measuring CD5+/CD3+ cells from the cells in step (a); (c) measuring or determining a percentage or amount of CD3+/TCRgd- cells from the cells in step (b), wherein the percentage or amount of CD3+/TCRgd- cells in step (c) indicates a percentage or amount of TCRab+ T cells present in the population of immune cells. In some examples, the one or more genetic modifications are at the TCRa locus. In some examples, the one or more genetic modifications are at the TRAC locus (TCRa chain constant region).

[0010] In another aspect, the present disclosure provides a method of measuring a percentage or amount of TCRab+ T cells in a population of immune cells, wherein the population of immune cells has been engineered to introduce one or more genetic modifications at the TCRa and/or TCRb locus to reduce or impair TCRab surface expression, the method comprising the steps of (a) obtaining or measuring viable CD45+ cells from the population of immune cells; (b) obtaining or measuring CD5+/CD3+ cells from the cells in step (a); and (c) measuring or determining a percentage or amount of CD3+/TCRgd- cells from the cells in step (b), wherein the percentage or amount of CD3+/TCRgd- cells in step (c) indicates the percentage or amount of TCRab+ T cells in the population of immune cells. In some examples, the one or more genetic modifications are at the TCRa locus. In some examples, the one or more genetic modifications are at the TRAC locus.

[0011] In some examples, the population of immune cells has been engineered to express a CAR. In some examples, the population of immune cells is CAR T cells. In some examples, the population of immune cells is peripheral blood mononuclear cells (PBMC). In some examples, the population of immune cells is a population of CD4+ and/or CD8+ T cells. In some examples, the percentage or amount of TCRab+ T cells is determined by subtracting the percentage or amount of CD3+/TCRgd+ cells from the population of CD5+/CD3+ cells in step (b). In certain examples, the method further comprises the step of determining CD3+/ TCRgd+ cells. In some examples, the CAR T cells are allogeneic CAR T cells.

[0012] In a further example, the present disclosure provides a method of analyzing CAR T cells comprising the step of measuring surface CD107 of the CAR T cells after antigen stimulation, wherein an increased level of surface CD107 as compared to a level before antigen stimulation indicates polyfunctional CAR T cells. In some examples, the

increased level of surface CD107 is an increased percentage or an increased mean/medium fluorescence intensities of surface CD107. In certain examples, the polyfunctional CAR T cells secret a higher level of TNFa after antigen stimulation as compared to CAR T cells that are not polyfunctional under the same conditions. In certain examples, the polyfunctional CAR T cells secret a higher level of IL2 after antigen stimulation as compared to CAR T cells that are not polyfunctional under the same conditions. In certain examples, the polyfunctional CAR T cells secret a higher level of IFNgafter antigen stimulation as compared to CAR T cells that are not polyfunctional under the same conditions. In some examples, the CAR T cells have been engineered to introduce one or more genetic modifications at the TCRa and/or TCRb locus to reduce or impair TCRab surface expression. In some examples, the one or more genetic modifications are at the TCRa locus. In some examples, the method does not require a step of measuring one or more effector cytokines. In some examples, the method further comprises a step of measuring one or more cytokines selected from the group consisting of INFg, TNFa, IL2, GM-CSF, CXCL1, IL-1b, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-17, IL-21, IL-22, IL-23, CXCL11, Mip1a, Mip1b, Mip3a, TNFb, Perforin, Granzyme A, Granzyme B, Granzyme H, CCL11, IP-10, CCL5, TGFb, sCD137, sCD40L, MCP-1, and MCP-4. In some examples, the CAR T cells is stimulated by co-culturing the CAR T cells with target cells expressing an antigen of the CAR. In some examples, wherein the target cells are tumor cells. In some examples, the level of surface CD107 is measured by flow cytometry. In some examples, the CD107 is CD107a and/or CD107b. In some examples, CD107 is measured about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, or about 10 hours after the start of antigen activation. In some examples, CD107 is measured about 4-8 hours, about 4-6 hours, about 6-8 hours, about 6-10 hours, or about 8-10 hours after the start of antigen activation. In some examples, the CAR T cells are autologous CAR T cells. In some examples, the CAR T cells are allogeneic CAR T cells.

[0012a] In another aspect, the present disclosure refers to a method of analyzing a population of immune cells that has been engineered to express a CAR, comprising the step of measuring surface CD107 of the CAR T cells after antigen stimulation, wherein an increased level of surface CD107 as compared to a level before antigen stimulation indicates polyfunctional CAR T cells.

[0012b] In another aspect, the present disclosure refers to a method of analyzing a population of immune cells, wherein the population of immune cells has been engineered to introduce one or more genetic modifications at the TCRa and/or TCRb locus to reduce or impair TCRab surface expression, and wherein the population of lymphocytes has been engineered to express a chimeric antigen receptor (CAR), the method comprising the steps of: (a) measuring or determining a percentage or amount of TCRab+ T cells in the population of immune cells according to the method as disclosed herein; and (b)measuring in the population of immune cells: (i) a percentage or amount of CAR+ T cells; and/or (j) level of surface CD107 after antigen stimulation.

[0013] In some examples of any aspects of the disclosure, the method further comprises a step of measuring CAR+ T cells. In some examples, the percentage or amount of CAR+ T cells is measured using an anti-id antibody. In some examples, the population of immune cells are obtained from a healthy donor. In some examples, the CAR T cells are allogeneic CAR T cells.

