donor meets the following criteria (inclusion criteria): (i) at least 16 years of age; and (ii) fit to undergo peripheral blood stem cell (PBSC) collection and two rounds of leukapheresis. In some embodiments, the donor meets the following criteria: (i) not positive for HIV-1, HIV-2, HTLV-1, HTLV-2, active hepatitis B, active hepatitis C, active syphilis, or active West Nile virus infection; and (ii) not at risk for Creutzfeldt-Jakob disease or Zika virus.

[0012] In some embodiments, the treatment does not cause limiting toxicities (DLTs), cytokine release syndrome (CRS), or neurotoxicity. In some embodiments, the neurotoxicity

is immune effector cell-associated neurotoxicity syndrome (ICANS). In some embodiments, haploidentical T cells persist in the subject for at least 100 days and/or at least 200 days after administration. In some embodiments, haploidentical T cells persist in the subject for at least 100 days after administration. In some embodiments, haploidentical T cells persist in the subject for at least 200 days after administration.

[0013] In some embodiments, the haploidentical T cells are characterized in that they inhibit growth of HA-1- and/or HA-2-pulsed T2 cells when co-incubated with the T2 cells in vitro. In some embodiments, the treatment increases donor chimerism, optionally where the subject achieves complete donor chimerism. In some embodiments, donor chimerism is measured in CD3+ and/or CD33+ cells. In some embodiments, after treatment, the subject is minimal residual disease (MRD) negative, optionally where the subject is MRD positive prior to treatment. In some embodiments, the treatment prevents and/or reduces the risk of relapse of the malignancy, optionally where relapse refers to a change from MRD negative to MRD positive. In some embodiments, the treatment increases subject survival time and/or expected survival time as compared to reference controls. In some embodiments, the malignancy is a TP53 mutated MDS.

[0014] In at least one aspect, the present disclosure encompasses a method of assessing the efficacy of a therapy for a malignancy selected from AML, ALL, or MDS, where cells of the malignancy are characterized by expression of HLA-A*02:01 and expression of HA-1 and/or HA-2, the method including: a) determining the presence or level of reactivity between T cells obtained from the subject and at least one immunogenic peptide selected from HA-1 and HA-2 or at least one stable MHC-peptide complex in which HLA-A*02:01 presents the immunogenic peptide, in a first sample obtained from the subject prior to providing at least a portion of the therapy, and b) determining the presence or level of reactivity between the at least one immunogenic peptide, or the at least one stable MHC-peptide complex, and T cells obtained from the subject present in a second sample obtained from the subject following provision of the therapy, where the presence or a higher level of reactivity in the second sample, relative to the first sample, is an indication that the therapy is efficacious for treating the malignancy, and/or where the absence or a reduced level of reactivity in the second sample, relative to the first sample, is an indication that the therapy is not efficacious for treating the malignancy.

[0015] In at least one aspect, the present disclosure encompasses a method of assessing the efficacy of a therapy for a malignancy selected from AML, ALL, or MDS, where cells of the malignancy are characterized by expression of HLA-A*02:01 and

expression of HA-1, the method including: a) determining the presence or level of reactivity between a first sample obtained from the subject and at least one TCR or at least one T cell expressing the TCR, where the first sample is obtained from the subject prior to providing at least a portion of the therapy, and b) determining the presence or level of reactivity between a second sample obtained from the subject and at least one TCR or at least one T cell expressing the TCR, where the second sample is obtained from the subject following provision of the therapy for the malignancy, where the absence or a reduced level of reactivity in the second sample, relative to the first sample, is an indication that the therapy is efficacious for treating the malignancy, and/or where the presence or an increased level of reactivity in the second sample, relative to the first sample, is an indication that the therapy is not efficacious, where the TCR includes: a TCR alpha chain variable domain including a CDR1 including the amino acid sequence of SEQ ID NO: 5, a CDR2 including the amino acid sequence of SEQ ID NO: 6, and a CDR3 including the amino acid sequence of SEQ ID NO: 7, and a TCR beta chain variable domain including a CDR1 including the amino acid sequence of SEQ ID NO: 11, a CDR2 including the amino acid sequence of SEQ ID NO: 12, and a CDR3 including the amino acid sequence of SEQ ID NO: 13, optionally where the at least one T cell is a population of T cells.

[0016] In some embodiments, the TCR alpha chain variable domain sequence includes an amino acid sequence having at least 90% identity with SEQ ID NO: 8 and/or the TCR beta chain variable domain sequence includes an amino acid sequence having at least 90% identity with SEQ ID NO: 14. In some embodiments, the TCR alpha chain variable domain sequence includes the amino acid sequence of SEQ ID NO: 8 and/or the TCR beta chain variable domain sequence includes the amino acid sequence of SEQ ID NO: 14. In some embodiments, the TCR includes a TCR alpha chain including an amino acid sequence having at least 90% identity with SEQ ID NO: 10, and/or a TCR beta chain including an amino acid sequence having at least 90% identity with SEQ ID NO: 16. In some embodiments, the TCR alpha chain includes the amino acid sequence of SEQ ID NO: 10, and/or the TCR beta chain includes the amino acid sequence of SEQ ID NO: 16. In some embodiments, the at least one T cell expresses CD8α, CD8β, and/or a selectable protein marker, optionally where the selectable protein marker is DHFR and further optionally where the CD8α, CD8β, and/or the selectable protein marker is fused to a CD34 enrichment tag.

[0017] In at least one aspect, the present disclosure encompasses a method of assessing the efficacy of a therapy for a malignancy selected from AML, ALL, or MDS,

where cells of the malignancy are characterized by expression of HLA-A*02:01 and expression of HA-2, the method including: a) determining the presence or level of reactivity between a first sample obtained from the subject and at least one TCR or at least one T cell expressing the TCR, where the first sample is obtained from the subject prior to providing at least a portion of the therapy, and b) determining the presence or level of reactivity between a second sample obtained from the subject and at least one TCR or at least one T cell expressing the TCR, where the second sample is obtained from the subject following provision of the therapy for the malignancy, where the absence or a reduced level of reactivity in the second sample, relative to the first sample, is an indication that the therapy is efficacious for treating the malignancy, and/or where the presence or an increased level of reactivity in the second sample, relative to the first sample, is an indication that the therapy is not efficacious, where the TCR includes: a TCR alpha chain variable domain including a CDR1 including the amino acid sequence of SEQ ID NO: 17, a CDR2 including the amino acid sequence of SEQ ID NO: 18, and a CDR3 including the amino acid sequence of SEQ ID NO: 19, and a TCR beta chain variable domain including a CDR1 including the amino acid sequence of SEQ ID NO: 23, a CDR2 including the amino acid sequence of SEQ ID NO: 24, and a CDR3 including the amino acid sequence of SEQ ID NO: 25, optionally where the at least one T cell is a population of T cells.

[0018] In some embodiments, the TCR alpha chain variable domain sequence includes an amino acid sequence having at least 90% identity with SEQ ID NO: 20 and/or the TCR beta chain variable domain sequence includes an amino acid sequence having at least 90% identity with SEQ ID NO: 26. In some embodiments, the TCR alpha chain variable domain sequence includes the amino acid sequence of SEQ ID NO: 20 and/or the TCR beta chain variable domain sequence includes the amino acid sequence of SEQ ID NO: 26. In some embodiments, the TCR includes a TCR alpha chain including an amino acid sequence having at least 90% identity with SEQ ID NO: 22, and/or a TCR beta chain including an amino acid sequence having at least 90% identity with SEQ ID NO: 28. In some embodiments, the TCR alpha chain includes the amino acid sequence of SEQ ID NO: 22, and/or the TCR beta chain includes the amino acid sequence of SEQ ID NO: 28. In some embodiments, the at least one T cell expresses CD8α, CD8β, and/or a selectable protein marker, optionally where the selectable protein marker is DHFR and further optionally where the CD8α, CD8β, and/or the selectable protein marker is fused to a CD34 enrichment tag.

[0019] In some embodiments, the level of reactivity is indicated by a) the presence of binding and/or b) T cell activation and/or effector function, optionally where the T cell activation or effector function is T cell proliferation, killing, or cytokine release. In some embodiments, the binding, activation, and/or effector function is detected using fluorescence activated cell sorting (FACS), enzyme linked immunosorbent assay (ELISA), radioimmune assay (RIA), immunochemically, Western blot, or intracellular flow assay. In some embodiments, the level of reactivity is indicated by a minimal residual disease (MRD) assay. In some embodiments, a reduction in MRD and/or conversion from MRD positive in the first sample to MRD negative in the second sample is an indication that the therapy is efficacious for treating the malignancy.

[0020] In some embodiments, MRD is assayed by next-generation sequencing (NGS), flow cytometry, or a combination thereof. In some embodiments, the MRD assay includes NGS, optionally where the assay is an AlloHeme assay. In some embodiments, the level of reactivity is indicated by a donor cell chimerism assay. In some embodiments, an increase in donor cell chimerism and/or conversion from partial donor cell chimerism in the first sample to complete donor cell chimerism in the second sample is an indication that the therapy is efficacious for treating the malignancy.

[0021] In at least one aspect, the present disclosure encompasses a method of assessing the efficacy of a therapy for a malignancy selected from AML, ALL, or MDS, where cells of the malignancy are characterized by expression of HLA-A*02:01 and expression of HA-1, the method including: a) determining minimal residual disease (MRD) and/or donor cell chimerism from a first sample obtained from the subject, where the first sample is obtained from the subject prior to providing at least a portion of the therapy, and b) determining the minimal residual disease (MRD) and/or donor cell chimerism from a second sample obtained from the subject, where the second sample is obtained from the subject following provision of the therapy for the malignancy, optionally where the therapy includes infusing a first dose of haploidentical T cells engineered to express a TCR that binds HA-1 (VLHDDLLEA; SEQ ID NO: 1) when presented by HLA-A*02:01, where the TCR includes: a TCR alpha chain variable domain including a CDR1 including the amino acid sequence of SEQ ID NO: 5, a CDR2 including the amino acid sequence of SEQ ID NO: 6, and a CDR3 including the amino acid sequence of SEQ ID NO: 7, and a TCR beta chain variable domain including a CDR1 including the amino acid sequence of SEQ ID NO: 11, a CDR2 including the amino acid sequence of SEQ ID NO: 12, and a CDR3 including the amino acid sequence of SEQ ID NO: 13, optionally where the at least one T cell is a population of T cells,

optionally where the subject has received or the therapy includes HCT, and optionally where the HCT is RIC-HCT. In some embodiments, the TCR alpha chain variable domain sequence includes an amino acid sequence having at least 90% identity with SEQ ID NO: 8 and/or the TCR beta chain variable domain sequence includes an amino acid sequence having at least 90% identity with SEQ ID NO: 14. In some embodiments, the TCR alpha chain variable domain sequence includes the amino acid sequence of SEQ ID NO: 8 and/or the TCR beta chain variable domain sequence includes the amino acid sequence of SEQ ID NO: 14. In some embodiments, the TCR includes a TCR alpha chain including an amino acid sequence having at least 90% identity with SEQ ID NO: 10, and/or a TCR beta chain including an amino acid sequence having at least 90% identity with SEQ ID NO: 16. In some embodiments, the TCR alpha chain includes the amino acid sequence of SEQ ID NO: 10, and/or the TCR beta chain includes the amino acid sequence of SEQ ID NO: 16. In some embodiments, the at least one T cell expresses CD8α, CD8β, and/or a selectable protein marker, optionally where the selectable protein marker is DHFR and further optionally where the CD8α, CD8β, and/or the selectable protein marker is fused to a CD34 enrichment tag.

[0022] In at least one aspect, the present disclosure encompasses a method of assessing the efficacy of a therapy for a malignancy selected from AML, ALL, or MDS, where cells of the malignancy are characterized by expression of HLA-A*02:01 and expression of HA-2, the method including: a) determining minimal residual disease (MRD) and/or donor cell chimerism from a first sample obtained from a subject, where the first sample is obtained from the subject prior to providing at least a portion of the therapy, and b) determining the minimal residual disease (MRD) and/or donor cell chimerism from a second sample obtained from the subject, where the second sample is obtained from the subject following provision of the therapy for the malignancy, optionally where the therapy includes infusing a first dose of haploidentical T cells engineered to express a TCR that binds HA-2 (YIGEVLVSV; SEQ ID NO: 2) when presented by HLA-A*02:01, where the TCR includes: a TCR alpha chain variable domain including a CDR1 including the amino acid sequence of SEQ ID NO: 17, a CDR2 including the amino acid sequence of SEQ ID NO: 18, and a CDR3 including the amino acid sequence of SEQ ID NO: 19, and a TCR beta chain variable domain including a CDR1 including the amino acid sequence of SEQ ID NO: 23, a CDR2 including the amino acid sequence of SEQ ID NO: 24, and a CDR3 including the amino acid sequence of SEQ ID NO: 25, optionally where the at least one T cell is a population of T cells,

optionally where the subject has received or the therapy includes HCT, and optionally where the HCT is RIC-HCT.

[0023] In some embodiments, the TCR alpha chain variable domain sequence includes an amino acid sequence having at least 90% identity with SEQ ID NO: 20 and/or the TCR beta chain variable domain sequence includes an amino acid sequence having at least 90% identity with SEQ ID NO: 26. In some embodiments, the TCR alpha chain variable domain sequence includes the amino acid sequence of SEQ ID NO: 20 and/or the TCR beta chain variable domain sequence includes the amino acid sequence of SEQ ID NO: 26. In some embodiments, the TCR includes a TCR alpha chain including an amino acid sequence having at least 90% identity with SEQ ID NO: 22, and/or a TCR beta chain including an amino acid sequence having at least 90% identity with SEQ ID NO: 28. In some embodiments, the TCR alpha chain includes the amino acid sequence of SEQ ID NO: 22, and/or the TCR beta chain includes the amino acid sequence of SEQ ID NO: 28. In some embodiments, the at least one T cell expresses CD8α, CD8β, and/or a selectable protein marker, optionally where the selectable protein marker is DHFR and further optionally where the CD8α, CD8β, and/or the selectable protein marker is fused to a CD34 enrichment tag.

[0024] In some embodiments, a reduction in MRD, and/or conversion from MRD positive in the first sample to MRD negative in the second sample, is an indication that the therapy is efficacious for treating the malignancy. In some embodiments, MRD is assayed by next-generation sequencing (NGS), flow cytometry, or a combination thereof. In some embodiments, the MRD assay includes NGS, optionally where the assay is an AlloHeme assay.

[0025] In some embodiments, an increase in donor cell chimerism, and/or conversion from partial donor cell chimerism in the first sample to complete donor cell chimerism in the second sample, is an indication that the therapy is efficacious for treating the malignancy. In some embodiments, a method includes repeating steps a) and b) at a subsequent point in time, optionally where the subject has undergone further treatment for the malignancy between the first point in time and the subsequent point in time.

[0026] In various embodiments of methods encompassed herein, T cells include CD8+ T cells and CD4+ T cells.

DEFINITIONS

[0027] For convenience, certain terms employed in the specification, examples, and appended claims are collected here.

[0028] The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.,* to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0029] As used herein, term "about", when used in reference to a value, refers to a value that is similar, in context to the referenced value. In general, those skilled in the art, familiar with the context, will appreciate the relevant degree of variance encompassed by "about" in that context. For example, in some embodiments, the term "about" may encompass a range of values that within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less of the referenced value. To provide another particular example, in the context of numbers of days, "about" refers to a range of a number of days that spans from three days less than a reference value to three days more than a reference value (*e.g.*, "about 21 days" refers to a range of 18-24 days).

[0030] As used herein, the term "administering", when applied to administration of T cells (e.g., engineered T cell products) refers to the physical introduction of a composition comprising the T cells to a subject by infusion.

[0031] As used here, the term "adult" refers to a person of at least 18 years of age.

[0032] As used herein, the term "antigen" refers to any natural or synthetic immunogenic substance, such as a protein, peptide, or hapten. An antigen may be an HA-1 antigen, a fragment of an HA-1 antigen, an HA-2 antigen, or a fragment of an HA-2 antigen, against which protective or therapeutic immune responses are desired.

[0033] The terms "complementarity determining region" and "CDR" are synonymous with "hypervariable region" or "HVR" and are known in the art to refer to non-contiguous sequences of amino acids within certain binding proteins, such as TCR variable regions, which confer antigen specificity and/or binding affinity. For TCRs, in general, there are three CDRs in each α-chain variable region (αCDR1, αCDR2, and αCDR3) and three CDRs in each β-chain variable region (βCDR1, βCDR2, and βCDR3). CDR3 is believed to be the main CDR responsible for recognizing processed antigen. CDR1 and CDR2 mainly interact with the MHC.

[0034] The term "coding region" refers to regions of a nucleotide sequence comprising codons that are translated into amino acid residues, whereas the term "non-coding

region" refers to regions of a nucleotide sequence that are not translated into amino acids (*e.g.,* 5' and 3' untranslated regions).

[0035] As used herein, the term "costimulate" with reference to activated immune cells includes the ability of a costimulatory molecule to provide a second, non-activating receptor mediated signal (a "costimulatory signal") that induces proliferation or effector function. For example, a costimulatory signal may result in cytokine secretion, *e.g.,* in a T cell that has received a T cell-receptor-mediated signal. Immune cells that have received a cell-receptor mediated signal, *e.g.,* via an activating receptor are referred to herein as "activated immune cells."

[0036] A "component of a TCR complex," as used herein, refers to a TCR chain (i.e ., TCRα, TCRβ, TCRγ or TCRδ), a CD3 chain (*i.e.,* CD3γ, CD3δ, CD3ε or CD3ζ), or a complex formed by two or more TCR chains or CD3 chains (*e.g.,* a complex of TCRα and TCRβ, a complex of TCRγ and TCRδ, a complex of CD3ε and CD3δ, a complex of CD3γ and CD3ε, or a sub-TCR complex of TCRα, TCRβ, CD3γ, CD3δ, and two CD3ε chains).

[0037] The term "determining a suitable treatment regimen for the subject" is taken to mean the determination of a treatment regimen (*i.e.,* a single therapy or a combination of different therapies that are used for the prevention and/or treatment of the viral infection in the subject) for a subject that is started, modified and/or ended based or essentially based or at least partially based on the results of the analysis according to the present invention. One example is starting an adjuvant therapy after surgery whose purpose is to decrease the risk of recurrence, another would be to modify the dosage of a particular chemotherapy. The determination can, in addition to the results of the analysis according to the present invention, be based on personal characteristics of the subject to be treated. In most cases, the actual determination of the suitable treatment regimen for the subject will be performed by the attending physician or doctor.

[0038] As used herein, the term "HA-1 antigen" or "HA-1 peptide antigen" or "HA-1-containing peptide antigen" or "HA-1 epitope" or "HA-1 peptide epitope" or "HA-1 peptide" refers to a naturally or synthetically produced peptide portion of a HMHA1 protein, which is also known as ARHGAP45, and is an intracellular protein expressed at high levels in all blood cells, but not in any other tissue. ARHGAP45 comes in two forms. In HA-1-positive individuals, the peptide has the sequence VL**H**DDLLEA (SEQ ID NO: 1) and, if the individual has an HLA A*02 serotype such as HLA type A*02:01 or HLA type A*02:06 (Torikai *et al.* (2007) *Bone Marrow Transplant.* 40:165-174), then the antigen is efficiently

displayed on the surface of blood cells. In HA-1-negative individuals, the peptide has the sequence VL**R**DDLLEA (SEQ ID NO: 3), and the HA-1 antigen is not displayed. Approximately 60% of people have the VL**H**DDLLEA (SEQ ID NO: 1) sequence and approximately 42% of people in the United States have the HLA type A*02:01, which means that approximately 25% of individuals in the United States are HA-1-positive and HLA type A*02:01 positive. Studies of patients receiving HCT have shown that in cases where the T cells of an HA-1-negative donor naturally develop a response to HA-1 in an HA-1-positive patient, the T cells mediate a specific graft-versus-leukemia (GvL) effect and the patient often experiences a long-term remission. In some embodiments, compositions and therapeutic methods described herein are based on this clinical observation and is designed to specifically cause this GvL effect in patients receiving HCT. HA-1 antigen protein can range in length from about 7 amino acids, about 8 amino acids, about 9 amino acids, about 10 amino acids, up to about 20 amino acids, and comprising the R139H substitution polymorphism), which can form a complex with a MHC (*e.g.*, HLA) molecule, and a binding protein of this disclosure specific for an HA-1 peptide:MHC (*e.g.*, HLA) complex can bind (*e.g.*, specifically bind) to such as complex. An exemplary HA-1 peptide antigen comprises a peptide having the amino acid VL**H**DDLLEA (SEQ ID NO: 1), wherein the bolded histidine in the sequence represents the R139H polymorphism. The term "HA-1^{R}" or "HA-1^{-}H" refers to HA-1 antigen with R139H.

