

cell therapies are needed, including to provide for an improved cell therapy product with high consistency, potency and persistence *in vivo*.

Summary

[0005] Provided herein are methods for enriching T cells, the method including performing a first selection, the first selection including removing CD57+ T cells from a biological sample containing primary human T cells, thereby generating a depleted population, the depleted population containing fewer CD57+ T cells than the biological sample; and performing a second selection on the cells from the depleted population, the second selection including enriching for CD3+ T cells from the depleted population, the enrichment thereby generating an enriched population of CD57-CD3+ T cells.

[0006] Also provided herein are methods for enriching T cells, the method including performing a first selection, the first selection including enriching for CD3+ T cells from a biological sample containing primary human T cells, thereby generating an enriched T cell population; and performing a second selection on the cells from the enriched T cell population, the second selection including removing CD57+ T cells from the enriched sample cell population, thereby generating a depleted population, wherein the depleted population contains fewer CD57+ T cells than the biological sample and/or than the enriched T cell population and is enriched for CD3+ T cells.

[0006a] In one aspect, the present disclosure refers to a method for enriching T cells, the method comprising: (a) performing a first selection, the first selection comprising enriching for either of CD57- or CD3+ T cells from a biological sample comprising primary human T cells, thereby generating an enriched T cell population; and (b) performing a second selection on the

cells from the enriched T cell population, wherein: the first selection comprises enriching for CD57⁻ T cells and the second selection comprises enriching for CD3⁺ T cells from the enriched population; or the first selection comprises enriching for CD3⁺ T cells and the second selection comprises removing CD57⁺ T cells from the enriched T cell population, wherein the method generates a depleted population that comprises fewer CD57⁺ T cells than the biological sample and is enriched for CD3⁺ T cells, and (c) introducing a heterologous polynucleotide encoding a chimeric antigen receptor into a population of T cells obtained from the depleted population, thereby generating an engineered population of T cells.

[0007] Also provided herein are methods for enriching T cells, the method including removing CD57⁺ T cells from a biological sample containing an apheresis product or a leukapheresis product containing primary human T cells, thereby generating a depleted population of T cells, wherein the depleted population contains fewer CD57⁺ T cells than the biological sample and wherein the depleted population contains at least one of the following: (i) less than at or about 5% CD57⁺ T cells; (ii) a frequency of CD57⁺ T cells that is less than at or about 35% of the frequency of CD57⁺ T cells present in the biological sample; and (iii) CD3⁺ T cells, wherein at least at or about 95% of the CD3⁺ T cells are CD57⁻.

[0008] In some of any such examples, the depleted population contains at least one of the following: (i) less than at or about 5% CD57⁺ T cells; (ii) a frequency of CD57⁺ T cells that is less than at or about 35% of the frequency of CD57⁺ T cells present in the biological sample;

thereby generating a second depleted population enriched for the one of (i) CD4+ T cells and (ii) CD8+ T cells and a non-selected population; and (c) performing a third selection, said third selection including enriching for the other of (i) CD4+ cells and (ii) CD8+ cells from the non-selected population, the enrichment thereby generating a third depleted population enriched for the other of the (i) CD4+ T cells and (ii) CD8+ T cells. In some of any such examples, the frequency of the CD57+ cells in the depleted population (optionally the first depleted population, second depleted population or third depleted population) is less than at or about 35%, 30%, 20%, 10%, 5%, 1%, or 0.1% of the frequency of CD57+ T cells in the biological sample. In some of any such examples, the frequency of the CD57+ T cells in the depleted population (optionally the first depleted population, second depleted population or third depleted population) is less than at or about 35%, 30%, 20%, 10%, 5%, 1%, or 0.1% of the frequency of CD57+ T cells in the biological sample. In some of any such examples, the depleted population (optionally the first depleted population, second depleted population or third depleted population) comprises less than at or about 3%, less than at or about 2%, less than at or about 1%, less than at or about 0.1%, or less than at or about 0.01% CD57+ T cells. In some of any such examples, the depleted population (optionally the first depleted population, second depleted population or third depleted population) is free or is essentially free of CD57+ T cells. In some examples, a depleted population that is essentially free of CD57+ T cells comprises less than about or about 3%, less than about or about 2%, less than about or about 1%, less than about or about 0.1% or less than about or about 0.01% CD57+ T cells.