[0014] In some examples, the immune cells express a CAR specific for EGFRvIII, WT-1, CD20, CD23, CD30, CD38, CD33, CD133, MHC-WT1, TSPAN10, MHC-PRAME, Liv1, ADAM10, CHRNA2, LeY, NKGD2D, CS1, CD44v6, ROR1, Claudin-18.2, Muc17, FAP alpha, Ly6G6D, c6orf23, G6D, MEGT1, NG25, CD19, BCMA, FLT3, CD70, DLL3, CD52 or CD34. In some examples, the CAR comprises an antigen binding domain that targets EGFRvIII, WT-1, CD20, CD23, CD30, CD38, CD33, CD133, MHC-WT1, TSPAN10, MHC-PRAME, Liv1, ADAM10, CHRNA2, LeY, NKGD2D, CS1, CD44v6, ROR1, Claudin-18.2, Muc17, FAP alpha, Ly6G6D, c6orf23, G6D, MEGT1, NG25, CD19, BCMA, FLT3, CD70, DLL3, CD52 or CD34.

[0015] In some examples, the method further comprises a step of filling the population of immune cells into one or more containers, if the amount or percentage of TCRab+ T cells is no more than a predetermined threshold and/or if the population of immune cells comprises polyfunctional CAR T cells optionally no less than a predetermined threshold, as determined herein. In some examples, the predetermined threshold for the percentage of TCRab+ T cells is about 3%, 2%, 1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2% or 0.1%. In some examples, the predetermined threshold for the percentage of polyfunctional CAR T cells is about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% of the total population of CAR T cells.

[0016] In a further example, the present disclosure provides a method of preparing a cell-based drug product comprising engineered immune cells, said method comprising the method of analyzing a population of immune cells as described herein and the step of filling the population of immune cells into one or more containers, if the amount or percentage of TCRab+ T cells is no more than a predetermined threshold and/or if the population of immune cells comprises polyfunctional CAR T cells, optionally no less than a predetermined threshold.

[0016a] In another aspect, the present disclosure refers to a method of preparing a drug product comprising engineered immune cells, said method comprising the method as disclosed herein.

[0017] In an additional aspect, the preset disclosure provides a kit or an article of manufacture for analyzing a CAR T cell comprising one or more reagents for the detection of CD3 and/or TCRgd.

[0018] In some examples, the kit or article of manufacture further comprise one or more reagents for detecting CD45, CD5, CD52, CD107 (CD107a and/or CD107b), and/or a CAR. In some examples, the one or more reagents comprise an antibody, optionally conjugated with a detectable label. In some examples, the detectable label is

selected from the group consisting of a fluorescent label, a photochromic compound, a proteinaceous fluorescent label, a magnetic label, a radiolabel, and a hapten. In some examples, the fluorescent label is selected from the group consisting of an Atto dye, an Alexafluor dye, quantum dots, Hydroxycoumarin, Aminocouramin, Methoxycourmarin, Cascade Blue, Pacific Blue, Pacific Orange, Lucifer Yellow, NBD, R-Phycoerythrin (PE), PE-Cy5 conjugates, PE-Cy7 conjugates, Red 613, PerCP, TruRed, FluorX, Fluorescein, BODIPY-FL, Cy2, Cy3, Cy3B, Cy3.5, Cy5, Cy5.5, Cy7, TRITC, X-Rhodamine, Lissamine Rhocamine B, Texas Red, Allophycocyanin (APC), APC-Cy7 conjugates, Indo-1, Fluo-3, Fluo-4, DCFH, DHR, SNARF, GFP (Y66H mutation), GFP (Y66F mutation), EBFP, EBFP2, Azurite, GFPuv, T-Sapphire, Cerulean, mCFP, mTurquoise2, ECFP, CyPet, GFP (Y66W mutation), mKeima-Red, TagCFP, AmCyan1, mTFP1, GFP (S65A mutation), Midorishi Cyan, Wild Type GFP, GFP (S65C mutation), TurboGFP, TagGFP, GFP (S65L mutation), Emerald, GFP (S65T mutation), EGFP, Azami Green, ZsGreen1, TagYFP, EYFP,Topaz, Venus, mCitrine, YPet, TurboYFP, ZsYellow1, Kusabira Orange, mOrange, Allophycocyanin (APC), mKO, TurboRFP, tdTomato, TagRFP, DsRed monomer, DsRed2 ("RFP"), mStrawberry, TurboFP602, AsRed2, mRFP1, J-Red, R-phycoerythrin (RPE), B-phycoeryhring (BPE), mCherry, HcRed1, Katusha, P3, Peridinin Chlorophyll (PerCP), mKate (TagFP635), TurboFP635, mPlum, and mRaspberry. In some examples, the one or more reagents are used for flow cytometry.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] **FIG. 1** shows quantitative results of flow cytometry analyses of residual % of TCRab+ cells gated by the TCRab cutoff set by donor cells determined pre-TCRab depletion from either donor 1 (ACC-1: assay control cells from donor 1) or donor 2 (ACC-2: assay control cells from donor 2). The results presented are triplicates with standard deviation (SD). See also Table 2. ALLO-501A: CD19 CAR T without a rituximab suicide switch.

[0020] **FIGs. 2A and 2B** depict panels of flow cytometry analysis showing the current gating in TCR ablated CAR T cells (FIG. 2A), and a correlation between CD3+ intracellular staining and CD5+ surface staining (FIG. 2B). See also Table 3.

[0021] **FIG. 3** panels A-I show the workflow of sequential FACS analyses of CD19 CAR T cells for determining residual % of TCRab+ T cells after TCR ablation using an internal biological gate, and the level of CD107a after CD19 positive target cell activation.