[0039] As used herein, the term "HA-2 antigen" or "HA-2 peptide antigen" or "HA-2-containing peptide antigen" or "HA-2 epitope" or "HA-2 peptide epitope" or "HA-2 peptide" refers to a naturally or synthetically produced peptide portion of a member of the class I myosin family known as MYO1G (Pierce *et al.* (2001) *J. Immunol.* 167:3223-3230). The gene is located on the short arm of chromosome 7 in humans and its expression is limited to cells of hematopoietic origin. MYO1G is a diallelic gene encoding two genetic variants, designated MYO1G(V) and MYO1G(M). MYO1G(V) encodes the YIGEVLVS**V** HA-2 epitope (SEQ ID NO: 2) and MYO1G(M) encodes the YIGEVLVS**M** HA-2 epitope (SEQ ID NO: 4). The single amino acid change between the two peptides has only a modest effects on peptide binding to the class I MHC-restricted element HLA-A (*e.g.*, HLA-A*0201) and on recognition by T cells when added exogenously to target cells. However, the peptide encoded by MYO1G(M) is believed not to be presented at the surface of cells that endogenously express the MYO1G(M) allele. HA-2 antigen protein can range in length from about 7 amino acids, about 8 amino acids, about 9 amino acids, about 10 amino acids, up to about 20 amino acids, and can form a complex with a MHC (*e.g.*, HLA) molecule such that a

binding protein of this disclosure specific for an HA-2 peptide:MHC (*e.g.*, HLA) complex can bind (*e.g.*, specifically and/or selectively) to such as complex. An exemplary HA-2 peptide antigen comprises a peptide having the amino acid YIGEVLVSV (SEQ ID NO: 2) or YIGEVLVSM (SEQ ID NO: 4). In some embodiments, the YIGEVLVSV peptide sequence (SEQ ID NO: 2) is referred to as "HA-2 REF" and the YIGEVLVSM peptide sequence (SEQ ID NO: 4) is referred to as "HA-2 SNP".

[0040] The term "immune response" includes T cell mediated and/or B cell mediated immune responses. Exemplary immune responses include T cell responses, *e.g.*, cytokine production and cellular cytotoxicity. In addition, the term immune response includes immune responses that are indirectly affected by T cell activation, *e.g.*, antibody production (humoral responses) and activation of cytokine responsive cells, *e.g.*, macrophages.

[0041] As used herein, the term "linked" refers to the association of two or more molecules. The linkage may be covalent or non-covalent. The linkage also may be genetic (*i.e.*, recombinantly fused). Such linkages may be achieved using a wide variety of art recognized techniques, such as chemical conjugation and recombinant protein production.

[0042] A "linker," in some embodiments, may refer to an amino acid sequence that connects two proteins, polypeptides, peptides, domains, regions, or motifs and may provide a spacer function compatible with interaction of the two sub-binding domains so that the resulting polypeptide retains a specific binding affinity *(e.g.,* scTCR) to a target molecule or retains signaling activity *(e.g.,* TCR complex). In some embodiments, a linker is comprised of about two to about 35 amino acids, for instance, or about four to about 20 amino acids or about eight to about 15 amino acids or about 15 to about 25 amino acids.

[0043] "Major histocompatibility complex" (MHC) refers to glycoproteins that deliver peptide antigens to a cell surface. MHC class I molecules are heterodimers having a membrane spanning a chain (with three a domains) and a non-covalently associated b2 microglobulin. MHC class II molecules are composed of two transmembrane glycoproteins, a and b, both of which span the membrane. Each chain has two domains. MHC class I molecules deliver peptides originating in the cytosol to the cell surface, where a peptide antigen-MHC (pMHC) complex is recognized by $CD8^+$ T cells. MHC class II molecules deliver peptides originating in the vesicular system to the cell surface, where they are recognized by $CD4^+$ T cells. Human MHC is referred to as human leukocyte antigen (HLA).

[0044] The terms "prevent," "preventing," "prevention," "prophylactic treatment," and the like refer to reducing the probability of developing a disease, disorder, or condition in

a subject, who does not have, but is at risk of or susceptible to developing a disease, disorder, or condition.

[0045] The term "prognosis" includes a prediction of the probable course and outcome of a viral infection or the likelihood of recovery from the disease. In some embodiments, the use of statistical algorithms provides a prognosis of a viral infection in an individual. For example, the prognosis may be surgery, development of a clinical subtype of a viral infection, development of one or more clinical factors, or recovery from the disease.

[0046] As used herein, "percent identity" between amino acid sequences is synonymous with "percent homology," which can be determined using the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264-2268, modified by Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877. The noted algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.* (1990) *J. Mol. Biol.* 215:403-410. BLAST nucleotide searches are performed with the NBLAST program, score=100, wordlength=12, to obtain nucleotide sequences homologous to a polynucleotide described herein. BLAST protein searches are performed with the XBLAST program, score=50, wordlength=3, to obtain amino acid sequences homologous to a reference polypeptide. To obtain gapped alignments for comparison purposes, Gapped BLAST is utilized as described in Altschul *et al.* (1997) *Nuc. Acids Res.* 25:3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) may be used.

[0047] The phrase "pharmaceutically-acceptable carrier" means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body.

[0048] The term "specific binding" refers to binding protein binding to a predetermined antigen. Typically, the binding protein binds with an affinity (K_D) of approximately less than or equal to about $5x10^{-4}$ M, less than or equal to about $1x10^{-4}$ M, less than or equal to about $5x10^{-5}$ M, less than or equal to about $1x10^{-5}$ M, less than or equal to about $5x10^{-6}$ M, less than or equal to about $1x10^{-6}$ M, less than or equal to about $5x10^{-7}$ M, less than or equal to about $1x10^{-7}$ M, less than or equal to about $5x10^{-8}$ M, less than or equal to about $1x10^{-8}$ M, less than or equal to about $5x10^{-9}$ M, less than or equal to about $1x10^{-9}$ M, less than or equal to about $5x10^{-10}$ M, less than or equal to about $1x10^{-10}$ M, less than or equal to about $5x10^{-11}$ M, less than or equal to about $1x10^{-11}$ M, less than or equal to about $5x10^{-12}$ M, less than or equal to about $1x10^{-12}$ M, or even lower, or any range in between, inclusive,

such as between about 1-50 micromolar, 1-100 micromolar, 0.1-500 micromolar, and the like,when determined by a binding assay, such as surface plasmon resonance (SPR) technology in a BIAcore™ assay instrument using an antigen of interest as the analyte and the binding protein as the ligand. In some embodiments, the binding protein binds to the predetermined antigen with an affinity that is at least 1.1-, 1.2-, 1.3-, 1.4-, 1.5-, 1.6-, 1.7-, 1.8-, 1.9-, 2.0-, 2.5-, 3.0-, 3.5-, 4.0-, 4.5-, 5.0-, 6.0-, 7.0-, 8.0-, 9.0-, or 10.0-fold or greater than its affinity for binding to a non-specific antigen (*e.g.,* BSA, casein) other than the predetermined antigen or a closely-related antigen. The phrases "a binding protein recognizing an antigen" and "a binding protein specific for an antigen" are used interchangeably herein with the term "a binding protein which binds specifically to an antigen." Selective binding is a relative term referring to the ability of a binding protein to discriminate the binding of one antigen over another, such as a particular family member or antigen target over a related family member or antigen target. For example, analytical data provided in the Examples section demonstrate that binding proteins described herein specifically bind HA-1 or HA-2 immunogenic epitopes and/or selectively bind a number of related epitopes (*e.g.,* HA-2 or HA-2 immunogenic epitopes and closely related sequences) discriminating such targets from the vast majority of other possible epitopes available in the human genome.

[0049] The term "subject" refers to any healthy animal, mammal or human, or any animal, mammal or human afflicted with a disorder, e.g., a hyperproliferative disorder, or a relapse of a hyperproliferative disorder, characterized by expression of an HA-1 antigen or an HA-2 antigen. The term "subject" is interchangeable with "patient."

[0050] The term "survival" includes all of the following: survival until mortality, also known as overall survival (wherein said mortality may be either irrespective of cause or tumor related); "recurrence-free survival" (wherein the term recurrence shall include both localized and distant recurrence); metastasis free survival; disease free survival (wherein the term disease shall include cancer and diseases associated therewith). The length of said survival may be calculated by reference to a defined start point (*e.g.,* time of diagnosis or start of treatment) and end point (*e.g.,* death, recurrence or metastasis). In addition, criteria for efficacy of treatment may be expanded to include response to chemotherapy, probability of survival, probability of metastasis within a given time period, and probability of tumor recurrence.

[0051] As used herein, the term "T cell-mediated response" refers to a response mediated by T cells, including effector T cells (*e.g.,* $CD8^+$ cells) and helper T cells (*e.g.,*

$CD4^+$ cells). T cell mediated responses include, for example, T cell cytotoxicity and proliferation.

[0052] A "T cell" is an immune system cell that matures in the thymus and produces T cell receptors (TCRs). T cells may be naive (not exposed to antigen; increased expression of CD62L, CCR7, CD28, CD3, CD 127, and CD45RA, and decreased expression of CD45RO as compared to T_{CM}), memory T cells (T_M) (antigen-experienced and long-lived), and effector cells (antigen-experienced, cytotoxic). T_M may be further divided into subsets of central memory T cells (T_{CM}, increased expression of CD62L, CCR7, CD28, CD127, CD45RO, and CD95, and decreased expression of CD54RA as compared to naive T cells) and effector memory T cells (T_{EM}, decreased expression of CD62L, CCR7, CD28, CD45RA, and increased expression of CD127 as compared to naive T cells or T_{CM}). Effector T cells (T_E) refers to antigen-experienced CD8+ cytotoxic T lymphocytes that have decreased expression of CD62L ,CCR7, CD28, and are positive for granzyme and perforin as compared to T_{CM}. Other exemplary T cells include regulatory T cells, such as $CD4^+$ $CD25^+$ ($Foxp3^+$) regulatory T cells and Treg17 cells, as well as Trl, Th3, $CD8^+CD28$, and Qa-1 restricted T cells.

[0053] Conventional T cells, also known as Tconv or Teffs, have effector functions (*e.g.*, cytokine secretion, cytotoxic activity, anti-self-recognition, and the like) to increase immune responses by virtue of their expression of one or more T cell receptors. Tcons or Teffs are generally defined as any T cell population that is not a Treg and include, for example, naïve T cells, activated T cells, memory T cells, resting Tcons, or Tcons that have differentiated toward, for example, the Th1 or Th2 lineages. In some embodiments, Teffs are a subset of non-Treg T cells. In some embodiments, Teffs are CD4+ Teffs or CD8+ Teffs, such as CD4+ helper T lymphocytes (*e.g.*, Th0, Th1, Tfh, or Th17) and CD8+ cytotoxic T lymphocytes. As described further herein, cytotoxic T cells are CD8+ T lymphocytes. "Naïve Tcons" are $CD4^+$ T cells that have differentiated in bone marrow, and successfully underwent a positive and negative processes of central selection in a thymus, but have not yet been activated by exposure to an antigen. Naïve Tcons are commonly characterized by surface expression of L-selectin (CD62L), absence of activation markers such as CD25, CD44 or CD69, and absence of memory markers such as CD45RO. Naïve Tcons are therefore believed to be quiescent and non-dividing, requiring interleukin-7 (IL-7) and interleukin-15 (IL- 15) for homeostatic survival (see, at least WO 2010/101870). The presence and activity of such cells are undesired in the context of suppressing immune responses. Unlike Tregs, Tcons are not anergic and can proliferate in response to antigen-

based T cell receptor activation (Lechler *et al.* (2001) *Philos. Trans. R. Soc. Lond. Biol. Sci.* 356:625-637).

[0054] "T effector" ("T_{eff}" or "T_E") cells refers to T cells (*e.g.*, CD4+ and CD8+ T cells) with cytolytic activities as well as T helper (Th) cells, which secrete cytokines and activate and direct other immune cells, but does not include regulatory T cells (Treg cells).

[0055] "T cell receptor" or "TCR" refers to an immunoglobulin superfamily member (having a variable binding domain, a constant domain, a transmembrane region, and a short cytoplasmic tail; *see, e.g.,* Janeway *et al.* (1997*) Curr. Biol. Publ.* 4:33) that is capable of binding (*e.g.*, specifically and/or selectively) to an antigen peptide bound to a MHC receptor. A TCR can be found on the surface of a cell or in soluble form and generally is comprised of a heterodimer having alpha and beta chains (also known as TCRα and TCRβ, respectively), or γ and δ chains (also known as TCRγ and TCRδ, respectively). Like immunoglobulins (*e.g.*, antibodies), the extracellular portion of TCR chains (*e.g.*, α-chain and β-chain) contain two immunoglobulin domains: a variable domain (*e.g.*, α-chain variable domain or V_α and β-chain variable domain or V_β; typically amino acids 1 to 116 based on Kabat numbering (Kabat *et al.* (1991) "Sequences of Proteins of Immunological Interest, US Dept. Health and Human Services, Public Health Service National Institutes of Health, 5th ed.) at the N-terminal end, and one constant domain (*e.g.*, α-chain constant domain or C_α, typically amino acids 117 to 259 based on Kabat, β-chain constant domain or C_β, typically amino acids 117 to 295 based on Kabat) at the C-terminal end and adjacent to the cell membrane. Also like immunoglobulins, the variable domains contain complementary determining regions ("CDRs", also called hypervariable regions or "HVRs") separated by framework regions ("FRs") *(see, e.g.,* Fores *et al.* (1990) *Proc. Natl. Acad Sci. US.A.* 87:9138; Chothia *et al.* (1988) *EMBO J.* 7:3745; Lefranc *et al.* (2003) *Dev. Comp. Immunol.* 27:55). In some embodiments, a TCR is found on the surface of a T cell (or T lymphocyte) and associates with the CD3 complex. The source of a TCR encompassed by the present invention may be from various animal species, such as a human, mouse, rat, rabbit or other mammal.

[0056] The term "T cell receptor" or "TCR" should be understood to encompass full TCRs as well as antigen-binding portions or antigen-binding fragments thereof. In some embodiments, the TCR is an intact or full-length TCR, including TCRs in the αβ form or γδ form. In some embodiments, the TCR is an antigen-binding portion that is less than a full-length TCR but that binds to a specific peptide bound in an MHC molecule, such as binds to an MHC-peptide complex. In some cases, an antigen-binding portion or fragment of a TCR

may contain only a portion of the structural domains of a full-length or intact TCR, but yet is able to bind the peptide epitope, such as MHC-peptide complex, to which the full TCR binds. In some cases, an antigen-binding portion contains the variable domains of a TCR, such as variable α chain and variable β chain of a TCR, sufficient to form a binding site for binding to a specific MHC-peptide complex. Generally, the variable chains of a TCR contain complementarity determining regions (CDRs) involved in recognition of the peptide, MHC and/or MHC-peptide complex.

[0057] Nomenclature established by the International Immunogenetics Information System (IMGT) (see also Scaviner and Lefranc (2000) *Exp. Clin. Immunogenet.* 17:83-96 and 97-106; Folch and Lefranc (2000) *Exp. Clin. Immunogenet,* 17:107-114; T Cell Receptor Factsbook", (2001) LeFranc and LeFranc, Academic Press, ISBN 0-12-441352-8). The IMGT provides unique sequences used to describe a TCR, and sequences described herein may be identified by reference to such unique sequences provided herein. TCR sequences are publicly available at the IMGT database at imgt.org.

[0058] As described above, native alpha/beta heterodimeric TCRs have an alpha chain and a beta chain. Broadly, each chain comprises variable, joining and constant regions, and the beta chain also usually contains a short diversity region between the variable and joining regions, but this diversity region is often considered as part of the joining region. Each variable region comprises three hypervariable CDRs (Complementarity Determining Regions) embedded in a framework sequence. CDR3 is well-known to be the main mediator of antigen recognition. There are several types of alpha chain variable (Vα) regions and several types of beta chain variable (Vβ) regions distinguished by their framework, CDR1 and CDR2 sequences, and by a partly defined CDR3 sequence. The Vα types are referred to in IMGT nomenclature by a unique TRAV number. For example, "TRAV4" defines a TCR Vα region having unique framework and CDR1 and CDR2 sequences, and a CDR3 sequence which is partly defined by an amino acid sequence which is preserved from TCR to TCR but which also includes an amino acid sequence which varies from TCR to TCR. Similarly, "TRBV2" defines a TCR Vβ region having unique framework and CDR1 and CDR2 sequences, but with only a partly defined CDR3 sequence. It is known that there are 54 alpha variable genes, of which 44 are functional, and 67 beta variable genes, of which 42 are functional, within the alpha and beta loci, respectively.

[0059] The joining regions of the TCR are similarly defined by the unique IMGT TRAJ and TRBJ nomenclature, and the constant regions by the IMGT TRAC and

TRBC nomenclature. The beta chain diversity region is referred to in IMGT nomenclature by the abbreviation TRBD, and, as mentioned, the concatenated TRBD/TRBJ regions are often considered together as the joining region.

[0060] The gene pools that encode the TCR alpha and beta chains are located on different chromosomes and contain separate V, (D), J and C gene segments, which are brought together by rearrangement during T cell development. This leads to a very high diversity of T cell alpha and beta chains due to the large number of potential recombination events that occur between the 54 TCR alpha variable genes and 61 alpha J genes or between the 67 beta variable genes, two beta D genes and 13 beta J genes. The recombination process is not precise and introduces further diversity within the CDR3 region. Each alpha and beta variable gene may also comprise allelic variants, designated in IMGT nomenclature as TRAVxx*01 and *02, or TRBVx-x*01 and *02 respectively, thus further increasing the amount of variation. In the same way, some of the TRBJ sequences have two known variations. (Note that the absence of a "*" qualifier means that only one allele is known for the relevant sequence). The natural repertoire of human TCRs resulting from recombination and thymic selection has been estimated to comprise approximately 10^6 unique beta chain sequences, determined from CDR3 diversity (Arstila *et al.* (1999) *Science* 286:958-961) and could be even higher (Robins *et al.* (2009) *Blood* 114:4099-4107). Each beta chain is estimated to pair with at least 25 different alpha chains, thus generating further diversity (Arstila *et al.* (1999) Science 286:958-961).

[0061] The term "TCR alpha variable domain" therefore refers to the concatenation of TRAV and TRAJ regions; a TRAV region only; or TRAV and a partial TRAJ region, and the term TCR alpha constant domain refers to the extracellular TRAC region, or to a C-terminal truncated or full length TRAC sequence. Likewise the term "TCR beta variable domain" refers to the concatenation of TRBV and TRBD/TRBJ regions; to the TRBV and TRBD regions only; to the TRBV and TRBJ regions only; or to the TRBV and partial TRBD and/or TRBJ regions, and the term TCR beta constant domain refers to the extracellular TRBC region, or to a C-terminal truncated or full length TRBC sequence. These TCR alpha variable domain and TCR beta variable domain nomenclature similarly applies to the variable domains of TCR gamma and TCR delta chains, respectively, for gamma/delta TCRs. An ordinarily skilled artisan can obtain TRAV, TRAJ, TRAC, TRBV, TRBJ, and TRBC gene sequences, such as through the publicly available IMGT database.

[0062] The term "TCR complex" refers to a complex formed by the association of CD3 with TCR. For example, a TCR complex may be composed of a CD3γ chain, a CD3δ chain, two CD3ε chains, a homodimer of CD3ζ chains, a TCRα chain, and a TCRβ chain. Alternatively, a TCR complex may be composed of a CD3γ chain, a CD3δ chain, two CD3ε chains, a homodimer of CD3ζ chains, a TCRγ chain, and a TCRδ chain.