[0017a] In one aspect, the present disclosure refers to a method for enriching T cells, the method comprising: (a) performing a first selection, said first selection comprising removing CD57+ T cells from a biological sample comprising primary human T cells, thereby generating a first depleted population, said first depleted population comprising fewer CD57+ T cells than the biological sample; (b) performing a second selection on the cells from the first depleted population, said second selection comprising enriching for one of (i) CD4+ T cells and (ii) CD8+ T cells from the first depleted population, the enrichment thereby generating a second depleted population enriched for the one of (i) CD4+ T cells and (ii) CD8+ T cells and a non-selected population; (c) performing a third selection, said third selection comprising enriching for the other of (i) CD4+ cells and (ii) CD8+ cells from the non-selected population, the enrichment thereby generating a third depleted population enriched for the other of the (i) CD4+ T cells and (ii) CD8+ T cells; and (d) introducing a heterologous polynucleotide encoding a chimeric antigen receptor into a population of T cells obtained from the depleted population, thereby generating an engineered population of T cells.

[0018] In some of any of such examples, at least at or about 95% of the CD4+ T cells of the depleted population (optionally the first depleted population, second depleted population or third depleted population) comprises CD57-CD4+ T cells. In some of any of such examples, at least at or about 95% of the CD8+ T cells of the depleted population (optionally the first depleted population, second depleted population or third depleted population) comprises CD57-CD8+ T cells. In some of any of such examples, at least at or about 95% of the CD4+ T cells and 95% of the CD8+ T cells of the depleted population (optionally the first depleted population, second depleted population or third depleted population) comprises CD57-CD4+ T cells and CD57-CD8+ T cells, respectively.

[0019] In some of any such examples, the frequency of the naïve-like cells in the depleted population (optionally the first depleted population, second depleted population or

90% CD57-CD4+ T cells; (iv) at least at or about 90% CD57-CD8+ T cells; and (v) at least at or about 90% CD57-CD3+ cells.

[0029] Also provided herein are methods for stimulating T cells, including incubating T cells of an input composition under stimulating conditions thereby generating a stimulated population of T cells, wherein the input composition contains at least one of the following: (i) less than at or about 5% CD57+ T cells; (ii) at least at or about 95% CD57- T cells; (iii) at least at or about 90% CD57-CD4+ T cells; (iv) at least at or about 90% CD57-CD8+ T cells; and (v) at least about or about 90% CD57-CD3+ T cells.

[0030] In some of any such examples, the input composition includes cells obtained from a biological sample containing an apheresis product or a leukapheresis product containing primary human T cells.

[0031] In some of any such examples, the method further includes removing CD57+ T cells from the biological sample, thereby generating an input composition containing a depleted population of T cells.

[0032] Also provided are methods for stimulating T cells, the method including: (a) performing a first selection, said first selection including removing CD57+ T cells from a biological sample containing primary human T cells, thereby generating a first depleted population, said first depleted population containing fewer CD57+ T cells than the biological sample; (b) performing a second selection on the cells from the first depleted population, said second selection including enriching for one of (i) CD4+ T cells and (ii) CD8+ T cells from the first depleted population, the enrichment thereby generating a second depleted population enriched for the one of (i) CD57-CD4+ T cells and (ii) CD57-CD8+ T cells and a non-selected population; (c) performing a third selection on the cells from the non-selected population, said third selection including enriching for the other of (i) CD4+ cells and (ii) CD8+ cells from the non-selected population, the enrichment thereby generating a third depleted population enriched for the other of the (i) CD57-CD4+ T cells and (ii) CD57-CD8+ T cells; and (d) incubating one or more input compositions under stimulating conditions, said one or more input compositions comprising T cells from the second depleted population or the third depleted population.

[0033] In some of any such examples, step (d) includes incubating an input composition comprising CD4+ T cells and CD8+ T cells from the second and third depleted populations. In some of any such examples, step (d) includes separately incubating a first input composition

Glu-Lys (SEQ ID NO: 69), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)₃-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 70), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)₂-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 77, e.g. SEQ ID NO:71) and Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)₂Gly-Gly-Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 89, also set forth SEQ ID NO:72). In some of any such examples, the substance is or includes biotin or a biologically active fragment, or a biotin analog or biologically active fragment.

[0050] In some of any such examples, the stimulating conditions include the presence of one or more recombinant cytokines. In some of any such examples, the stimulating conditions include the presence of one or more of recombinant IL-2, IL-7, and IL-15.