[0063] The term "therapeutic effect" refers to a local or systemic effect in animals, particularly mammals, and more particularly humans, caused by a pharmacologically active substance. The term thus means any substance intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease or in the enhancement of desirable physical or mental development and conditions in an animal or human.

[0064] The terms "therapeutically effective amount" and "effective amount" means that amount of a substance that produces some desired effect, such as a desired local or systemic therapeutic effect, in at least a sub-population of cells in an animal at a reasonable benefit/risk ratio applicable to any treatment. In some embodiments, a therapeutically effective amount of a substance will depend on the substance's therapeutic index, solubility, pharmacokinetics, half-life, and the like. Toxicity and therapeutic efficacy of subject compounds may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} and the ED_{50}. In some embodiments, compositions that exhibit large therapeutic indices are used. In some embodiments, the LD_{50} (lethal dosage) may be measured and may be, for example, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000% or more reduced for the agent relative to no administration of the agent. Similarly, the ED_{50} (*i.e.*, the concentration which achieves a half-maximal inhibition of symptoms) may be measured and may be, for example, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000% or more increased for the agent relative to no administration of the agent. Also, similarly, the IC_{50} may be measured and may be, for example, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000% or more increased for the agent relative to no administration of the agent. In some embodiments, T cell immune response in an assay may be increased by at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or even 100%. In another embodiment, at least about a 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%,

50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or even 100% decrease in a viral load may be achieved.

[0065] The term "treat" refers to the therapeutic management or improvement of a condition (*e.g.*, a disease or disorder) of interest. Treatment may include, but is not limited to, infusing an agent or composition (*e.g.*, a pharmaceutical composition) to a subject. Treatment is typically undertaken in an effort to alter the course of a disease (which term is used to indicate any disease, disorder, syndrome or undesirable condition warranting or potentially warranting therapy) in a manner beneficial to the subject. The effect of treatment may include reversing, alleviating, reducing severity of, delaying the onset of, curing, inhibiting the progression of, and/or reducing the likelihood of occurrence or recurrence of the disease or one or more symptoms or manifestations of the disease. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. A therapeutic agent may be administered to a subject who has a disease or is at increased risk of developing a disease relative to a member of the general population. In some embodiments, a therapeutic agent may be administered to a subject who has had a disease but no longer shows evidence of the disease. The agent may be administered *e.g.*, to reduce the likelihood of recurrence of evident disease. A therapeutic agent may be administered prophylactically, *i.e.*, before development of any symptom or manifestation of a disease. "Prophylactic treatment" refers to providing medical and/or surgical management to a subject who has not developed a disease or does not show evidence of a disease in order, *e.g.*, to reduce the likelihood that the disease will occur or to reduce the severity of the disease should it occur. The subject may have been identified as being at risk of developing the disease (*e.g.*, at increased risk relative to the general population or as having a risk factor that increases the likelihood of developing the disease.

[0066] The term "variable region" or "variable domain" refers to the domain of an immunoglobulin superfamily binding protein (*e.g.*, a TCR α-chain or β-chain (or γ chain and δ chain for γδ TCRs)) that is involved in binding of the immunoglobulin superfamily binding protein (*e.g.*, TCR) to antigen. The variable domains of the α-chain and β-chain (V_α and V_β, respectively) of a native TCR generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three CDRs. The V_α domain is encoded by two

separate DNA segments, the variable gene segment and the joining gene segment (V-J); the V_β domain is encoded by three separate DNA segments, the variable gene segment, the diversity gene segment, and the joining gene segment (V-D-J). A single V_α or V_β domain may be sufficient to confer antigen-binding specificity. Furthermore, TCRs that bind a particular antigen may be isolated using a V_α or V_β domain from a TCR that binds the antigen to screen a library of complementary V_α or V_β domains, respectively.

[0067] The term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. In some embodiments, a vector is an episome, *i.e.,* a nucleic acid capable of extra-chromosomal replication. In some embodiments, vectors are those capable of autonomous replication and/or expression of nucleic acids to which they are linked. Vectors capable of directing the expression of genes to which they are operatively linked are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of "plasmids" which refer generally to circular double stranded DNA loops, which, in their vector form are not bound to the chromosome. In the present specification, "plasmid" and "vector" are used interchangeably as the plasmid is the most commonly used form of vector. However, as will be appreciated by those skilled in the art, the present invention is intended to include such other forms of expression vectors that serve equivalent functions and which become subsequently known in the art.

[0068] There is a known and definite correspondence between the amino acid sequence of a particular protein and the nucleotide sequences that can code for the protein, as defined by the genetic code (shown below). Likewise, there is a known and definite correspondence between the nucleotide sequence of a particular nucleic acid and the amino acid sequence encoded by that nucleic acid, as defined by the genetic code.

GENETIC CODE	
Alanine (Ala, A)	GCA, GCC, GCG, GCT
Arginine (Arg, R)	AGA, ACG, CGA, CGC, CGG, CGT
Asparagine (Asn, N)	AAC, AAT
Aspartic acid (Asp, D)	GAC, GAT
Cysteine (Cys, C)	TGC, TGT
Glutamic acid (Glu, E)	GAA, GAG
Glutamine (Gln, Q)	CAA, CAG

Glycine (Gly, G)	GGA, GGC, GGG, GGT
Histidine (His, H)	CAC, CAT
Isoleucine (Ile, I)	ATA, ATC, ATT
Leucine (Leu, L)	CTA, CTC, CTG, CTT, TTA, TTG
Lysine (Lys, K)	AAA, AAG
Methionine (Met, M)	ATG
Phenylalanine (Phe, F)	TTC, TTT
Proline (Pro, P)	CCA, CCC, CCG, CCT
Serine (Ser, S)	AGC, AGT, TCA, TCC, TCG, TCT
Threonine (Thr, T)	ACA, ACC, ACG, ACT
Tryptophan (Trp, W)	TGG
Tyrosine (Tyr, Y)	TAC, TAT
Valine (Val, V)	GTA, GTC, GTG, GTT
Termination signal (end)	TAA, TAG, TGA

[0069] An important and well-known feature of the genetic code is its redundancy, whereby, for most of the amino acids used to make proteins, more than one coding nucleotide triplet may be employed (illustrated above). Therefore, a number of different nucleotide sequences may code for a given amino acid sequence. Such nucleotide sequences are considered functionally equivalent since they result in the production of the same amino acid sequence in all organisms (although certain organisms may translate some sequences more efficiently than they do others). Moreover, occasionally, a methylated variant of a purine or pyrimidine may be found in a given nucleotide sequence. Such methylations do not affect the coding relationship between the trinucleotide codon and the corresponding amino acid.

[0070] For polypeptide amino acid sequence, corresponding nucleotide sequences that can encode the polypeptide can be deduced from the genetic code (which, because of its redundancy, will produce multiple nucleic acid sequences for any given amino acid sequence). Thus, description and/or disclosure herein of a nucleotide sequence which encodes a polypeptide should be considered to also include description and/or disclosure of the amino acid sequence encoded by the nucleotide sequence. Similarly, description and/or disclosure of a polypeptide amino acid sequence herein should be considered to also include description and/or disclosure of all possible nucleotide sequences that can encode the amino acid sequence.

BRIEF DESCRIPTION OF THE DRAWINGS

[0071] FIG. 1 is a schematic illustration of a vector that encodes a promoter is operably linked with a coding sequence that encodes TCRα and TCRβ chains, as well as CD8α and CD8β. The TCRα and TCRβ chains encode the chains of TCR of TSC-100 or TSC-101, and the amino acid sequences of the chains are separated by a self-cleaving peptide (P2A). CD8α and CD8β coreceptors allow CD4+ helper T cells to participate in target recognition and cytotoxicity, boosting CD8+ T cell function. CD8α and CD8β are likewise separated by a self-cleaving peptide (P2A), and the CD8α polypeptide includes an N-terminal tag that enables purification of engineered T cells as well as tracking in the patient.

[0072] FIG. 2 includes graphs of panels A and B, showing that TSC-100 and TSC-101, respectively, inhibit growth of antigen-pulsed T2 cells. T2 cells pulsed with HA-1 or HA-2 peptides containing the antigenic sequences were incubated *in vitro* withTSC-100 or TSC-101 T cells, respectively. Graphs show the ratio of the number of cells in each population relative to the starting number of cells at time 0.

[0073] FIG. 3 is a schematic showing that treated subjects receive both HCT from a selected donor and T cell therapy (TSC-100 or TSC-101) using T cells from the same donor. In the pictured, non-limiting embodiments, the subject is positive for the antigen targeted by the engineered T cells and for the presenting HLA protein (HLA-A*02:01), whereas the donor cells are negative for the antigen. The T cell therapy targets residual cancer cells but not donor cells, eliminating residual recipient cells and preventing relapse following HCT.

[0074] FIG. 4 is a schematic representing distribution of subjects into three arms (two treatment arms and a control standard of care (SOC) arm) for clinical evaluation of TSC-100 and TSC-101.

[0075] FIG. 5 is a chart showing that the presence or absence of HLA-A*02:01 in subjects undergoing RIC-HCT does not significantly impact outcomes of malignancy. Relapse, overall survival, and acute GvHD frequency at 6 months were not found to be significantly different.

[0076] FIG. 6 is a schematic showing an exemplary, non-limiting timeline for treatment of subjects in TSC-100 or TSC-101 treatment groups. A first dose is given to all subjects, and second doses can be infused in accordance with treatment assignments and protocols.

[0077] FIG. 7 is a schematic detailing treatment regimens and dosing levels for treatment and control arms. All does levels include the same first dose. Dose Level 2

includes a second dose at the same level, while Dose Level 3 includes a second dose at four-fold the level of the first dose.

[0078] FIG. 8 is a chart showing non-limiting examples of application of a treatment protocol to clinical subjects for the TSC-100 treatment group, the TSC-101 treatment group, and the control group.

[0079] FIG. 9 includes panels A and B, which are respectively a chart and a graph relating to adverse events. Panel A is a chart showing adverse events relating to cytokine release syndrome (CRS) or graft-versus-host disease (GvHD). Panel B is a graph showing levels of C-Reactive Protein (CRP) after transplantation in treatment arms (TSC-100 or TSC-101, at various dosing levels (DLs), or in controls. Data show that CRS and GvHD are similar across TSC and control arms. No cytokine release syndrome or neurotoxicity was observed after TSC-100 treatment or TSC-101 treatment, and minimal changes in CRP (a laboratory marker of CRS) were observed, consistent with the general safety of the engineered TCR T cell therapies (TSC-100 and TSC-101).

[0080] FIG. 10 includes panels A and B, each of which is a chart relating to adverse events. Panel A is a chart showing the types and highest grades of adverse events of at least Grade 2 in both the treatment arms (TSC-100 and/or TSC-101 treatment arms) or control arm. Adverse events having a grade equal to or greater than Grade 2 were similar in all arms. Panel B is a chart showing particular examples of adverse events, as well as their grade, time of occurrence, and whether related to treatment with engineered T cell therapy.

[0081] FIG. 11 is a series of four graphs showing that TSC-100 cells demonstrated early expansion (percent of T cells), proliferation (Ki67 positive), activation (granzyme B positive) and shift to CD4+ subsets that can persist long-term, respectively, in subjects.

[0082] FIG. 12 is a series of four graphs showing that TSC-101 cells demonstrated early expansion (percent of T cells), proliferation (Ki67 positive), activation (granzyme B positive) and shift to CD4+ subsets that can persist long-term, respectively, in subjects.

[0083] FIG. 13 is a pair of graphs showing that each of TSC-1001 and TSC-101 persisted in peripheral blood of treatment group recipients for over 200 days, and at higher levels in subjects that received two doses.

[0084] FIG. 14 is a chart showing particular examples of control subjects and subjects receiving engineered T cell therapy with TSC-100 or TSC-101. Data shown indicated the time of T cell therapy administration (where relevant), status of donor chimerism (mixed or complete), and whether the subject required clinical intervention for increasing mixed chimerism or relapsed. Donor chimerism detected by high-sensitivity next-generation

sequencing (NGS) assay (AlloHeme) with limit of detection of 0.13%. *Measurements taken at indicated day post HCT ±3 days.

[0085] FIG. 15 is a pair of graphs showing the percentage of chimerism in control and treatment arm subjects, respectively, after HCT. All relapses or adverse outcomes occurred with mixed chimerism. Median post-transplant follow-up in treatment arms: 8 months (range 3-12 months); median follow-up in control arm: 7 months (range 2-15 months).

[0086] FIG. 16 is a graph showing particular examples of control subjects and subjects receiving engineered T cell therapy with TSC-100 or TSC-101. Data shown indicated the time of T cell therapy administration (where relevant), presence or absence of MRD, and whether the subject required clinical intervention for increasing mixed chimerism or relapsed. *MRD determined by NGS (lower limit of detection 0.05-0.1%). ‡ Dose did not meet target dose criteria in supplemental cohorts.

[0087] FIG. 17 is chart showing results from a control subject and a subject receiving engineered T cell therapy with TSC-101, each subject having TP53-mutated MDS. Data shown indicated the time of T cell therapy administration (where relevant), presence or absence of MRD, status of chimerism (mixed or complete), and whether the subject relapsed or succumbed to disease. MRD and chimerism determined by NGS (lower limits of detection 0.1% and 0.13%, respectively).

[0088] FIG. 18 is a schematic representation of a construct for expression of TCR set forth in Table 1.

[0089] FIG. 19 is a schematic representation of the TSC-100 npDNA Transposon (SEQ ID NO: 29).

[0090] FIG. 20 is a schematic representation of the TSC-101 npDNA Transposon (SEQ ID NO: 30).

[0091] FIG. 21 is a set of panels providing information regarding a phase 1 trial of TSC-100 and TSC-101, engineered T cell therapies that target minor histocompatibility antigens to eliminate residual disease after hematopoietic cell transplantation.

[0092] FIG. 22 is a set of panels providing information regarding a phase 1 trial of TSC-100 and TSC-101, engineered T cell therapies that target minor histocompatibility antigens to eliminate residual disease after hematopoietic cell transplantation.

[0093] FIG. 23 is a set of panels providing information regarding a phase 1 trial of TSC-100 and TSC-101, engineered T cell therapies that target minor histocompatibility antigens to eliminate residual disease after hematopoietic cell transplantation.

[0094] FIG. 24 is a chart showing particular representative examples of control subjects and subjects receiving engineered T cell therapy with TSC-100 or TSC-101. Data shown indicated the time of T cell therapy administration (where relevant), status of donor chimerism (mixed or complete), and whether the subject required clinical intervention for increasing mixed chimerism or relapsed. FIG. 24 shows that all 11 TSC-treated patients remained relapse-free with no detectable cancer. Shown are patients that were ≥ 60 days post-HCT at the time of data cutoff. Donor chimerism was detected by high-sensitivity next-generation sequencing (NGS) assay (AlloHeme) with limit of detection of 0.2%. ‡ Measurements taken at indicated day post HCT ± 3 days.

[0095] FIG. 25 is a graph showing particular representative examples of control subjects and subjects receiving engineered T cell therapy with TSC-100 or TSC-101. Data shown indicated the time of T cell therapy administration (where relevant), presence or absence of MRD, and whether the subject required clinical intervention for increasing mixed chimerism or relapsed. FIG. 25 shows that all TSC-treated subjects remained MRD-negative. MRD was determined by flow cytometry (lower limit of detection 0.1-1%) or NGS (lower limit of detection 0.05-0.1% in myeloid and 0.001-0.01% in lymphoid malignancies). ‡ Dose did not meet target dose criteria in supplemental cohorts.

[0096] FIG. 26 provides a summary of a multi-arm Phase 1 trial for TSC-100 and TSC-101 in subjects with AML, ALL, and MDS.

[0097] FIG. 27 provides a summary of the subjects enrolled in the Phase I trial and indicates that a majority of subjects in the treatment and control arms are at high risk for relapse. "*" refers to control subjects that received transplant are included in the safety data cohort. "**" refers to adverse risk defined as having either a IPSS-M mutation if the subject has MDS or European Leukemia Network (ELN) high risk genetics or cytogenetics for AML; ELN 2022 high risk genetics/ cytogenetics include mutated ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, ZRSR2, TP53, -5/ del(5q)/, -7,-17/ abn(17p), t(6;9), t(v;11q23.3), t(9;22), t(8;16), inv(3) or t(3;3), t(3q26.2;v), monosomal or complex karyotype (for AML); IPSS-M mutations are reported in Bernard *et al.* (2022) *NEJM Evid.* 1(7) (for MDS).

[0098] FIG. 28 provides a summary chart demonstrating that subjects have been treated at all three dose levels and no dose-limiting toxicities have been observed.

[0099] FIG. 29 provides graphs demonstrating that TSC-100 and TSC-101 TCR-T cells are detected for greater than one year with increased persistence seen at highest dose level, DL3. "*" refers to the area under the curve (AUC) of TSC-100/TSC-101 between Day

90-180 (Geometric mean(geometric CV)): DL1: 2.26(47.2%); DL2 and sDL2: 8.15(42.2%); DL3 and sDL3: 34.47(97.7%). Dose did not meet target dose criteria in supplemental cohorts (sDL)

[0100] FIG. 30 provides a summary demonstrating that adverse events of special interest were low-grade and manageable. Common terminology criteria for adverse events (CTCAE) were used. Balanced Grade 2-4 acute graft-versus-host disease (GvHD) was observed between treatment and control arms. No moderate or severe chronic GvHD was observed. Two episodes of low-grade cytokine release syndrome (CRS) was reported post-TSC infusions, including one Grade 1 event (TSC-100) and one Grade 2 event (TSC-101). No cases of immune effector cell-associated neurotoxicity syndrome (ICANS) were observed.

[0101] FIG. 31 provides a summary demonstrating that Grade ≥3 treatment emergent adverse events are consistent with transplantation.

[0102] FIG. 32 provides a summary demonstrating complete donor chimerism achieved in all patients after initial TSC infusion. Donor chimerism results were measured using a commercially available short tandem repeat (STR) assay with LOD of 1-2% at indicated times post-HCT ± 3 days in patients at least 60 days post-HCT as of a given data cut. "‡" refers to a dose that did not meet target dose criteria in supplemental cohorts.

[0103] FIG. 33 provides a summary demonstrating that minimal residual disease (MRD)-negativity was achieved in all treatment-arm subjects.

[0104] FIG. 34 provides a graph demonstrating that TCR-T treatment reduces the probability of relapse. CoxPH Ratio = 0.275, CI = (0.05, 1.502), p = 0.136; Log–rank p = 0.1105. For the two subjects who relapsed following TSC infusion, unusual, non-representative circumstances occurred. In the TSC-101 dose level 3 case, the subject was a 65 year-old male with KMT2A-rearranged AM, who did not respond to induction chemotherapy (4% blasts), who was taken to transplant after reinduction chemotherapy without achieving CR, and passed away on Day 129 post-transplant with suspected relapse. In the TSC-100 dose level 3 case, the subject was a 59 year-old male with FLT3-TKD AML wherein donor apheresis for manufacturing occurred after G-CSF due to manufacturing slot shortage and manufacturing was challenging due to high granulocytes; repeat manufacturing required such that both infusions were delayed (Day 41 and Day 97) and relapse was observed in CNS at Day 139 post-transplant with no systemic relapse.

[0105] FIG. 35 provides a graph demonstrating that event-free survival (EFS) favors the treatment arm. Event defined as relapse, clinical intervention for impending relapse (non-

TSC), or death. Cox PH Ratio = 0.304, CI = (0.096, 0.966, p = 0.0435); Log-rank p = 0.0321.

[0106] FIG. 36 provides a graph demonstrating that TSC-101 alone captures almost all patients who are HLA-A*02:01 positive and obviating a need for TSC-100 or a companion diagnostic.

[0107] FIG. 37 provides a summary for an efficient study design with TSC-101-only and CIBMTR external control.

[0108] FIG. 38 provides a summary indicating how the efficient study design described in FIG. 37 can trigger a change in clinical practice and increase addressable patient populations.

[0109] FIG. 39 provides representative dosing regimens.

DETAILED DESCRIPTION

[0110] The present disclosure encompasses TCRs as set forth in Table 1 that bind (*e.g.*, specifically and/or selectively) to a peptide-MHC (pMHC) complex comprising an HA-1 immunogenic peptide or HA-2 peptide in the context of an HLA-A*02:01. The present disclosure further encompasses T cells that encode and/or express TCRs. In various embodiments, administration of cells that encode and/or express TCRs that recognize HA-1 or HA-2 to subjects can treat malignancy and/or prevent relapse. The present disclosure encompasses, among other things, methods of treating a malignancy selected from the group consisting of acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), and myelodysplastic disorder (MDS) in an adult subject that previously received reduced intensity conditioning (RIC) hematopoietic cell therapy (HCT), where the treatment includes infusing a first dose of haploidentical T cells engineered to express a TCR that binds HA-1 (VLHDDLLEA; SEQ ID NO: 1) or HA-2 (YIGEVLVSV; SEQ ID NO: 2) when presented by HLA-A*02:01, where the TCR includes sequence (e.g. CDRs, variable domains, and/or chains) set forth in Table 1. In some embodiments, cells of the malignancy encode and/or express each of HA-1 and HLA-A*02:01, the HCT includes administration of hematopoietic cells from a donor that (i) does not encode and/or express HA-1; and/or (ii) does not encode and/or express an HLA-A*02 allele, and the first dose is selected from the group consisting of about 5x10^6 cells/kg of the haploidentical T cells, about 0.8 billion of the haploidentical T cells, and about 1.2 billion of the haploidentical T cells, and the first dose is infused at a time that is between about 14 and about 42 days after the subject received HCT, optionally at a time that is about 21 days after the subject received HCT. In some embodiments, cells of

the malignancy encode and/or express each of HA-2 and HLA-A*02:01, the HCT includes administration of hematopoietic cells from a donor that (i) does not encode and/or express HA-2; and/or (ii) does not encode and/or express an HLA-A*02 allele, and the first dose is selected from the group consisting of about 5x10^6 cells/kg of the haploidentical T cells, about 0.8 billion of the haploidentical T cells, and about 1.2 billion of the haploidentical T cells, and the first dose is infused at a time that is between about 14 and about 42 days after the subject received HCT, optionally at a time that is about 21 days after the subject received HCT. In some embodiments, cells of the malignancy encode and/or express each of HA-2 and HLA-A*02:01, the HCT includes administration of hematopoietic cells from a donor that does not encode and/or express an HLA-A*02 allele, and the first dose is selected from the group consisting of about 5x10^6 cells/kg of the haploidentical T cells, about 0.8 billion of the haploidentical T cells, and about 1.2 billion of the haploidentical T cells, and the first dose is infused at a time that is between about 14 and about 42 days after the subject received HCT, optionally at a time that is about 21 days after the subject received HCT. In various embodiments a subject further receives a second dose as described herein.

In various embodiments, at least 80% of cells of a dose (*e.g.*, a first dose and/or a second dose) of haploidentical T cells express a marker, optionally wherein the marker is Qtag-CD34 (*e.g.*, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%). In various embodiments, the percentage of cells of a dose of haploidentical T cells that express a marker, optionally wherein the marker is Qtag-CD34, is between 80% and 85%, 80% and 90%, 80% and 95%, or 80% and 100%. In various embodiments, the percentage of cells of a dose of haploidentical T cells that express a marker, optionally wherein the marker is Qtag-CD34, is between 90% and 95%, 90% and 96%, 90% and 97%, 90% and 98%, 90% and 99%,or 90% and 100%. In various embodiments, engineered cells are selected by tag-based purification.

TCRs and Engineered T Cells

[0111] TCRs of the present disclosure include the two TCRs for which CDR, variable domain, constant domain, and full chain sequences are set forth in Table 1 below. In various embodiments, the present disclosure provides a T cell, or population of T cells, engineered to encode and express a TCR set forth in Table 1. Engineered T cell products of the present

disclosure include products referred to as TSC-100 and TSC-101. Engineered T cell products of the present disclosure can include CD8+ and/or CD4+ T cells.

[0112] In various embodiments, TSC-100 includes engineered T cells that encode and/or express a TCR that includes an alpha chain CDR1 according to SEQ ID NO: 5, an alpha chain CDR2 according to SEQ ID NO: 6, and an alpha chain CDR3 according to SEQ ID NO: 7, e.g., in an alpha chain variable domain, and includes a beta chain CDR1 according to SEQ ID NO: 11, a beta chain CDR2 according to SEQ ID NO: 12, and a beta chain CDR3 according to SEQ ID NO: 13, *e.g.*, in a beta chain variable domain.

[0113] In various embodiments, TSC-100 includes engineered T cells that encode and/or express a TCR that includes an alpha chain variable domain according to SEQ ID NO: 8 and a beta chain variable domain according to SEQ ID NO: 14. In various embodiments, TSC-100 includes engineered T cells that encode and/or express a TCR that includes an alpha chain variable domain having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity with SEQ ID NO: 8 and a beta chain variable domain having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity with SEQ ID NO: 14. In various embodiments, TSC-100 includes engineered T cells that encode and/or express a TCR that includes an alpha chain variable domain having no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid differences with SEQ ID NO: 8 and a beta chain variable domain having no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid differences with SEQ ID NO: 14.

[0114] In various embodiments, TSC-100 includes engineered T cells that encode and/or express a TCR that includes an alpha chain according to SEQ ID NO: 10 and a beta chain according to SEQ ID NO: 16. In various embodiments, TSC-100 includes engineered T cells that encode and/or express a TCR that includes an alpha chain having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity with SEQ ID NO: 10 and a beta chain having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity with SEQ ID NO: 16. In various embodiments, TSC-100 includes engineered T cells that encode and/or express a TCR that includes an alpha chain having no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid differences with SEQ ID NO: 10 and a beta chain having no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid differences with SEQ ID NO: 16.

[0115] In various embodiments, TSC-100 includes engineered T cells that encode and/or express CD8α and CD8β. Expression of CD8α and CD8β coreceptors by TSC-100 engineered T cells can allow CD4+ helper T cells to participate in target recognition and cytotoxicity, boosting CD8+ T cell function.

[0116] TSC-100 also encompasses products disclosed under that designation in International Application Publication No.: WO 2022/099100 and corresponding US Application Publication No.: 2023/0398217, which is incorporated herein by reference in its entirety, with respect to its disclosure of TCRs and uses thereof, and in particular with respect to TSC-100 (see "HA1-TSC-100 CDTM").

[0117] In various embodiments, TSC-101 includes engineered T cells that encode and/or express a TCR that includes an alpha chain CDR1 according to SEQ ID NO: 17, an alpha chain CDR2 according to SEQ ID NO: 18, and an alpha chain CDR3 according to SEQ ID NO: 19, e.g., in an alpha chain variable domain, and includes a beta chain CDR1 according to SEQ ID NO: 23, a beta chain CDR2 according to SEQ ID NO: 24, and a beta chain CDR3 according to SEQ ID NO: 25, e.g., in a beta chain variable domain.

[0118] In various embodiments, TSC-101 includes engineered T cells that encode and/or express a TCR that includes an alpha chain variable domain according to SEQ ID NO: 20 and a beta chain variable domain according to SEQ ID NO: 26. In various embodiments, TSC-101 includes engineered T cells that encode and/or express a TCR that includes an alpha chain variable domain having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity with SEQ ID NO: 20 and a beta chain variable domain having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity with SEQ ID NO: 26. In various embodiments, TSC-101 includes engineered T cells that encode and/or express a TCR that includes an alpha chain variable domain having no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid differences with SEQ ID NO: 20 and a beta chain variable domain having no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid differences with SEQ ID NO: 26.

[0119] In various embodiments, TSC-101 includes engineered T cells that encode and/or express a TCR that includes an alpha chain according to SEQ ID NO: 22 and a beta chain according to SEQ ID NO: 28. In various embodiments, TSC-101 includes engineered T cells that encode and/or express a TCR that includes an alpha chain having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity with SEQ ID NO: 22 and a beta chain having at least 90%,

at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity with SEQ ID NO: 28. In various embodiments, TSC-101 includes engineered T cells that encode and/or express a TCR that includes an alpha chain having no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid differences with SEQ ID NO: 22 and a beta chain having no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid differences with SEQ ID NO: 28.

[0120] In various embodiments, TSC-100 includes engineered T cells that encode and/or express CD8α and CD8β. Expression of CD8α and CD8β coreceptors by TSC-100 engineered T cells can allow CD4+ helper T cells to participate in target recognition and cytotoxicity, boosting CD8+ T cell function.

[0121] TSC-101 also encompasses products disclosed under that designation in International Application Publication No.: WO 2022/221478, which is incorporated herein by reference in its entirety, with respect to its disclosure of TCRs and uses thereof, and in particular with respect to TSC-101 (see "HA2-MJ14-DP317 MGTM").

Table 1. Exemplary TCR Amino Acid Sequences

Agent	Alpha/ Beta	Element	Sequence	SEQ ID NO:
TSC-100 HA1-TSC-100 CDTM	Alpha TRAV21/TRAJ40	CDR1	**DSAIYN**	5
		CDR2	**IQSSQRE**	6
		CDR3	**CAVRGGTSGTYKYIF**	7
		V_α	METLLGLLILWLQLQWVSSKQEVTQIPAALSVPEGE NLVLNCSFT**DSAIYN**LQWFRQDPGKGLTSLLL**IQSS QRE**QTSGRLNASLDKSSGRSTLYIAASQPGDSATYL **CAVRGGTSGTYKYIF**GTGTRLKVLAN	8
		C_α	iqnpdpavyqlrdskssdksvclftdfdsqtnvsqskdsdvyitdktvldmrs mdfksnsavawsnksdfacanafnnsiipedtffpssdvpcdvklveksfetd tnlnfqnllvivlrilllkvagfnllmtlrlwss	9
		Chain	METLLGLLILWLQLQWVSSKQEVTQIPAALSVPEGE NLVLNCSFT**DSAIYN**LQWFRQDPGKGLTSLLL**IQSS QRE**QTSGRLNASLDKSSGRSTLYIAASQPGDSATYL **CAVRGGTSGTYKYIF**GTGTRLKVLANiqnpdpavyqlrd skssdksvclftdfdsqtnvsqskdsdvyitdktvldmrsmdfksnsavawsn ksdfacanafnnsiipedtffpssdvpcdvklveksfetdtnlnfqnllvivlrilll kvagfnllmtlrlwss	10
	Beta	CDR1	**SEHNR**	11

		CDR2	**FQNEAQ**	12
	TRBV7-9/	CDR3	**CASSRTAGDTQYF**	13
	TRBJ2-3	V_β	MGTSLLCWMALCLLGADHADTGVSQDPRHKITKR GQNVTFRCDPI**SEHNR**LYWYRQTLGQGPEFLTY**FQ NEAQ**LEKSRLLSDRFSAERPKGSFSTLEIQRTEQGDS AMYL**CASSRTAGDTQYF**GPGTRLTVLE	14
		C_β	dlnkvfppevavfepskaeiahtqkatlvclatgffpdhvelswwvngkevhs gvstdpqplkeqpalndsryclssrlrvsatfwqnprnhfrcqvqfyglsende wtqdrakpvtqivsaeawgradcgitsasyhqgvlsatilyeillgkatlyavlvs alvlmamvkrkdf	15
		Chain	MGTSLLCWMALCLLGADHADTGVSQDPRHKITKR GQNVTFRCDPI**SEHNR**LYWYRQTLGQGPEFLTY**FQ NEAQ**LEKSRLLSDRFSAERPKGSFSTLEIQRTEQGDS AMYL**CASSRTAGDTQYF**GPGTRLTVLEdlnkvfppeva vfepskaeiahtqkatlvclatgffpdhvelswwvngkevhsgvstdpqplke qpalndsryclssrlrvsatfwqnprnhfrcqvqfyglsendewtqdrakpvtq ivsaeawgradcgitsasyhqgvlsatilyeillgkatlyavlvsalvlmamvkr kdf	16
TSC-101 HA2- MJ14- DP317 MGTM	Alpha TRAV38- 2DV8/ TRAJ11/ TRAC	CDR1	**TSESDYY**	17
		CDR2	**QEAYKQQN**	18
		CDR3	**CAYRSERYSTLTF**	19
		V_α	MACPGFLWALVISTCLEFSMAQTVTQSQPEMSVQE AETVTLSCTYD**TSESDYY**LFWYKQPPSRQMILVIR**Q EAYKQQN**ATENRFSVNFQKAAKSFSLKISDSQLGD AAMYF**CAYRSERYSTLTF**GKGTMLLVSPD	20
		C_α	iqnpdpavyqlrdskssdksvclftfdsqtnvsqskdsdvyitdktvldmrsm dfksnsavawsnksdfacanafnnsiipedtffpssdvpcdvklveksfetdtn lnfqnllvivlrilllkvagfnllmtlrlwss	21
		Chain	MACPGFLWALVISTCLEFSMAQTVTQSQPEMSVQE AETVTLSCTYD**TSESDYY**LFWYKQPPSRQMILVIR**Q EAYKQQN**ATENRFSVNFQKAAKSFSLKISDSQLGD AAMYF**CAYRSERYSTLTF**GKGTMLLVSPDiqnpdpav yqlrdskssdksvclftfdfsqtnvsqskdsdvyitdktvldmrsmdfksnsav awsnksdfacanafnnsiipedtffpssdvpcdvklveksfetdtnlnfqnllvi vlrilllkvagfnllmtlrlwss	22
	Beta TRBV30/	CDR1	**GTSNPN**	23
		CDR2	**SVGIG**	24
		CDR3	**CAWSVPYLAGGKRPDTQYF**	25

	TRBJ2-3/ TRBC1	V_β	MLCSLLALLLGTFFGVRSQTIHQWPATLVQPVGSPL SLECTVE**GTSNPN**LYWYRQAAGRGLQLLFY**SVGIG** QISSEVPQNLSASRPQDRQFILSSKKLLLSDSGFYL**C** **AWSVPYLAGGKRPDTQYF**GPGTRLTVLE	26
		C_β	dlnkvfppevavfepskaeiahtqkatlvclatgffpdhvelswwvngkevhs gvstdpqplkeqpalndsryclssrlrvsatfwqnprnhfrcqvqfyglsende wtqdrakpvtqivsaeawgradcgitsasyhqgvlsatilyeillgkatlyavlvs alvlmamvkrkdf	27
		Chain	MLCSLLALLLGTFFGVRSQTIHQWPATLVQPVGSPL SLECTVE**GTSNPN**LYWYRQAAGRGLQLLFY**SVGIG** QISSEVPQNLSASRPQDRQFILSSKKLLLSDSGFYL**C** **AWSVPYLAGGKRPDTQYF**GPGTRLTVLEdlnkvfppe vavfepskaeiahtqkatlvclatgffpdhvelswwvngkevhsgvstdpqpl keqpalndsryclssrlrvsatfwqnprnhfrcqvqfyglsendewtqdrakpv tqivsaeawgradcgitsasyhqgvlsatilyeillgkatlyavlvsalvlmamv krkdf	28

Nucleic Acids and Vectors

[0122] In an aspect encompassed by the present invention, provided herein are nucleic acid molecules that encode that encode a TCR of Table 1. In particular, the present disclosure encompasses nucleic acid vectors that include constructs as shown in **FIG. 1**, where the sequences encoding the TCRα and TCRβ chains encode the TCR chains of a TCR of Table 1.

[0123] As shown in **FIG. 1**, a promoter is operably linked with the coding sequence that encodes TCRα and TCRβ chains, as well as CD8α and CD8β. The TCRα and TCRβ chains encode the chains of TCR of TSC-100 or TSC-101, and the amino acid sequences of the chains are separated by a self-cleaving peptide (P2A). CD8α and CD8β coreceptors allow CD4+ helper T cells to participate in target recognition and cytotoxicity, boosting CD8+ T cell function. CD8α and CD8β are likewise separated by a self-cleaving peptide (P2A), and the CD8α polypeptide includes an N-terminal tag that enables purification of engineered T cells as well as tracking in the subject.

[0124] In some embodiments, a vector encoded and/or expressed by engineered T cells designated TSC-100 has the sequence set forth in SEQ ID NO: 29, referred to as the "TSC-100 npDNA Transposon" and further illustrated in **FIGS. 18-19**.

[0125] The TSC-100 npDNA Transposon is a 5,709-bp supercoiled, Nanoplasmid™ DNA (npDNA) carrying the minimal R6K bacterial replication origin and anti-sense RNA-

based antibiotic-free selection marker (RNA-OUT). The terminal inverted repeats (TIRs) of the armyworm *Spodoptera frugiperda* transposon (required for transposase-mediated integration) are connected to the two ends of the exogenous DNA sequence. The TSC-100 HA-1-specific TCR α- and β-chain genes and the codon-diversified CD8 α- and β-chain genes are placed under the control of the murine stem cell virus (MSCV) promoter. The constant regions of the TCR α- and β-chains contain amino-acid substitutions that stabilize their expression on the surface of engineered T cells. These four open reading frames are encoded on a single mRNA molecule along with woodchuck post-transcriptional regulatory element (WPRE), which is known to enhance gene expression (Loeb, 1999). The ribosomal skipping that takes places on the self-cleaving peptide P2A leads to production of the four separate polypeptides. The CD34 epitope recognized by the mouse monoclonal antibody QBend/10 is fused to the N-terminus of the exogenous CD8 α-chain, allowing for purification of transgene-expressing cells to increase the safety and potency of the drug substance. The Nanoplasmid vector backbone (<500 base pairs) is substantially smaller than conventional plasmids (>1500 base pairs). It contains no antibiotic-resistant gene, and its replication origin (R6K) necessary for plasmid preparation can support propagation in a narrower range of bacterial species than a commonly used origin (pUC).

[0126] In some embodiments, a vector encoded and/or expressed by engineered T cells designated TSC-101 has the sequence set forth in SEQ ID NO: 30, referred to as the "TSC-101 npDNA Transposon" and further illustrated in **FIGS. 18 and 20**.

[0127] The TSC-101 npDNA Transposon is a 5,721-bp supercoiled, Nanoplasmid™ DNA (npDNA) carrying the minimal R6K bacterial replication origin and anti-sense RNA-based antibiotic-free selection marker (RNA-OUT). The inverted terminal repeats (ITRs) of the armyworm Spodoptera frugiperda transposon (required for transposase-mediated integration) are connected to the two ends of the exogenous DNA sequence. The TSC-101 HA-2-specific TCR α- and β-chain genes and the codon-diversified CD8 α- and β-chain genes are placed under the control of the murine stem cell virus (MSCV) promoter. The constant regions of the TCR α- and β-chains contain amino-acid substitutions that stabilize their expression on the surface of engineered T cells and help prevent mispairing with the α- and β-chains of the endogenous TCRs. These four open reading frames (TCRβ, TCRα, CD8α, and CD8β) are encoded on a single mRNA molecule along with the open reading frame-disabled woodchuck post-transcriptional regulatory element (WPRE), which is known to enhance gene expression (Loeb, 1999). The ribosomal skipping that takes places on the self-cleaving peptide P2A leads to production of the four separate polypeptides. The 16-

amino acid CD34 epitope recognized by the mouse monoclonal antibody QBend/10 is fused to the N-terminus of the exogenous CD8 α-chain, allowing for purification of transgene-expressing cells to increase the safety and potency of the drug substance. The Nanoplasmid vector backbone (<500 base pairs) is substantially smaller than conventional plasmids (>1500 base pairs). It contains no antibiotic-resistant gene, and its replication origin (R6K) necessary for plasmid preparation supports propagation in a narrower range of bacterial species than the commonly used origin pUC.