[0051] In some of any such examples, the method further includes introducing a heterologous polynucleotide encoding a recombinant receptor into a population of T cells obtained from the depleted population or the input composition, or the stimulated population, thereby generating a an engineered population of T cells.

[0052] Also provided are methods for genetically engineering T cells, the method including introducing a heterologous polynucleotide encoding a recombinant receptor into a stimulated population of T cells generated by incubating T cells of an input composition under stimulating conditions if the percentage of CD57⁺ T cells in the input composition is below a threshold percentage of CD57⁺ T cells, wherein the threshold is no more than at or about 30%. In some of any such examples, the threshold is no more than at or about 25%, 20%, 15%, 10%, 5%, or 1%.

[0053] Also provided are methods for genetically engineering T cells, the method including introducing a heterologous polynucleotide encoding a recombinant receptor into a population of T cells from the T cell composition produced by the method of any of the methods herein, thereby generating an engineered population of T cells. Also provided are methods for genetically engineering T cells, the method including introducing a heterologous polynucleotide encoding a recombinant receptor into a population of T cells from any of the stimulated T cell populations provided herein, thereby generating an engineered population of T cells.

[0053a] In one aspect, the present disclosure refers to a method of genetically engineering T cells, the method comprising introducing a heterologous polynucleotide encoding a chimeric antigen receptor into a population of T cells from the stimulated T cell population disclosed herein, thereby generating an engineered population of T cells.

[0054] Also provided are methods for genetically engineering T cells, including introducing a heterologous polynucleotide encoding a recombinant receptor into T cells from an input composition, wherein the input composition contains one or more of: (i) less than at or about 5%

[0104] Also provided are uses of a population engineered T cells produced by any method described herein or an expanded population of T cells produced by any method described herein for the treatment of a disease, disorder, or condition in a subject.

[0104a] In one aspect, the present disclosure refers to a population of engineered T cells produced by the method disclosed herein or the composition disclosed herein for use in the treatment of a cancer.

[0104b] In one aspect, the present disclosure refers to the use of a population of engineered T cells produced by the method disclosed herein or the composition disclosed herein for the manufacture of a medicament for the treatment of a cancer.

[0105] Also provided are uses of a population engineered T cells produced by any method described herein or an expanded population of T cells produced by any method described herein for the manufacture of a medicament for the treatment of a disease, disorder, or condition in a subject.

[0106] Also provided are articles of manufacture, including: (i) one or more reagents for immunoaffinity-based selection of cells specific for CD57, CD4 and/or CD8; and (ii) instructions for use of the one or more reagents for performing any methods described herein.

[0106a] In one aspect, the present disclosure refers to an article of manufacture, comprising: (i) one or more reagents for immunoaffinity-based selection of cells specific for CD57 and one or more of CD3, CD4 and/or CD8; and (ii) instructions for use of the one or more reagents for performing the methods disclosed herein.

[0107] Also provided are articles of manufacture, including: (i) one or more reagents for immunoaffinity-based selection of cells specific for CD57, CD4 and/or CD8; (ii) one or more stimulatory reagents capable of activating one or more intracellular signaling domains of one or more components of a TCR complex and one or more intracellular signaling domains of one or more costimulatory molecules; and (iii) instructions for use of the one or more reagents for performing any methods described herein.

[0107a] In one aspect, the present disclosure refers to an article of manufacture, comprising: (i) one or more reagents for immunoaffinity-based selection of cells specific for CD57 and one or more of CD3, CD4 and/or CD8; (ii) one or more stimulatory reagents capable of activating one or more intracellular signaling domains of one or more components of a TCR complex and one or more intracellular signaling domains of one or more costimulatory molecules; and (iii) instructions for use of the one or more reagents for performing the methods disclosed herein.

[0107b] In another aspect, the present disclosure refers to an article of manufacture, comprising: (i) the composition disclosed herein; and (ii) instructions for administering the composition to a subject.

[0108] In some of any such examples, the reagent for immunoaffinity-based selection is or includes an antibody capable of specifically binding to CD57, CD4 or CD8. In some of any such examples, the reagent for immunoaffinity-based selection is or includes an antibody capable of specifically binding to CD57. In some of any such examples, the antibody is immobilized on a magnetic particle or is immobilized on or attached to an affinity chromatography matrix.