Vector: TSC-100 npDNA Transposon: (SEQ ID NO: 29; FIG. 29)

AATTAATTGCTTGCAATTAACCCTTTAACGGTTATAAGGATCTAGATGAGATAGA
AAGATTTGGTTTTCGGATTTGTGTTACATAAGATGCCTAAAATAAAAATTGAGAT
TCAATTTTTTTTAAACTTTTTTTTAATTGGTGGTAAGAATATTCCCTCTACCTGTTT
GAGAGTAATGAAATTGTAGTATGATTTTTCAACAAACTAAAAAAACAACATAAA
TCTCACATAATAACTTTATTTCAATCACACAATTGAATACCAATATTAATTAATG
GTTGACAGTACTTACCAGCCTGCAGG**TGAAAGACCCCACCTGTAGGTTTGGCA**
AGTTAGCTTAAGTAACGCCATTTTGCAAGGCATGGAAAATACATAACTGAGA
ATAGAGAAGTTCAGATCAAGGTTAGGAACAGAGAGACAGCAGAATATGGGC
CAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAA
CAGATGGTCCCCAGATGCGGTCCCGCCCTCAGCAGTTTCTAGCGAACCATCA
GATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAA
CTAACCAATCAGTTTGCTTCTTGCTTCTGTTTGTGTGCTTCTGCTCCCTGAGC
TCAATAAAAGAGCCCACAACCCCTCACTTGGTGGGCCAGTCCTCTGATAGAC
TGTGTCCCCTGGATACCCGTACGGTACCGCTAGCGCCACC*ATGGGCACCAGCCT*
CCTCTGCTGGATGGCCCTGTGTCTCCTGGGGGCAGATCACGCAGATACTGGAGTCT
CCCAGGACCCCAGACACAAGATCACAAAGAGGGGACAGAATGTTACTTTCAGGTGT
GATCCAATTTCTGAACACAACCGCCTTTATTGGTACCGCCAGACCCTGGGGCAGGG
CCCAGAGTTTCTGACTTACTTCCAGAATGAAGCTCAACTTGAAAAATCAAGGCTGC
TCAGTGATCGGTTCTCTGCAGAGAGGCCTAAGGGATCTTTCTCCACCTTGGAGATC
CAGCGCACAGAGCAGGGGGACTCTGCCATGTATCTCTGTGCCAGCAGCCGCACTGC
TGGAGATACTCAGTATTTTGGCCCAGGCACCCGGCTGACAGTGCTCGAAGATCTGA
ACAAGGTGTTCCCTCCAGAGGTGGCCGTGTTCGAGCCTTCTAAGGCCGAGATCGCC
CACACACAAAAAGCCACCCTCGTGTGCCTGGCCACCGGCTTTTTCCCCGACCACGT
GGAACTGTCTTGGTGGGTCAACGGCAAAGAGGTGCACTCCGGCGTGTCAACGGAT
CCCCAGCCTCTGAAAGAACAGCCTGCCCTGAACGACAGCCGGTACTGCCTGAGCTC
CAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCCCGGAACCACTTCAGATGCC
AGGTGCAGTTTTACGGCCTGAGCGAGAACGACGAGTGGACCCAGGACAGAGCCAA
GCCCGTGACACAAATCGTGTCTGCCGAAGCCTGGGGAAGAGCCGATTGCGGCATC
ACCAGCGCCTCCTATCACCAGGGCGTGCTGAGCGCCACAATCCTGTACGAAATCCT
GCTGGGCAAGGCCACCCTGTACGCCGTGCTGGTGTCTGCTCTGGTGCTGATGGCC
ATGGTCAAGCGGAAGGACTTTGGCAGCGGCAGAGCCAAAAGGTCCGGGAGCGGT
GCGACAAACTTTAGCCTGTTGAAACAAGCCGGCGACGTTGAAGAGAACCCCGGA
CCT**ATGGAAACCCTCTTGGGCCTGCTTATCCTTTGGCTGCAGCTGCAATGGGTG**
AGCAGCAAACAGGAGGTGACTCAGATTCCTGCAGCTCTGAGTGTCCCAGAAG
GAGAAAACTTGGTTCTCAACTGCAGTTTCACTGATAGCGCTATTTACAACCT
CCAGTGGTTTAGGCAGGACCCTGGGAAAGGCCTCACATCTCTGTTGCTTATT
CAGTCAAGTCAGAGAGAGCAAACAAGTGGACGCCTTAATGCCTCTCTGGAT

AAATCATCAGGACGCAGTACTCTTTACATTGCAGCTTCTCAGCCTGGTGATTC
AGCCACCTACCTGTGCGCTGTGAGGGGTGGTACCTCAGGAACCTACAAATACAT
CTTTGGAACAGGCACCAGGCTGAAGGTTCTTGCAAACATCCAGAACCCCGA
CCCCGCCGTGTACCAGCTGAGGGACTCCAAGTCCAGCGACAAGAGCGTGTG
TCTGTTTACGGACTTCGACAGCCAGACCAACGTGAGTCAAAGCAAGGACAG
CGACGTCTACATAACGGATAAGACCGTGCTGGACATGCGGAGCATGGACTT
CAAGAGCAACAGCGCCGTGGCCTGGTCCAACAAGAGCGACTTCGCCTGCGC
CAACGCCTTCAACAACAGCATCATCCCCGAGGACACCTTCTTCCCCAGCAGC
GACGTGCCCTGCGACGTGAAACTGGTGGAGAAGTCCTTCGAGACAGACACC
AATCTGAACTTTCAGAACCTGCTGGTGATCGTGCTGCGGATTCTGCTGCTGAAA
GTGGCCGGCTTCAATCTGCTGATGACCCTGCGGCTGTGGAGCAGCAGGGCTA
AGAGGTCCGGCAGCGGAGCCACCAATTTTTCCCTGCTGAAACAGGCTGGTGACG
TGGAAGAAAACCCTGGCCCC*ATGGCGCTGCCCGTCACCGCGCTGCTGCTGCCCCTG*
*GCGCTGCTGTTACACGCCGCTCGGCCA*GAGCTTCCCACCCAGGGCACATTCTCCAAC
*GTGTCCACCAATGTGTCGGGAGGCGGCGGATCG*TCCCAGTTCAGAGTGTCCCCTCTG
GACCGCACCTGGAACCTGGGCGAGACCGTGGAGCTGAAATGTCAGGTCCTGCTGAGC
AACCCGACCTCCGGGTGCAGTTGGCTGTTCCAGCCGCGTGGTGCTGCCGCAAGCCCT
ACGTTCCTGCTTTACCTGAGCCAGAACAAGCCCAAGGCGGCCGAGGGCCTGGACACC
CAGAGATTCTCCGGCAAGCGCCTGGGGGACACATTCGTGCTTACTTTGAGCGATTTCC
GCAGAGAGAACGAGGGCTACTATTTCTGTTCGGCGCTGAGCAATTCCATCATGTATTTC
AGCCACTTTGTGCCAGTGTTCCTGCCTGCCAAGCCTACCACAACACCAGCTCCCCGTC
CCCCGACTCCGGCGCCTACCATCGCGAGTCAACCGTTGAGCCTGAGGCCTGAGGCTT
GTCGGCCCGCTGCGGGGGGTGCCGTCCACACCAGGGGCCTCGACTTTGCGTGCGAC
ATCTATATTTGGGCGCCTCTGGCGGGTACCTGCGGGGTGCTGCTGCTGTCATTGGTGA
TTACCCTGTACTGCAATCACCGCAACCGCCGGCGGGTCTGTAAGTGCCCACGGCCTG
*TGGTCAAGTCCGGTGACAAACCGTCGCTCTCGGCTCGCTACGTG*CGCGCTAAGCGCA
GCGGTTCCGGGGCCACCAACTTTTCATTGCTGAAGCAGGCCGGTGATGTGGAGG
AGAATCCAGGGCCCATGCGCCCCAGGCTTTGGCTCCTTCTTGCTGCTCAGCTCAC
TGTCTTGCATGGCAACTCCGTTCTGCAGCAGACTCCCGCCTACATCAAGGTGCAG
ACGAACAAGATGGTGATGCTGTCATGCGAGGCCAAGATCTCTCTTTCAAATATGA
GAATTTATTGGCTACGACAGCGCCAGGCCCCCTCCAGCGACAGCCACCACGAGTT
CCTGGCGCTTTGGGATTCTGCTAAAGGCACCATCCATGGAGAGGAGGTGGAACA
GGAGAAGATAGCTGTCTTCCGCGACGCATCCCGCTTCATCCTGAACCTGACCAGC
GTGAAGCCGGAGGACAGCGGCATCTACTTCTGTATGATCGTTGGCTCCCCCGAGC
TGACCTTCGGCAAAGGCACCCAGCTGTCCGTGGTGGACTTCCTGCCCACCACAGC
CCAGCCAACCAAGAAATCCACCCTCAAGAAGCGCGTGTGCCGACTGCCCCGCCC
TGAAACCCAGAAGGGCCCTCTGTGCTCCCCCATCACCCTTGGACTGCTGGTGGCG
GGAGTCCTGGTGCTGCTCGTATCTCTGGGTGTCGCCATCCACCTGTGCTGCCGCC
GCCGCCGCGCCCGCCTGAGGTTTATGAAACAGTTTTACAAGTGATAAATCGATAG
ATCCTAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAAC
TATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCT
ATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCT
CTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGT
TTGCTGACGCAACCCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCTTTCC
GGGACTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACTCATCGCCGCCTGCCT
TGCCCGCTGCTGGACAGGGGCTCGGCTGTTGGGCACTGACAATTCCGTGGTGTTG
TCGGGGAAATCATCGTCCTTTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCT
GCGCGGGACGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCTT
CCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAG
ACGAGTCGGATCTCCCTTTGGGCCGCCTCCCCGCCTGAGATCCTTTAAGACCAAT

GCTGGAGTTCTTCGCCCACCCCAACTTGTTTATTGCAGCTTATAATGGTTACAAAT
AAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAG
TTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGTATACAGGTTACCT
CAGTCTCCTAGGTACGTCTTATATCTATGAAAAAACATTCAAAAGCACAACATCT
AGAAGAACTTACCTTTTTTCACCACTCTATTGCAAAGATATGTACCGATTTCTCTC
GAAGTACAAAAAACCGCTAGTTTTCAAATTCACCTCAAGACTTTGAAAAAAAATT
GAATCTGTCAATGTCAAATAAAATCAGAAACAAATGTCATAATGTTACGTTAATG
TTGTCAGGTCGAAAAATAAAATTGCAAATAGAAATTTTGTTCCTTTTTTATTGGTTTTT
ATTGGTGGGAAAAATATTCCCTCTAACTGCAAAAGGGTTAATTATGTTAGAGGTAG
AGTCGACGCTAGCTGGCTTGTTGTCCACAACCATTAAACCTTAAAAGCTTTAAAA
GCCTTATATATTCTTTTTTTTCTTATAAAACTTAAAACCTTAGAGGCTATTTAAGT
TGCTGATTTATATTAATTTTATTGTTCAAACATGAGAGCTTAGTACGTGAAACAT
GAGAGCTTAGTACATTAGCCATGAGAGCTTAGTACATTAGCCATGAGGGTTTAGT
TCATTAAACATGAGAGCTTAGTACATTAAACATGAGAGCTTAGTACATACTATCA
ACAGGTTGAACTGCTGATCTGTACAGTAGAATTGGTAAAGAGAGTTGTGTAAAAT
ATTGAGTTCGCACATCTTGTTGTCTGATTATTGATTTTTGGCGAAACCATTTGATC
ATATGACAAGATGTGTATCTACCTTAACTTAATGATTTTGATAAAAATCATTAGG
TACC

MSCV promoter is in bold. Beta chain is annotated using bold and italic text. Alpha chain is annotated using bold and underlined text. CD34-enrichment tag (Q tag) is annotated using italic and underlined text. CD8-alpha is in italic. CD8-beta is underlined.

TSC-100 TCR-T cells (*e.g.*, helper ($CD4^+$, now $CD8^+/CD4^+$) and cytotoxic ($CD8^+$) T cells) as described in the examples were engineered by transposon/transposase-mediated gene delivery of the TSC-100 npDNA transposon as shown immediately above, to express (1) a recombinant TCR (*e.g.*, the recombinant TCR specific to the HA-1 peptide (VLHDDLLEA; SEQ ID NO: 1) presented on HLA-A*02:01, (2) recombinant CD8α and CD8β co-receptors to maximize the efficacy of the therapeutic product, and (3) a CD34-derived epitope tag fused on the N-terminus of CD8α to facilitate tracking of engineered cells *in vitro* and *in vivo*. Such cells can be manufactured using known techniques and, for the present example, were generated through isolation of peripheral blood mononuclear cells (PBMC) from a fresh apheresis product, delivery of transposase mRNA and vector transposon npDNA by electroporation, T cell activation and culture, culture expansion, and culture wash, formulation, and cryopreservation.

Vector: TSC-101 npDNA Transposon: (SEQ ID NO: 30)

AATTAATTGCTTGCAATTAACCCTTTAACGGTTATAAGGATCTAGATGAGATAGA
AAGATTTGGTTTTCGGATTTGTGTTACATAAGATGCCTAAAATAAAAATTGAGAT
TCAATTTTTTTTTAAACTTTTTTTTAATTGGTGGTAAGAATATTCCCTCTACCTGTTT
GAGAGTAATGAAATTGTAGTATGATTTTTCAACAAACTAAAAAAACAACATAAA

TCTCACATAATAACTTTATTTCAATCACACAATTGAATACCAATATTAATTAATG
GTTGACAGTACTTACCAGCCTGCAGGTGAAAGACCCCACCTGTAGGTTTGGCA
AGTTAGCTTAAGTAACGCCATTTTGCAAGGCATGGAAAATACATAACTGAGA
ATAGAGAAGTTCAGATCAAGGTTAGGAACAGAGAGACAGCAGAATATGGGC
CAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAA
CAGATGGTCCCCAGATGCGGTCCCGCCCTCAGCAGTTTCTAGCGAACCATC
AGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGA
ACTAACCAATCAGTTTGCTTCTTGCTTCTGTTTGTGTGCTTCTGCTCCCTGAG
CTCAATAAAAGAGCCCACAACCCCTCACTTGGTGGGCCAGTCCTCTGATAGA
CTGTGTCCCCTGGATACCCGTACGGTACCGCTAGCGCCACC*ATGCTGTGTAGCC*
TGCTGGCCCTGCTCCTGGGCACATTTTTTGGCGTCAGATCCCAGACCATCCATCAG
TGGCCTGCCACACTGGTGCAGCCTGTTGGATCTCCCCTGTCTCTGGAATGTACCGT
GGAAGGCACAAGCAACCCCAACCTCTACTGGTATAGGCAGGCCGCTGGAAGAGGA
CTGCAGCTGCTGTTTTACAGCGTCGGCATCGGCCAGATCAGCAGCGAGGTTCCACA
GAATCTGAGCGCCTCCAGGCCTCAGGACAGACAGTTTATCCTGAGCAGCAAGAAG
CTGCTGCTGAGCGACTCCGGCTTTTACCTCTGTGCTTGGAGCGTTCCATACCTGGC
CGGTGGCAAACGCCCTGACACTCAATATTTCGGCCCAGGCACCCGGCTGACAGTG
CTCGAAGATCTGAACAAGGTGTTCCCTCCAGAGGTGGCCGTGTTCGAGCCTTCTAA
GGCCGAGATCGCCCACACACAAAAAGCCACCCTCGTGTGCCTGGCCACCGGCTTTT
TCCCCGACCACGTGGAACTGTCTTGGTGGGTCAACGGCAAAGAGGTGCACTCCGG
CGTGTCAACGGATCCCCAGCCTCTGAAAGAACAGCCTGCCCTGAACGACAGCCGG
TACTGCCTGAGCTCCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCCCGGAA
CCACTTCAGATGCCAGGTGCAGTTTTACGGCCTGAGCGAGAACGACGAGTGGACC
CAGGACAGAGCCAAGCCCGTGACACAAATCGTGTCTGCCGAAGCCTGGGGAAGAG
CCGATTGCGGCATCACCAGCGCCTCCTATCACCAGGGCGTGCTGAGCGCCACAATC
CTGTACGAAATCCTGCTGGGCAAGGCCACCCTGTACGCCGTGCTGGTGTCTGCTCT
*GGTGCTGATGGCCATGGTCAAGCGGAAGGACTTT*GGCAGCGGCAGAGCCAAAAG
GTCCGGGAGCGGTGCGACAAACTTTAGCCTGTTGAAACAAGCCGGCGACGTTGA
AGAGAACCCCGGACCT**ATGGCCTGTCCTGGCTTCCTGTGGGCCCTTGTGATC**
AGCACTTGCCTGGAATTCAGCATGGCTCAGACAGTCACCCAGTCTCAGCCC
GAAATGAGCGTCCAAGAGGCTGAAACCGTGACTCTGTCTTGTACCTACGAC
ACCTCCGAGAGCGATTACTACCTCTTTTGGTATAAGCAACCGCCGTCCAGGC
AAATGATCCTCGTGATCCGGCAAGAAGCTTACAAACAGCAGAATGCTACCG
AAAACCGGTTCTCCGTCAATTTTCAGAAAGCCGCTAAGAGCTTTAGCCTGAA
AATCTCCGACTCTCAGCTCGGCGACGCTGCTATGTATTTCTGTGCCTACCGC
AGTGAGCGCTACAGCACTTTGACTTTCGGGAAGGGGACTATGCTTCTAGTCT
CTCCAGACATCCAGAACCCCGACCCCGCCGTGTACCAGCTGAGGGACTCCA
AGTCCAGCGACAAGAGCGTGTGTCTGTTTACGGACTTCGACAGCCAGACCA
ACGTGAGTCAAAGCAAGGACAGCGACGTCTACATAACGGATAAGACCGTGC
TGGACATGCGGAGCATGGACTTCAAGAGCAACAGCGCCGTGGCCTGGTCCA
ACAAGAGCGACTTCGCCTGCGCCAACGCCTTCAACAACAGCATCATCCCCG
AGGACACCTTCTTCCCCAGCAGCGACGTGCCCTGCGACGTGAAACTGGTGG
AGAAGTCCTTCGAGACAGACACCAATCTGAACTTTCAGAACCTGCTGGTGAT
CGTGCTGCGGATTCTGCTGCTGAAAGTGGCCGGCTTCAATCTGCTGATGAC
CCTGCGGCTGTGGAGCAGCAGGGCTAAGAGGTCCGGCAGCGGAGCCACCAATT
TTTCCCTGCTGAAACAGGCTGGTGACGTGGAAGAAAACCCTGGCCCC*ATGGCGCT*
GCCCGTCACCGCGCTGCTGCTGCCCCTGGCGCTGCTGTTACACGCCGCTCGGCCA<u>*GA*</u>
GCTTCCCACCCAGGGCACATTCTCCAACGTGTCCACCAATGTGTCGGGAGGCGGCGG
<u>*ATCGT*</u>*CCCAGTTCAGAGTGTCCCCTCTGGACCGCACCTGGAACCTGGGCGAGACCGT*
GGAGCTGAAATGTCAGGTCCTGCTGAGCAACCCGACCTCCGGGTGCAGTTGGCTGTT

CCAGCCGCGTGGTGCTGCCGCAAGCCCTACGTTCCTGCTTTACCTGAGCCAGAACAA
GCCCAAGGCGGCCGAGGGCCTGGACACCCAGAGATTCTCCGGCAAGCGCCTGGGGG
ACACATTCGTGCTTACTTTGAGCGATTTCCGCAGAGAGAACGAGGGCTACTATTTCTGT
TCGGCGCTGAGCAATTCCATCATGTATTTCAGCCACTTTGTGCCAGTGTTCCTGCCTGC
CAAGCCTACCACAACACCAGCTCCCCGTCCCCCGACTCCGGCGCCTACCATCGCGAG
TCAACCGTTGAGCCTGAGGCCTGAGGCTTGTCGGCCCGCTGCGGGGGGTGCCGTCC
ACACCAGGGGCCTCGACTTTGCGTGCGACATCTATATTTGGGCGCCTCTGGCGGGTA
CCTGCGGGGTGCTGCTGCTGTCATTGGTGATTACCCTGTACTGCAATCACCGCAACCG
CCGGCGGGTCTGTAAGTGCCCACGGCCTGTGGTCAAGTCCGGTGACAAACCGTCGCT
*CTCGGCTCGCTACGTGC*GCGCTAAGCGCAGCGGTTCCGGGGCCACCAACTTTTCA
TTGCTGAAGCAGGCCGGTGATGTGGAGGAGAATCCAGGGCCCATGCGCCCCAGG
CTTTGGCTCCTTCTTGCTGCTCAGCTCACTGTCTTGCATGGCAACTCCGTTCTGCA
GCAGACTCCCGCCTACATCAAGGTGCAGACGAACAAGATGGTGATGCTGTCATG
CGAGGCCAAGATCTCTCTTTCAAATATGAGAATTTATTGGCTACGACAGCGCCAG
GCCCCCTCCAGCGACAGCCACCACGAGTTCCTGGCGCTTTGGGATTCTGCTAAAG
GCACCATCCATGGAGAGGAGGTGGAACAGGAGAAGATAGCTGTCTTCCGCGACG
CATCCCGCTTCATCCTGAACCTGACCAGCGTGAAGCCGGAGGACAGCGGCATCT
ACTTCTGTATGATCGTTGGCTCCCCCGAGCTGACCTTCGGCAAAGGCACCCAGCT
GTCCGTGGTGGACTTCCTGCCCACCACAGCCCAGCCAACCAAGAAATCCACCCTC
AAGAAGCGCGTGTGCCGACTGCCCCGCCCTGAAACCCAGAAGGGCCCTCTGTGC
TCCCCCATCACCCTTGGACTGCTGGTGGCGGGAGTCCTGGTGCTGCTCGTATCTCT
GGGTGTCGCCATCCACCTGTGCTGCCGCCGCCGCCGCGCCCGCCTGAGGTTTATG
AAACAGTTTTACAAGTGATAAATCGATAGATCCTAATCAACCTCTGGATTACAAA
ATTTGTGAAAGATTGACTGGTATTCTTAACTATGTTGCTCCTTTTACGCTATGTGG
ATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTT
CTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTG
TCAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCTGACGCAACCCCCACTGGTTG
GGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTTCGCTTTCCCCCTCCCTA
TTGCCACGGCGGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCG
GCTGTTGGGCACTGACAATTCCGTGGTGTTGTCGGGGAAATCATCGTCCTTTCCTT
GGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCCTTCTGCTACGTC
CCTTCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGC
GGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGCC
GCCTCCCCGCCTGAGATCCTTTAAGACCAATGCTGGAGTTCTTCGCCCACCCCAA
CTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTC
ACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAA
TGTATCTTATCATGTCTGTATACAGGTTACCTCAGTCTCCTAGGTACGTCTTATAT
CTATGAAAAAACATTCAAAAGCACAACATCTAGAAGAACTTACCTTTTTTCACCA
CTCTATTGCAAAGATATGTACCGATTTCTCTCGAAGTACAAAAAACCGCTAGTTT
TCAAATTCACCTCAAGACTTTGAAAAAAAATTGAATCTGTCAATGTCAAATAAAA
TCAGAAACAAATGTCATAATGTTACGTTAATGTTGTCAGGTCGAAAAATAAAATT
GCAAATAGAAATTTTGTTCCTTTTTTATTGGTTTTTATTGGTGGGAAAAATATTCC
CTCTAACTGCAAAAGGGTTAATTATGTTAGAGGTAGAGTCGACGCTAGCTGGCTT
GTTGTCCACAACCATTAAACCTTAAAAGCTTTAAAAGCCTTATATATTCTTTTTTT
TCTTATAAAACTTAAAACCTTAGAGGCTATTTAAGTTGCTGATTTATATTAATTTT
ATTGTTCAAACATGAGAGCTTAGTACGTGAAACATGAGAGCTTAGTACATTAGCC
ATGAGAGCTTAGTACATTAGCCATGAGGGTTTAGTTCATTAAACATGAGAGCTTA
GTACATTAAACATGAGAGCTTAGTACATACTATCAACAGGTTGAACTGCTGATCT
GTACAGTAGAATTGGTAAAGAGAGTTGTGTAAAATATTGAGTTCGCACATCTTGT

TGTCTGATTATTGATTTTTGGCGAAACCATTTGATCATATGACAAGATGTGTATCT
ACCTTAACTTAATGATTTTGATAAAAATCATTAGGTACC

MSCV promoter is in bold. Beta chain is annotated using bold and italic text. Alpha chain is annotated using bold and underlined text. CD34-enrichment tag (Q tag) is annotated using italic and underlined text. CD8-alpha is in italic. CD8-beta is underlined.

TSC-101 TCR-T cells (*e.g.*, helper ($CD4^+$, now $CD8^+/CD4^+$) and cytotoxic ($CD8^+$) T cells) as described in the examples were engineered by transposon/transposase-mediated gene delivery of the TSC-101 npDNA transposon as shown immediately above, to express (1) a recombinant TCR (*e.g.*, the recombinant TCR specific to the HA-2 (YIGEVLVSV; SEQ ID NO: 2) when presented on HLA-A*02:01, (2) recombinant CD8α and CD8β co-receptors to maximize the efficacy of the therapeutic product, and (3) a CD34-derived epitope tag fused on the N-terminus of CD8α to facilitate tracking of engineered cells *in vitro* and *in vivo*. Such cells can be manufactured using known techniques and, for the present example, were generated through isolation of peripheral blood mononuclear cells (PBMC) from a fresh apheresis product, delivery of transposase mRNA and vector transposon npDNA by electroporation, T cell activation and culture, culture expansion, and culture wash, formulation, and cryopreservation.

Pharmaceutical Compositions

[0128] In another aspect encompassed by the present invention, pharmaceutical compositions of TSC-100 or TSC-101 include a pharmaceutically acceptable carrier, diluent, or excipient. Methods for producing and formulating engineered TCR T cells are known in the art. Exemplary formulation components and excipients can include, for example, CryoStor®, Plasmalyte and HAS, which can be included to promote product quality and stability during cryopreservation, long-term storage (*e.g.*, under liquid nitrogen), freezing and/or thawing, and/or clinical use.

Treatment of AML, ALL, and MDS

[0129] In an aspect encompassed by the present disclosure, provided herein are methods for treating acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), and myelodysplastic disorder (MDS). In certain embodiments, myelodysplastic disorder (MDS) can be, for example, MDS with single lineage dysplasia, MDS with ring sideroblasts

(MDS-RS), MDS-RS and single lineage dysplasia, MDS-RS and multilineage dysplasia, MDS with multilineage dysplasia, MDS with excess blasts, MDS with isolated del(5q), MDS, unclassifiable, provisional entity: Refractory cytopenia of childhood, and Myeloid neoplasms with germ line predisposition. In certain embodiments, myelodysplastic disorder (MDS) can be, for example, a myelodysplastic/myeloproliferative neoplasms (MDS/MPN), e.g., selected from chronic myelomonocytic leukemia (CMML), atypical chronic myeloid leukemia (aCML), BCR-ABL1–, juvenile myelomonocytic leukemia (JMML), MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T), and MDS/MPN, unclassifiable. In certain embodiments, myelodysplastic disorder (MDS) can be , for example, refractory cytopenia with unilineage dysplasia (refractory anemia, refractory neutropenia, and refractory thrombocytopenia), refractory anemia with ring sideroblasts (RARS), refractory anemia with ring sideroblasts - thrombocytosis (RARS-t), refractory cytopenia with multinieage dysplasia (RCMD), refractory cytopenia with multinieage dysplasia and ring sideroblasts (RCMD-RS), refractory anemia with excess blasts (RAEB), myelodysplasia unclassifiable, refractory cytopenia of childhood, MDS with isolated del(5q), and other well-known MDS subtypes (see, for example, 2016 World Health Organization MDS classification available on the World Wide Web at nbci.nlm.nih.gov/pmc/articles/PMC655460/). In various embodiments, cells of the hematopoietic malignancy are characterized by expression of an HA-1 antigen and/or an HA-1 or HA-2 antigen, and/or for inducing an immune response against a cell of interest, such as a hyperproliferative cell, expressing an HA-1 antigen and/or an HA-1 or HA-2 antigen. In some embodiments, the method comprises infusing a subject with a therapeutically effective amount of a composition comprising cells expressing at least one binding protein described herein.

EXAMPLES

[0130] The present Examples illustrate the use of a TCR of Table 1 in the treatment of acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), and myelodysplastic disorder (MDS)in subjects that were previously treated by hematopoietic stem cell therapy. The present Examples provide a Phase I clinical trial demonstrating methods and results from administration of TCRs of the present disclosure to human subjects (see also **FIGS. 21-25**).

Example 1: Use of TSC-100 and TSC-101 TCR T cells to Prevent Relapse in Certain Hematopoietic Malignancies

[0131] There are approximately 35,000 cases of AML, MDS, and ALL in the United States every year. Of these 35,000 cases, about 7,350 undergo allogeneic hematopoietic cell transplant (HCT). Allogeneic HCT is the standard of care for most of these patients, and is likely to remain the standard of care. However, about 33% of patients relapse after HCT within one year (and about 40% within two years) due to residual malignance cells that persist after HCT, with greater than 90% mortality within 1 year after relapse. In Reduced intensity conditioning (RIC) HCT (RIC-HCT) utilizes conditioning regimens for HCT that are reduced in intensity as compared to conventional myeloblative conditioning (MAC) regimens, which were inherently toxic and often too stringent for older or less fit patients. Examples of common characteristics of RIC include inclusion of the purine analog fludarabine and reductions in dosage of alkylating agents and/or total body irradiation (TBI). The present disclosure specifically includes several RIC regimens: (i) an RIC regimen comprising fludarabine, cyclophosphamide, and total-body irradiation, wherein the total-body irradiation comprises a dosage of 200 cGy or 400 cGy; (ii) an RIC regimen comprising fludarabine and total-body irradiation, wherein the total-body irradiation comprises a dosage of 200 cGy, optionally wherein the RIC regimen comprises melphalan; and (iii) an RIC regimen comprising thiotepa, busulfan and fludarabine. The present Example relates to the use of TSC-100 and TSC-101 engineered TCR T cells to eliminate residual recipient blood cells post-RIC-HCT, thus supporting treatment and preventing relapse.

[0132] Cells genetically reprogrammed to express engineered TCRs engage natural mechanisms used by the human body to fight cancer. TCR T cells can target both intra-cellular and extra-cellular protein targets. Although T cell therapies targeting the lineage-specific antigens CD19 (B cells) or BCMA (plasma cells) are highly effective in patients with lymphoid malignancies, and feasible because depleting normal B cells or plasma cells can be tolerated by patients, this approach is not effective for myeloid malignancies, since depleting normal myeloid cells like neutrophils would lead to serious complications such as febrile neutropenia. Malignancies such as acute myeloid leukemia (AML), myelodysplastic syndrome (MDS) and exemplary acute lymphoblastic leukemias (ALL) have not been fully addressed by CAR-T therapies, at least in part due to a lack of targets specific for malignant myeloid cells that spare normal myeloid cells. Hematopoietic lineage-specific minor histocompatibility antigens (MiHAs) can be targeted by T cell receptors (TCRs), but not chimeric antigen receptors, because they most frequently represent single-amino acid changes

in intracellular proteins that are presented on the cell surface by human leukocyte antigens (HLA). TCRs directed against MiHAs HA-1 and HA-2 are especially useful for treatment of AML, MDS, and ALL. TCRs of the present disclosure target peptides expressed in the context of HLA-A*02:01, an HLA type found in 42% of people in the United States, 47% of Europeans, and 19% of Asians.

[0133] In the present Example, TSC-100 and TSC-101 engineered TCR T cells are used to target hematopoietic cells of subjects that have received allogeneic, haploidentical RIC-HCT, but not the transplanted donor hematopoietic cells. Because engineered T cell products TSC-100 and TSC-101 target MiHAs HA-1 and HA-2 respectively when presented by HLA-A*02:01, the engineered T cells can target all recipient hematopoietic cells while leaving donor hematopoietic cells untouched in subjects receiving HCT from donors who are mismatched for either the MiHA or HLA-A*02:01. Administration of TSC-100 or TSC-101 to AML, ALL and MDS subjects following RIC-HCT can therefore eliminate residual hematopoietic cells after HCT and prevent disease relapse. Donor hematopoietic cells negative for MiHAs or HLA-A*02:01 that presents these MiHAs are untouched by TCR T cells, thereby sparing normal blood cells post-RIC-HCT.

TCR Vector and Engineered T Cells

The present TCRs targeting HA-1 and HA-2 were expressed from a vector construct illustrated by the schematic of **FIG. 1**. A promoter is operably linked with a coding sequence that encodes TCRα and TCRβ chains, as well as CD8α and CD8β. The TCRα and TCRβ chains encode the chains of TCR of TSC-100 or TSC-101, and the amino acid sequences of the chains are separated by a self-cleaving peptide (P2A). CD8α and CD8β coreceptors allow CD4+ helper T cells to participate in target recognition and cytotoxicity, boosting CD8+ T cell function. CD8α and CD8β are likewise separated by a self-cleaving peptide (P2A), and the CD8α polypeptide includes an N-terminal tag that enables purification of engineered T cells as well as tracking in the subject. In some embodiments, the tag can be a Q-tag.

Elimination of Target Cells by TSC-100 and TSC-101

[0134] To demonstrate elimination of cells that express HA-1 or HA-2, respectively, when presented by HLA-A*02:01, engineered T cells were assayed for their ability to inhibit growth of peptide-pulsed T2 cells. Data demonstrate dose-dependent *in-vitro* elimination of the antigen-pulsed cells, with an EC_{50} value of 9.8 pg/mL for TSC100 engineered cells for

HA-1-pulsed T2 cells and an EC_{50} value of 15.9 pg/mL for TSC101 engineered cells for HA-2-pulsed T2 cells (**FIG. 2**).

Subjects and Treatment Criteria

[0135] Engineered T cell products TSC-100 and TSC-101 target MiHAs HA-1 and HA-2 respectively when presented by HLA-A*02:01. After RIC-HCT, AML, ALL, and MDS subjects can harbor both residual malignant cells that present HA-1 and/or HA-2 in the context of HLA proteins, and healthy donor cells that can differ in antigen and/or HLA expression. In the present Example, TSC-100 and TSC-101 engineered T cells were infused to subjects that express both the relevant target antigen (HA-1 or HA-2) and the required HLA (HLA-A*02:01), where the donor does not express the targeted antigen, does not express HLA-A*02:01, or does not express either. This allows TSC-100 and TSC-101 T cell products to target residual malignant cells but not healthy donor cells from HCT (see **FIG. 3**).

[0136] Accordingly, subjects were genotyped to determine whether they were positive for HLA-A*02:01. Because TSC-100 and TSC-101 target HLA-A*02:01 presented MiHAs, subjects positive for HLA-A*02:01 (~40% prevalence) were assigned to the treatment group for TSC100 or TSC101 treatment, and all others (~60% prevalence) were assigned to the control group (see **FIG. 4**). Within the treatment arm, subjects were genotyped for HA-1 and/or HA-2 (see, *e.g.*, Dossa 2018 *Blood* 131(1):108-120 and Bleakley 2011 *Immunol Cell Biol* 89(3):396-407, each of which is incorporated herein by reference with respect to genotyping). Subjects positive for HA-1 (~60% prevalence) received TSC-100 in addition to standard of care treatment including RIC-HCT, and subjects positive for HA-2 (~40% prevalence) received TSC-101 in addition to standard of care treatment including RIC-HCT. In the control arm, all subjects received standard of care treatment including RIC-HCT. Thus, eligible subjects were assigned to treatment or control arms depending on their HLA and HA-1/ HA-2 genotypes, and received TSC-100/101+ SOC or SOC alone. Separation of the treatment group and control group based on HLA alleles is supported by data, in that prior analysis have confirmed that HLA-type is not associated with a difference in outcomes for AML, MDS, and ALL (**FIG. 5**).

[0137] For the present Example, inclusion and exclusion criteria were applied to all subjects. The present Example was limited to subjects with AML, MDS and ALL planned to receive HCT with reduced intensity conditioning (RIC). All subjects were required have a diagnosis of AML, ALL or MDS, to be at least 18 years of age, to have an Eastern

Cooperative Oncology Group performance status (ECOG-PS) score of at least two at any time during screening, to be eligible for reduced intensity conditioning (RIC) and haploidentical donor HCT, and agree to a 15-year follow up term. ECOG-PS is framework for measuring how disease impacts a subject's daily living abilities, which can be referred to by physicians and researchers as performance status. ECOG-PS is commonly considered in the study of cancer therapy and is available online, e.g., at hypertext transfer protocol ecog-acrin.org/resources/ecog-performance-status/, which is incorporated herein by reference. Subjects were excluded from this Example if levels of donor-specific HLA antibodies were high enough to warrant desensitization protocols and the subject did not have access to alternate donors. In various embodiments, the presence or absence of levels of donor-specific HLA antibodies high enough to warrant desensitization protocols was determined at the discretion of an attending medical practitioner such as a physician. Subjects were also excluded if there was evidence of clinically significant infection or uncontrolled viral reactivation of cytomegalovirus (CMV), Epstein-Barr virus (EBV), Adenovirus, BK virus (BKV), or human herpesvirus 6 (HHV-6). Subjects were also excluded if they had received prior allogeneic HCT. Subjects were further excluded from the treatment group in this Example if they were HLA-A*02:07 positive. In various embodiments, the presence or absence of evidence of clinically significant infection or uncontrolled viral reactivation was determined at the discretion of an attending medical practitioner such as a physician.

[0138] For the treatment group additional exclusion criteria related to the HCT donor. Donors were required to be at least 16 years of age. Donors were required to be able to undergo peripheral blood stem cell (PBSC) collection and two rounds of leukapheresis. Treatment group HCT donors for subjects receiving TSC-100 were required to be negative for all HLA-A*02 alleles or negative of HA-1, while HCT donors for subjects receiving TSC-101 were required to be negative for all HLA-A*02 alleles or negative of HA-2. In some embodiments, HCT donors for subjects receiving TSC-101 were required to be negative for all HLA-A*02 alleles (regardless of HA-2 status). Subjects were also excluded if receiving HCT from donors who tested positive for: HIV-1, HIV-2, HTLV-1, HTLV-2 or had active hepatitis B or hepatitis C, syphilis, or West Nile virus infection, or screened positive for risk of Creutzfeldt-Jakob disease or Zika virus.

[0139] Additional restriction criteria apply to the treatment received by subjects. Subject eligibility depended upon subjects receiving standard of care according to particular guidelines. RIC regimens of eligible subjects were limited to (1) an RIC regimen of fludarabine, cyclophosphamide, and total-body irradiation (200 or 400 cGy); (2) an RIC

regimen of fludarabine and melphalan with or without total-body irradiation (200 cGy); (3) an RIC regimen of thiotepa, busulfan and fludarabine; or (4) an RIC regimen of fludarabine, melphalan and thiotepa. For all subjects, prophylaxis against graft-versus-host disease (GvHD) from donor HCT included post-transplant cyclophosphamide, mycophenolate mofetil (MMF), and tacrolimus. However, in the event of acute or chronic GvHD treatment, any appropriate therapy was acceptable. Maintenance therapies of eligible subjects could include FLT3 and/or BCR/Abl, as well as IDH inhibitors administered no sooner than 60 days after T cell therapy or after day 100 of the protocol, but other anti-leukemia agents (*e.g.*, oral azacytidine) were not allowed.

Protocol and Administration

[0140] The present Example relates to a multi-arm study of TSC-100 and TSC-101 in which subjects were enrolled in the control group (control subjects receiving RIC-HCT but not engineered T cells who are either 1) HLA-A*02:01 negative subjects or 2) positive for HLA-A*02:01, but lack a mismatched donor) or either of two experimental groups (subjects expressing HLA-A*02:01 and HA-1 or HA-2, receiving both RIC-HCT and an engineered T cell therapy). Within each experimental group, three dosage levels were tested: a single dose of 5x10^6 engineered T cells (infused about 14 days to about 42 days after HCT, *e.g.*, about 21 days after HCT; "Dose Level 1"); two doses of 5x10^6 engineered T cells/kg (a first dose infused about 14 days to about 42 days after HCT, *e.g.*, about 21 days after HCT, and a second dose infused about 54 days to about 82 days after HCT, *e.g.*, about 61 days after HCT; "Dose Level 2"); or two doses including a first dose of 5x10^6 engineered T cells/kg (infused about 14 days to about 42 days after HCT, *e.g.*, about 21 days after HCT) and a second four-fold greater dose of 2x10^7 engineered T cells/kg (infused about 54 days to about 82 days after HCT, *e.g.*, about 61 days after HCT)("Dose Level 3").

[0141] Subjects in all groups underwent RIC conditioning therapy (days 1-6) followed by hematopoietic stem cell infusion (day 0) and then post-transplant cyclophosphamide (PTCy; days 3 and 4). Upon count recovery (about 14 days to about 42 days after HCT, *e.g.*, about 21 days after HCT; measured as an absolute neutrophil count >500 cells/microliter), subjects in treatment groups receive a single or first dose of TSC-100 or TSC-101 at Dose Level 1. Subjects were escalated to Dose Level 2 or Dose Level 3 unless excessive toxicity was observed at Dose Level 1 and engineered T cell persistence was less than 3% of total T cells, and followed interval 3+3 design1 (i3+3) with 3-12 subjects per cohort (Liu *et al.*, (2020) *J. Biopharm. Stat.* 30:294-304). When infused, the second dose was

delivered about or at least 40 days after the first dose (**FIG. 7**). Exemplary application of the protocol can be seen in **FIG. 8**.

[0142] For each subject in the treatment arms, the same donor was used to provide cells for both HCT and production of engineered T cells (TSC-100 or TSC-101). Accordingly, donors for subjects in treatment arms underwent two rounds of leukapheresis. The first was before G-CSF mobilization and was used to manufacture TSC-100/101. The second was after G-CSF mobilization and was for standard peripheral blood stem cell (PBSC) collection.

Results

[0143] In this Example, primary endpoints of interest included measures of safety (adverse event profiles, dose limiting toxicities, and GvHD rates and grades). Secondary endpoints included rate of relapse (at 6 months, 1 year, and 2 years). Exploratory endpoints included donor chimerism, minimal residual disease (MRD), and persistence of engineered T cells in subjects.

[0144] MRD was measured by a combination of next-generation sequencing (NGS) alone or with flow cytometry. The combination of next-generation sequencing (NGS) with flow cytometry is more sensitive than flow cytometry alone. MRD is detected in many AML, ALL and MDS subjects following RIC and/or HCT, e.g., in about 40% of AML patients after RIC. Where MRD is detected pre-transplant after RIC, the risk of relapse is about 67%. Where MRD is detected post-transplant, the risk of relapse can be up to 90%. In the present Example, MRD was detected in pre- and post-transplant bone marrow biopsies using a combination of next-generation sequencing (NGS) with flow cytometry. In subjects in whom MRD is detected, a positive outcome can be demonstrated by conversion of subjects from MRD positive to MRD negative resulting from administration of engineered T cells, providing an early indicator of efficacy.

[0145] Mixed donor chimerism was measured by a standard STR-based assay, or alternatively by an NGS-based assay (AlloHeme test; see Kothari 2022 *Transplant Cell Ther* 28(3): supplement, page S438, abstract #565, which is incorporated herein by reference with respect to measuring chimerism and in its entirety). The standard STR-based assay is a clinically validated metric that can be measured in all subjects and predicts approximately 60% risk of relapse. The NGS-based assay utilizes about 400 SNPs, with a limit of detection of about 0.04%, but its predictive value had not yet been established as it was the subject of an ongoing clinical trial (NCT04635384; see information available online at hypertext

transfer protocol clinicaltrials.gov/study/NCT04635384, which is herein incorporated by reference). Chimerism was detected in bone marrow and whole blood, as well as CD3 and CD33 subsets, using both the standard STR and novel NGS assays. MRD or mixed donor cell chimerism clearance and/or kinetics can be early indicators of biological activity and early surrogates of efficacy.

[0146] *Results - Adverse Events*: No evidence of cytokine release syndrome (CRS) or neurotoxicity was observed following administration of engineered T cell therapy (TSC100 or TSC-101) as measured by clinical or laboratory monitoring (CRP as a marker of CRS; ferritin), as of present writing (**FIGS. 9A** and **9B**). Adverse events of Grade 2 or greater were similar in all trial arms, as graded by CTCAE version 5.0 (Exemplary data shown in **FIG. 10A**). Serious adverse events were reported after transplant in each arm, based on a median post-transplant follow-up in TSC-100/101 arms of 193 days (34-291 days) and in the control arm of 249 days (97-398 days) (**FIG. 10B**). Together, data do not demonstrate a safety concern for TSC-100 or TSC-101.

[0147] *Results –Engineered T cell persistence*: Both TSC-101 (**FIG. 11**) and TSC-100 (**FIG. 12**) demonstrated early expansion (percent of T cells), proliferation (Ki67 positive), activation (granzyme B positive) and shift to CD4+ subsets that can persist long-term (Melenhorst *et al.* (2022) *Nature* 602:503-509). TSC-100 and TSC-101 engineered T cells persisted in peripheral blood of treatment group recipients for over 200 days, and at higher levels in subjects that received two doses (**Fig. 13**).

[0148] *Results – Donor Chimerism*: Complete donor chimerism in CD3+ or CD33+ subsets was achieved in 8 of 8 (100%) treated subjects, with no instances of relapse (**FIGS. 14-15**). Multiple instances of relapse were observed in the control arm.

[0149] *Results - MRD*: All treated subjects achieved MRD negativity, including multiple subjects in which conversion from MRD+ to MRD- was observed (**FIG. 16**). MRD was measured by next-generation sequencing (limit of detection 0.05-0.1%) and chimerism (limit of detection 0.13%) before and after hematopoietic cell transplantation (HCT).

[0150] *Results – TP53-mutated MDS*: Data support use of TSC-101, and TSC-100, for treatment of malignancy characterized by TP53 mutation. TP53 mutated myelodysplastic syndrome (MDS) has >80% risk of relapse or death after HCT. Consistent with this statistic, a control subject with TP53 mutated MDS was observed to relapse, leading to death (**FIG. 17**). However, in another subject with TP53 mutated MDS receiving TSC-101 in the treatment group, the subject turned from MRD(+) to MRD(-) after HCT and TSC-101, achieved complete chimerism, and did not relapse (**FIG. 17**). These results demonstrate

support for treatment of malignancy characterized by TP53 mutation by TSC-101 as well as TSC-100, and in particular for treatment by TSC-101, *e.g.*, for TP53 mutated MDS.

Example 2: Further Confirmatory Characterization of TSC-100 and TSC-101 TCR T cells to Treat and Prevent Relapse in Certain Hematopoietic Malignancies

[0151] This representative Example further confirms the data and results presented in Example 1 and continue to be based, in part, on the recognition that adaptive cell transfer with genetically engineered T cells holds great promise for preventing relapse in certain hematopoietic malignancies. Patients positive for particular HLA alleles of interest, such as HLA-A*02:01, are amendable to treatment with TCRs recognizing epitopes of a given target presented by such HLAs, such as HA-1 and HA-2. Specifically, this Example further describes results from the multi-arm study of TSC-100 and TSC-101 in which subjects were enrolled in the control group (control subjects receiving RIC-HCT but not engineered T cells who are either 1) HLA-A*02:01 negative subjects or 2) positive for HLA-A*02:01, but lack a mismatched donor) or either of two experimental groups (subjects expressing HLA-A*02:01 and HA-1 or HA-2, receiving both RIC-HCT and an engineered T cell therapy). These data further confirm that TSC-100 and TSC-101 demonstrate the potential to reduce relapse rates and increase relapse-free survival in patients with AML, ALL, or MDS undergoing allogeneic hematopoietic cell transplantation (HCT) with reduced intensity conditioning (RIC).

[0152] Disease relapse is the leading cause of death in patients after allo-HCT for AML, ALL and MDS, affecting up to 40% of patients. TSC-100 and TSC-101 are donor-derived T-cell receptor engineered T-cells targeting HA-1 and HA-2 on hematopoietic cells, respectively. By choosing patients who are positive and donors who are negative for HA-1/HA-2, TSC-100 and TSC-101 are designed to selectively eliminate residual patient blood cells post-HCT, while sparing donor-derived cells, thereby preventing relapse.

[0153] This example further describes the multi-arm, biologically controlled, study evaluating TSC-100 and TSC-101 in adults with AML, ALL or MDS undergoing RIC HCT. Antigen-positive subjects received either one or two infusions of TSC-100 or TSC-101 after count recovery (~days 21 and 61 post-HCT). Control subjects received RIC HCT per standard of care. Primary endpoints were dose limiting toxicities and safety; others included assessments of efficacy, chimerism and minimal residual disease (MRD).

[0154] As of the present writing, 27 subjects enrolled and underwent HCT. Subject demographics are detailed in Table 2 (*e.g.*, 16 in the treatment arm [TSC-101 (8), TSC-100

(8)] and 11 in the control arm; Three subjects had ALL (all TSC), 9 had MDS (4 TSC, 5 controls) and 15 had AML (9 TSC, 6 controls). Median age was 69 years in the TSC arm and 70 years in controls. Most subjects had high-risk molecular or cytogenetic abnormalities, including 4 with mTP53 (2 TSC, 2 controls). Median follow-up was 5.8 months (TSC) vs 5.3 months (control).

[0155] Table 2: Demographics

		TSC-100	TSC-101	Any TSC	Control
Subjects Assigned to TSC or control (Efficacy Set)		8	8	16	11
Subjects Infused (Safety Set)		5	7	12	N/A
Median Time of Follow Up, months		5.8 (0.2-15.8)	6.2 (0.9-16.9)	5.8 (0.2-16.9)	5.3 (0.4-20.5)
Age, Median (Range)		72 (52-76)	67 (52-74)	69 (52-76)	70 (23-74)
Sex, Male (n, %)		5 (63%)	5 (63%)	10 (63%)	6 (55%)
Underlying Disease	ALL	1	2	3	0
	AML	6	3	9	6
	MDS	1	3	4	5
Genetics/ cytogenetics	TP53	2	1	3	2
	FLT3	1	0	1	1
	ELN/ IPSS-M Adverse Risk	Y=4 N=4	Y=6 N=2	Y=10 N=6	Y=5 N=6
Pre-HCT MRD Positive		Y=6 N=2	Y=4 N=4	Y=10 N=6	Y=6 N=4 Pending=1

[0156] No dose limiting toxicities (DLTs), cytokine release syndrome (CRS), or immune effector cell-associated neurotoxicity syndrome (ICANS) occurred following engineered T cell (TSC) infusions. Safety was similar in the treatment and control arms and the TSC safety profile was consistent with post-HCT adverse events. Grade II-IV acute graft-versus-host disease (GvHD) was similar in TSC (2 GII events) and control arms (1 GII, 1 GIII). Similarly, one mild chronic GvHD event occurred in the TSC and control arm each. No cytokine release syndrome or neurotoxicity occurred after TSC-100/TSC-101 infusions and no TSC-related deaths occurred. Two non-relapse deaths occurred in the TSC arm: one prior to any TSC infusion and related to an adenoviral infection, and one 15 months post-HCT from a wound infection. Three deaths occurred in the control arm: one non-relapse (septic shock) and two relapse-related.

[0157] No relapses have occurred in 16 TSC arm subjects versus three in 11 control subjects. Median time to relapse I not evaluable in TSC-treated subjects, where no relapses occurred, versus 159 days in control arm subjects. Additionally, there was a higher probability of being relapse-free at one year post-HCT in TSC arm vs control arm (p=0.047). A hazard ratio 0.09 for EFS (p=0.025) was observed at one year and was associated with a shorter time to death or relapse event in the control arm (p=0.0049).

[0158] Translational analysis was performed in 18 subjects (11 TSC, 7 controls) who were ≥60-days post-HCT at data cut-off. Peak TSC expansion occurred 7-14 days post-dosing, with persistence >360 days in all 5 subjects with >1-year data. The highest dose level (DL3) had substantially higher blood TSC levels. High-sensitivity chimerism analysis with next generation sequencing (NGS) in whole blood, CD33, or CD3+ cells showed complete donor chimerism (>99.8%) at Day 42 in all cells in all 11 TSC subjects (100%) compared with two of seven control subjects (29%) (**FIG. 24**). Post-HCT MRD by NGS (LOD <0.1% in myeloid, <0.01% in lymphoid cancers) was and has remained negative in all 11 TSC subjects post-TSC infusion, and positive in two of seven control subjects post-HCT (**FIG. 25**). Five TSC subjects reached 1-year follow-up with no relapse, no detectable MRD and complete donor chimerism in the malignant lineage, including a subject with mTP53/del5q MDS and other subjects with high-risk genetics/cytogenetics.

[0159] In summary, no DLTs occurred after TSC infusions and with post-infusion safety generally consistent with HCT. All TSC subjects remained relapse-free, MRD-negative, and with full donor chimerism in the malignant lineage, consistent with elimination of residual diseased cells post-HCT. The data support the safety and potential of TSC-100/TSC-101 to reduce relapses and increase event-free and relapse-free survival in patients with AML, ALL and MDS undergoing RIC HCT.

[0160] In addition, **FIGs 26-35** provide additional data confirming that infusions with TSC-100 and TSC-101 were well-tolerated with no DLTs, and adverse events (*e.g.*, toxicities) generally consistent with HCT. TSC-100 and TSC-101 TCR-T cells have been detected > 1-year post-infusion and have a clear dose-persistence relationship. Two of 22 (9%) TSC infused subjects relapsed after TSC infusion as compared to 4 of 12 (33%) control arm subjects. The median time to relapse was not evaluable in TSC-treated subjects (where no relapses occurred) vs 160 days in control arm subjects. TSC-treated subjects trend towards lower probability of relapse (HR=0.28) and EFS strongly favors the treatment arm (HR=0.30). These data further support TSC-100 and TSC-101 as adjuvant TCR-T cells for treatment of (*e.g.*, treating residual disease), and preventing relapse in, certain subjects (*e.g.*,

subjects with AML, ALL, or MDS post RIC-HCT, such as undergoing all-HCT with haploidentical donors, matched unrelated donors (MUD), or mismatched unrelated donors (MMUD). Relapse-free survival (RFS) is an appropriate primary end-point. In addition, **FIGs 36-38** provide an efficient study design based on TSC-101 only and allows for increased patient populations to be treated.

[0161] Given that infusions at all dose levels (*e.g.*, DL1, DL2, DL3, etc.) during the studies were well-tolerated without DLTs, yielded minimal adverse events (*e.g.*, toxicities), and suggested increased efficacy with dose escalation, additional analyses of dosing options were conducted. **FIG. 39** provides representative proposed dosing (*e.g.*, including DL4 and DL4'*) regimens considering the determinations described above, such as considering increasing the total number of cells at infusion, optimizing time to infusion after HCT, and optimizing manufacturing efficiencies by using fixed-unit dosing.

References

1. Al Malki M *et al., Mol Ther.* 2023, 31 (Supplement 1): S1-794
2. Craddock C *et al., J. Clin. Oncol.* 2021, 39, 768-78
3. Hourigan CS *et al., J. Clin. Oncol.* 2020, 38, 1273-83
4. Jongen-Lavrencic M *et al., N. Engl. J. Med.* 2018, 378, 1189-99
5. Koreth J *et al,. Biol. Blood Marrow Transplant.* 2014, 20, 1516-21
6. Legrand F *et al., Blood* 2016, 128 (22): 3417.
7. Liu M *et al., J. Biopharm. Stat.* 2020, 30, 294-304
8. Loke CT *et al., Blood* 2021, 138 (Supplement 1): 415
9. Melenhorst JJ, *Nature* 2022 Feb;602(7897):503-509.
10. Reshef R *et al., Biol. Blood Marrow Transplant.* 2014, 20, 1758-66

OTHER EMBODIMENTS

[0162] It will be appreciated that the scope of the present disclosure is to be defined by that which may be understood from the disclosure and claims rather than by the specific embodiments that have been presented by way of example. Elements described with respect to one aspect or embodiment of the present disclosure are also contemplated with respect to other aspects or embodiments of the present disclosure. Moreover, recitation of claim elements in connection with a particular independent claim support recitation of such elements in connection with other independent claims. Throughout the disclosure and claims, where compositions or methods are described as having, including, or comprising specific

elements, compositions and methods that consist essentially of, consist of, or do not comprise the recited elements are likewise hereby disclosed. All references cited herein are hereby incorporated by reference.

CLAIMS

What is claimed is:

1. A method of treating a malignancy selected from the group consisting of acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), and myelodysplastic disorder (MDS) in an adult human subject that previously received reduced intensity conditioning (RIC) hematopoietic cell therapy (HCT), the treatment comprising infusing a first dose of haploidentical T cells engineered to express a TCR that binds HA-1 (VLHDDLLEA; SEQ ID NO: 1) when presented by HLA-A*02:01,

wherein the TCR comprises:

a TCR alpha chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 5, a CDR2 comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 7, and

a TCR beta chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 11, a CDR2 comprising the amino acid sequence of SEQ ID NO: 12, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 13,

wherein cells of the malignancy encode and/or express each of HA-1 and HLA-A*02:01,

wherein the HCT comprises administration of hematopoietic cells from a donor that:

(i) does not encode and/or express HA-1; and/or

(ii) does not encode and/or express an HLA-A*02 allele, and

wherein the first dose is selected from the group consisting of about 5x10^6 cells/kg of the haploidentical

T cells, about 0.8 billion of the haploidentical T cells, and about 1.2 billion of the haploidentical T cells, and the first dose is infused at a time that is between about 14 and about 42 days after the subject received HCT, optionally at a time that is about 21 days after the subject received HCT,

optionally wherein at least 80% of cells of the first dose of haploidentical T cells express a marker, optionally wherein the marker is Qtag-CD34.

2. The method of claim 1, wherein the TCR alpha chain variable domain sequence comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 8 and/or the TCR beta chain variable domain sequence comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 14.

3. The method of claim 1 or 2, wherein the TCR alpha chain variable domain sequence comprises the amino acid sequence of SEQ ID NO: 8 and/or the TCR beta chain variable domain sequence comprises the amino acid sequence of SEQ ID NO: 14.

4. The method of any one of claims 1-3, wherein the TCR comprises a TCR alpha chain comprising an amino acid sequence having at least 90% identity with SEQ ID NO: 10, and/or a TCR beta chain comprising an amino acid sequence having at least 90% identity with SEQ ID NO: 16.

5. The method of claim 4, wherein the TCR alpha chain comprises the amino acid sequence of SEQ ID NO: 10, and/or the TCR beta chain comprises the amino acid sequence of SEQ ID NO: 16.

6. A method of treating a malignancy selected from the group consisting of acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), and myelodysplastic disorder (MDS) in an adult human subject that previously received reduced intensity conditioning (RIC) hematopoietic cell therapy (HCT), the treatment comprising infusing a first dose of haploidentical T cells engineered to express a TCR that binds HA-2 (YIGEVLVSV; SEQ ID NO: 2) when presented by HLA-A*02:01,

wherein the TCR comprises:

a TCR alpha chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 17, a CDR2 comprising the amino acid sequence of SEQ ID NO: 18, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 19, and

a TCR beta chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 23, a CDR2 comprising the amino acid sequence of SEQ ID NO: 24, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 25,

wherein cells of the malignancy encode and/or express each of HA-2 and HLA-A*02:01,

wherein the HCT comprises administration of hematopoietic cells from a donor that:

(i) does not encode and/or express HA-1; and/or

(ii) does not encode and/or express an HLA-A*02 allele, and

optionally wherein the HCT comprises administration of hematopoietic cells from a donor that does not encode and/or express an HLA-A*02 allele, and

wherein the first dose is selected from the group consisting of about 5x10^6 cells/kg of the haploidentical

T cells, about 0.8 billion of the haploidentical T cells, and about 1.2 billion of the haploidentical T cells, and the first dose is infused at a time that is between about 14 and about 42 days after the subject received HCT, optionally at a time that is about 21 days after the subject received HCT,

optionally wherein at least 80% of cells of the first dose of haploidentical T cells express a marker, optionally wherein the marker is Qtag-CD34.

7. The method of claim 6, wherein the TCR alpha chain variable domain sequence comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 20 and/or the TCR beta chain variable domain sequence comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 26.

8. The method of claim 6 or 7, wherein the TCR alpha chain variable domain sequence comprises the amino acid sequence of SEQ ID NO: 20 and/or the TCR beta chain variable domain sequence comprises the amino acid sequence of SEQ ID NO: 26.

9. The method of any one of claims 6-8, wherein the TCR comprises a TCR alpha chain comprising an amino acid sequence having at least 90% identity with SEQ ID NO: 22, and/or a TCR beta chain comprising an amino acid sequence having at least 90% identity with SEQ ID NO: 28.

10. The method of claim 9, wherein the TCR alpha chain comprises the amino acid sequence of SEQ ID NO: 22, and/or the TCR beta chain comprises the amino acid sequence of SEQ ID NO: 28.

11. The method of any one of claims 1-10, wherein the cell expresses CD8α, CD8β, and/or a selectable protein marker, optionally wherein the selectable protein marker is DHFR and further optionally wherein the CD8α, CD8β, and/or the selectable protein marker is fused to a CD34 enrichment tag.

12. The method of claim 11, wherein cells are enriched using the CD34 enrichment tag.

13. The method of any one of claims 1-12, wherein the cell comprises a vector encoding the TCR, optionally wherein i) the vector is a cloning vector, expression vector, or viral vector.

14. The method of claim 13, wherein the vector further comprises a nucleic acid sequence encoding CD8α, CD8β, and/or a selectable protein marker, optionally wherein the selectable protein marker is dihydrofolate reductase (DHFR).

15. The method of claim 14, wherein the nucleic acid sequence encoding CD8α, CD8β, and/or the selectable protein marker is operably linked to a nucleic acid encoding a tag.

16. The method of claim 14 or 15, wherein the nucleic acid encoding a tag is at the 5' upstream of the nucleic acid sequence encoding CD8 α, CD8β, and/or the selectable protein marker such that the tag is fused to the N-terminus of CD8α, CD8β, and/or the selectable protein marker.

17. The method of claim 15 or 16, wherein the tag is a CD34 enrichment tag.

18. The method of any one of claims 13-17, wherein the nucleic acid encoding the binding protein, the nucleic acid sequence encoding TCRα, TCRβ, CD8α, CD8β, and/or the selectable protein marker are interconnected with an internal ribosome entry site or a nucleic acid sequence encoding a self-cleaving peptide.

19. The method of claim 18, wherein the self-cleaving peptide is P2A, E2A, F2A or T2A.

20. The method of any one of claims 1-19, wherein the haploidentical T cells engineered to express the TCR are derived from the same donor from which the hematopoietic T cells are derived.

21. The method of claim 20, wherein the T cells engineered to express the TCR are produced from T cells derived from the donor by leukapheresis 6 to 13 days prior to HCT.

22. The method of any one of claims 1-21, wherein the HCT comprises hematopoietic stem cells derived from a donor one day prior to HCT by leukapheresis, optionally wherein the donor received a G-CSF mobilization treatment between 6 days before HCT and 2 days before HCT.

23. The method of any one of claims 1-22, wherein the RIC comprises:

(i) an RIC regimen comprising fludarabine, cyclophosphamide, and total-body irradiation, wherein the total-body irradiation comprises a dosage of 200 cGy or 400 cGy;

(ii) an RIC regimen comprising fludarabine and total-body irradiation, wherein the total-body irradiation comprises a dosage of 200 cGy, optionally wherein the RIC regimen comprises melphalan; or

(iii) an RIC regimen comprising thiotepa, busulfan and fludarabine.

24. The method of any one of claims 1-23, wherein the RIC is administered between 6 days before and 1 day before HCT.

25. The method of any one of claims 1-24, wherein the subject is infused with a second dose of the engineered haploidentical T cells if infusion of the first dose does not result in excessive toxicity and if persistence of the engineered haploidentical T cells infused in the first dose in the subject is greater than 3% of total T cells,

optionally wherein at least 80% of cells of the second dose of haploidentical T cells express a marker, optionally wherein the marker is Qtag-CD34.

26. The method of any one of claims 1-25, wherein the subject is infused a second dose of the engineered haploidentical T cells at a time that is between about 54 days and about 82 days after the subject received HCT, optionally wherein the second dose of the engineered

haploidentical T cells is infused at a time that is about 61 days after the subject received HCT.

27. The method of any one of claims 1-26, wherein the subject receives a second dose of the engineered haploidentical T cells at a time that is between about 40 days (or about 41 days) and about 55 days after the first dose, optionally wherein the second dose of the engineered haploidentical T cells is infused at a time that is about 40 days after the first dose.

28. The method of any one of claims 1-27, wherein the second dose is selected from the group consisting of about 5x10^6 cells/kg of the haploidentical T cells, about 2x10^7 cells/kg of the haploidentical T cells, about 1.2 billion of the haploidentical T cells, and about 1.6 billion of the haploidentical T cells.

29. The method of any one of claims 1-28, comprising administering to the subject a prophylactic regimen against graft-versus-host disease (GvHD) after HCT, wherein the prophylactic regimen comprises cyclophosphamide (PTCy), mycophenolate mofetil (MMF), and/or tacrolimus, optionally wherein the prophylactic regimen comprises cyclophosphamide.

30. The method of claim 29, wherein the prophylactic regimen is administered on the third and fourth days after HCT.

31. The method of any one of claims 1-30, comprising administering to the subject a maintenance therapy comprising one or more of FLT3, BCR/Abl, and IDH inhibitors.

32. The method of any one of claims 1-31, wherein the subject is not administered a further anti-leukemia agent, optionally wherein the further anti-leukemia agent is oral azacytidine.

33. The method of any one of claims 1-32, wherein the subject meets the following criteria (inclusion criteria):

(i) diagnosed as a candidate for RIC and haploidentical donor HCT; and

(ii) Eastern Cooperative Oncology Group performance status (ECOG-PS) score of two or more during at least one screening.

34. The method of any one of claims 1-33, wherein the subject meets the following criteria:

(i) does not encode and/or express HLA-A*02:07;

(ii) does not have levels of donor-specific HLA antibodies high enough to warrant desensitization protocols;

(iii) not suffering a clinically significant infection or uncontrolled viral reactivation of cytomegalovirus (CMV), Epstein-Barr virus (EBV), Adenovirus, BK virus (BKV), or human herpesvirus 6 (HHV-6); and

(iv) not a prior recipient of allogeneic HCT.

35. The method of any one of claims 1-34, wherein the donor meets the following criteria (inclusion criteria):

(i) at least 16 years of age; and

(ii) fit to undergo peripheral blood stem cell (PBSC) collection and two rounds of leukapheresis.

36. The method of any one of claims 1-35, wherein the donor meets the following criteria:

(i) not positive for HIV-1, HIV-2, HTLV-1, HTLV-2, active hepatitis B, active hepatitis C, active syphilis, or active West Nile virus infection; and

(ii) not at risk for Creutzfeldt-Jakob disease or Zika virus.

37. The method of any one of claims 1-36, wherein the treatment does not cause limiting toxicities (DLTs), cytokine release syndrome (CRS), or neurotoxicity; optionally wherein the neurotoxicity is immune effector cell-associated neurotoxicity syndrome (ICANS).

38. The method of any one of claims 1-37, wherein haploidentical T cells persist in the subject for at least 100 days and/or at least 200 days after administration.

39. The method of any one of claims 1-38, wherein haploidentical T cells persist in the subject for at least 100 days after administration.

40. The method of any one of claims 1-39, wherein haploidentical T cells persist in the subject for at least 200 days after administration.

41. The method of any one of claims 1-40, wherein the haploidentical T cells are characterized in that they inhibit growth of HA-1- and/or HA-2-pulsed T2 cells when co-incubated with the T2 cells *in vitro*.

42. The method of any one of claims 1-41, wherein the treatment increases donor chimerism, optionally wherein the subject achieves complete donor chimerism.

43. The method of claim 42, wherein donor chimerism is measured in CD3+ and/or CD33+ cells.

44. The method of any one of claims 1-43, wherein, after treatment, the subject is minimal residual disease (MRD) negative, optionally wherein the subject is MRD positive prior to treatment.

45. The method of any one of claims 1-44, wherein the treatment prevents and/or reduces the risk of relapse of the malignancy, optionally wherein relapse refers to a change from MRD negative to MRD positive.

46. The method of any one of claims 1-45, wherein the treatment increases subject survival time and/or expected survival time as compared to reference controls.

47. The method of any one of claims 1-46, wherein the malignancy is a TP53 mutated MDS.

48. A method of assessing the efficacy of a therapy for a malignancy selected from AML, ALL, or MDS, wherein cells of the malignancy are characterized by expression of HLA-A*02:01 and expression of HA-1 and/or HA-2, the method comprising:

a) determining the presence or level of reactivity between T cells obtained from the subject and at least one immunogenic peptide selected from HA-1 and HA-2 or at least one stable MHC-peptide complex in which HLA-A*02:01 presents the immunogenic peptide, in a first sample obtained from the subject prior to providing at least a portion of the therapy, and

b) determining the presence or level of reactivity between the at least one immunogenic peptide, or the at least one stable MHC-peptide complex, and T cells obtained

from the subject present in a second sample obtained from the subject following provision of the therapy,

wherein the presence or a higher level of reactivity in the second sample, relative to the first sample, is an indication that the therapy is efficacious for treating the malignancy, and/or wherein the absence or a reduced level of reactivity in the second sample, relative to the first sample, is an indication that the therapy is not efficacious for treating the malignancy.

49. A method of assessing the efficacy of a therapy for a malignancy selected from AML, ALL, or MDS, wherein cells of the malignancy are characterized by expression of HLA-A*02:01 and expression of HA-1, the method comprising:

a) determining the presence or level of reactivity between a first sample obtained from the subject and at least one TCR or at least one T cell expressing the TCR, wherein the first sample is obtained from the subject prior to providing at least a portion of the therapy, and

b) determining the presence or level of reactivity between a second sample obtained from the subject and at least one TCR or at least one T cell expressing the TCR, wherein the second sample is obtained from the subject following provision of the therapy for the malignancy,

wherein the absence or a reduced level of reactivity in the second sample, relative to the first sample, is an indication that the therapy is efficacious for treating the malignancy, and/or wherein the presence or an increased level of reactivity in the second sample, relative to the first sample, is an indication that the therapy is not efficacious,

wherein the TCR comprises:

a TCR alpha chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 5, a CDR2 comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 7, and

a TCR beta chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 11, a CDR2 comprising the amino acid sequence of SEQ ID NO: 12, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 13,

optionally wherein the at least one T cell is a population of T cells.

50. The method of claim 49, wherein the TCR alpha chain variable domain sequence comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 8 and/or the TCR beta chain variable domain sequence comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 14.

51. The method of claim 49 or 50, wherein the TCR alpha chain variable domain sequence comprises the amino acid sequence of SEQ ID NO: 8 and/or the TCR beta chain variable domain sequence comprises the amino acid sequence of SEQ ID NO: 14.

52. The method of any one of claims 49-51, wherein the TCR comprises a TCR alpha chain comprising an amino acid sequence having at least 90% identity with SEQ ID NO: 10, and/or a TCR beta chain comprising an amino acid sequence having at least 90% identity with SEQ ID NO: 16.

53. The method of claim 52, wherein the TCR alpha chain comprises the amino acid sequence of SEQ ID NO: 10, and/or the TCR beta chain comprises the amino acid sequence of SEQ ID NO: 16.

54. The method of any one of claims 49-53, wherein the at least one T cell expresses CD8α, CD8β, and/or a selectable protein marker, optionally wherein the selectable protein marker is DHFR and further optionally wherein the CD8α, CD8β, and/or the selectable protein marker is fused to a CD34 enrichment tag.

55. A method of assessing the efficacy of a therapy for a malignancy selected from AML, ALL, or MDS, wherein cells of the malignancy are characterized by expression of HLA-A*02:01 and expression of HA-2, the method comprising:

a) determining the presence or level of reactivity between a first sample obtained from the subject and at least one TCR or at least one T cell expressing the TCR, wherein the first sample is obtained from the subject prior to providing at least a portion of the therapy, and

b) determining the presence or level of reactivity between a second sample obtained from the subject and at least one TCR or at least one T cell expressing the TCR, wherein the second sample is obtained from the subject following provision of the therapy for the malignancy,

wherein the absence or a reduced level of reactivity in the second sample, relative to the first sample, is an indication that the therapy is efficacious for treating the malignancy, and/or wherein the presence or an increased level of reactivity in the second sample, relative to the first sample, is an indication that the therapy is not efficacious,

wherein the TCR comprises:

a TCR alpha chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 17, a CDR2 comprising the amino acid sequence of SEQ ID NO: 18, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 19, and

a TCR beta chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 23, a CDR2 comprising the amino acid sequence of SEQ ID NO: 24, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 25,

optionally wherein the at least one T cell is a population of T cells.

56. The method of claim 55, wherein the TCR alpha chain variable domain sequence comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 20 and/or the TCR beta chain variable domain sequence comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 26.

57. The method of claim 55 or 56, wherein the TCR alpha chain variable domain sequence comprises the amino acid sequence of SEQ ID NO: 20 and/or the TCR beta chain variable domain sequence comprises the amino acid sequence of SEQ ID NO: 26.

58. The method of any one of claims 55-57, wherein the TCR comprises a TCR alpha chain comprising an amino acid sequence having at least 90% identity with SEQ ID NO: 22, and/or a TCR beta chain comprising an amino acid sequence having at least 90% identity with SEQ ID NO: 28.

59. The method of claim 58, wherein the TCR alpha chain comprises the amino acid sequence of SEQ ID NO: 22, and/or the TCR beta chain comprises the amino acid sequence of SEQ ID NO: 28.

60. The method of any one of claims 55-59, wherein the at least one T cell expresses CD8α, CD8β, and/or a selectable protein marker, optionally wherein the selectable protein marker is DHFR and further optionally wherein the CD8α, CD8β, and/or the selectable protein marker is fused to a CD34 enrichment tag.

61. The method of any one of claims 48-60, wherein the level of reactivity is indicated by a) the presence of binding and/or b) T cell activation and/or effector function, optionally wherein the T cell activation or effector function is T cell proliferation, killing, or cytokine release.

62. The method of any one of claims 48-61, wherein the binding, activation, and/or effector function is detected using fluorescence activated cell sorting (FACS), enzyme linked immunosorbent assay (ELISA), radioimmune assay (RIA), immunochemically, Western blot, or intracellular flow assay.

63. The method of any one of claims 48-62, wherein the level of reactivity is indicated by a minimal residual disease (MRD) assay.

64. The method of claim 63, wherein a reduction in MRD and/or conversion from MRD positive in the first sample to MRD negative in the second sample is an indication that the therapy is efficacious for treating the malignancy.

65. The method of claim 63 or 64, wherein MRD is assayed by next-generation sequencing (NGS), flow cytometry, or a combination thereof.

66. The method of claim 65, wherein the MRD assay comprises NGS, optionally wherein the assay is an AlloHeme assay.

67. The method of any one of claims 48-66, wherein the level of reactivity is indicated by a donor cell chimerism assay.

68. The method of claim 67, wherein an increase in donor cell chimerism and/or conversion from partial donor cell chimerism in the first sample to complete donor cell

chimerism in the second sample is an indication that the therapy is efficacious for treating the malignancy.

69. A method of assessing the efficacy of a therapy for a malignancy selected from AML, ALL, or MDS, wherein cells of the malignancy are characterized by expression of HLA-A*02:01 and expression of HA-1, the method comprising:

a) determining minimal residual disease (MRD) and/or donor cell chimerism from a first sample obtained from the subject, wherein the first sample is obtained from the subject prior to providing at least a portion of the therapy, and

b) determining the minimal residual disease (MRD) and/or donor cell chimerism from a second sample obtained from the subject, wherein the second sample is obtained from the subject following provision of the therapy for the malignancy,

optionally wherein the therapy comprises infusing a first dose of haploidentical T cells engineered to express a TCR that binds HA-1 (VLHDDLLEA; SEQ ID NO: 1) when presented by HLA-A*02:01,

wherein the TCR comprises:

a TCR alpha chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 5, a CDR2 comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 7, and

a TCR beta chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 11, a CDR2 comprising the amino acid sequence of SEQ ID NO: 12, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 13,

optionally wherein the at least one T cell is a population of T cells,

optionally wherein the subject has received or the therapy comprises HCT, and

optionally wherein the HCT is RIC-HCT.

70. The method of claim 69, wherein the TCR alpha chain variable domain sequence comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 8 and/or the TCR beta chain variable domain sequence comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 14.

71. The method of claim 69 or 70, wherein the TCR alpha chain variable domain sequence comprises the amino acid sequence of SEQ ID NO: 8 and/or the TCR beta chain variable domain sequence comprises the amino acid sequence of SEQ ID NO: 14.

72. The method of any one of claims 69-71, wherein the TCR comprises a TCR alpha chain comprising an amino acid sequence having at least 90% identity with SEQ ID NO: 10, and/or a TCR beta chain comprising an amino acid sequence having at least 90% identity with SEQ ID NO: 16.

73. The method of claim 72, wherein the TCR alpha chain comprises the amino acid sequence of SEQ ID NO: 10, and/or the TCR beta chain comprises the amino acid sequence of SEQ ID NO: 16.

74. The method of any one of claims 69-73, wherein the at least one T cell expresses CD8α, CD8β, and/or a selectable protein marker, optionally wherein the selectable protein marker is DHFR and further optionally wherein the CD8α, CD8β, and/or the selectable protein marker is fused to a CD34 enrichment tag.

75. A method of assessing the efficacy of a therapy for a malignancy selected from AML, ALL, or MDS, wherein cells of the malignancy are characterized by expression of HLA-A*02:01 and expression of HA-2, the method comprising:

a) determining minimal residual disease (MRD) and/or donor cell chimerism from a first sample obtained from a subject, wherein the first sample is obtained from the subject prior to providing at least a portion of the therapy, and

b) determining the minimal residual disease (MRD) and/or donor cell chimerism from a second sample obtained from the subject, wherein the second sample is obtained from the subject following provision of the therapy for the malignancy,

optionally wherein the therapy comprises infusing a first dose of haploidentical T cells engineered to express a TCR that binds HA-2 (YIGEVLVSV; SEQ ID NO: 2) when presented by HLA-A*02:01,

wherein the TCR comprises:

a TCR alpha chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 17, a CDR2 comprising the amino acid

sequence of SEQ ID NO: 18, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 19, and

a TCR beta chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 23, a CDR2 comprising the amino acid sequence of SEQ ID NO: 24, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 25,

optionally wherein the at least one T cell is a population of T cells,

optionally wherein the subject has received or the therapy comprises HCT, and

optionally wherein the HCT is RIC-HCT.

76. The method of claim 75, wherein the TCR alpha chain variable domain sequence comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 20 and/or the TCR beta chain variable domain sequence comprises an amino acid sequence having at least 90% identity with SEQ ID NO: 26.

77. The method of claim 75 or 76, wherein the TCR alpha chain variable domain sequence comprises the amino acid sequence of SEQ ID NO: 20 and/or the TCR beta chain variable domain sequence comprises the amino acid sequence of SEQ ID NO: 26.

78. The method of any one of claims 75-77, wherein the TCR comprises a TCR alpha chain comprising an amino acid sequence having at least 90% identity with SEQ ID NO: 22, and/or a TCR beta chain comprising an amino acid sequence having at least 90% identity with SEQ ID NO: 28.

79. The method of claim 78, wherein the TCR alpha chain comprises the amino acid sequence of SEQ ID NO: 22, and/or the TCR beta chain comprises the amino acid sequence of SEQ ID NO: 28.

80. The method of any one of claims 75-79, wherein the at least one T cell expresses CD8α, CD8β, and/or a selectable protein marker, optionally wherein the selectable protein marker is DHFR and further optionally wherein the CD8α, CD8β, and/or the selectable protein marker is fused to a CD34 enrichment tag.

81. The method of any one of claims 69-80, wherein a reduction in MRD, and/or conversion from MRD positive in the first sample to MRD negative in the second sample, is an indication that the therapy is efficacious for treating the malignancy.

82. The method of claim 81, wherein MRD is assayed by next-generation sequencing (NGS), flow cytometry, or a combination thereof.

83. The method of claim 82, wherein the MRD assay comprises NGS, optionally wherein the assay is an AlloHeme assay.

84. The method of any one of claims 69-83, wherein an increase in donor cell chimerism, and/or conversion from partial donor cell chimerism in the first sample to complete donor cell chimerism in the second sample, is an indication that the therapy is efficacious for treating the malignancy.

85. The method of any one of claims 48-84, further comprising repeating steps a) and b) at a subsequent point in time, optionally wherein the subject has undergone further treatment for the malignancy between the first point in time and the subsequent point in time.

86. The method of any one of claims 1-85, wherein the T cells comprise CD8+ T cells and CD4+ T cells.