[0109] In some of any such examples, the stimulatory reagent includes (i) a primary agent that specifically binds to a member of a TCR complex, optionally that specifically binds to CD3 and (ii) a secondary agent that specifically binds to a T cell costimulatory molecule, optionally wherein the costimulatory molecule is selected from CD28, CD137 (4-1-BB), OX40, or ICOS. In some of any such examples, the one or both of the primary and secondary agents include an antibody or an antigen-binding fragment thereof. In some of any such examples, the primary and secondary agents include an antibody, optionally wherein the stimulatory reagent includes incubation with an anti-CD3 antibody and an anti-CD28 antibody, or an antigen-binding fragment thereof. In some of any such examples, the primary agent

and secondary agent are present or attached on the surface of a solid support. In some of any such examples, the solid support is or includes a bead, optionally a paramagnetic bead. In some of any such examples, the primary agent and secondary agent are reversibly bound on the surface of an oligomeric particle reagent including a plurality of streptavidin or streptavidin mutein molecules.

[0110] Also provided are articles of manufacture, including (i) any composition described herein; and (ii) instructions for administering the composition to a subject.

[0111] Also provided herein are therapeutic T cell compositions including CD4⁺ T cells expressing a recombinant receptor and CD8⁺ T cells expressing a recombinant receptor, wherein at least 80% or of the total receptor⁺/CD8⁺ cells in the composition are CD57⁻ and at least 80% of the total receptor⁺/CD4⁺ cells in the composition are CD57⁻.

[0112] In some of any such examples, at least or at least about 80%, at least or at least about 85%, at least or at least about 90%, at least or at least about 95%, at least or at least about 96%, at least or at least about 97%, at least or at least about 98%, at least or at least about 99%, about 100%, or 100% of the cells in the composition are CD4⁺ T cells and CD8⁺ T cells. In some of any such examples, at least 80% or of the total receptor⁺/CD3⁺ cells in the composition are CD57⁻. In some of any such examples, at least or at least about 80%, at least or at least about 85%, at least or at least about 90%, at least or at least about 95%, at least or at least about 96%, at least or at least about 97%, at least or at least about 98%, at least or at least about 99%, about 100%, or 100% of the cells in the composition are CD3⁺ T cells. In some of any such examples, the ratio of receptor⁺/CD4⁺ T cells to receptor⁺/CD8⁺ T cells in the composition is between about 1:3 and about 3:1. In some of any such examples, the ratio of receptor⁺/CD4⁺ T cells to receptor⁺/CD8⁺ T cells in the composition is at or about 1:1.

[0112a] In one aspect, the present disclosure refers to a therapeutic T cell composition comprising CD4⁺ T cells expressing a chimeric antigen receptor and CD8⁺ T cells expressing a chimeric antigen receptor, wherein at least 80% or of the total receptor⁺/CD8⁺ cells in the composition are CD57⁻ and at least 80% of the total receptor⁺/CD4⁺ cells in the composition are CD57⁻, optionally wherein at least or at least about 80%, at least or at least about 85%, at least or at least about 90%, at least or at least about 95%, at least or at least about 96%, at least or at least about 97%, at least or at least about 98%, at least or at least about 99%, about 100%, or 100% of the cells in the composition are CD4⁺ T cells and CD8⁺ T cells.

[0112b] In another aspect, the present disclosure refers to a therapeutic T cell composition comprising CD3⁺ T cells expressing a chimeric antigen receptor, wherein at least 80% or of the total receptor⁺/CD3⁺ cells in the composition are CD57⁻, optionally wherein at least or at least about 80%, at least or at least about 85%, at least or at least about 90%, at least or at least about 95%, at least or at least about 96%, at least or at least about 97%, at least or at least about 98%, at least or at least about 99%, about 100%, or 100% of the cells in the composition are CD3⁺ T cells.

[0113] In some of any such examples, the recombinant protein is or contains recombinant receptor that is capable of binding to a target protein that is associated with, specific to, and/or expressed on a cell or tissue of a disease, disorder or condition. In some of any such examples, the recombinant protein is a chimeric antigen receptor (CAR). In some of any such examples, the number of viable T cells in the composition is between at or about 10 x 10⁶ cells and at or about 200 x 10⁶ cells, optionally wherein the number of viable T cells in the composition is between at or about 10 x 10⁶ cells and at or about 100 x 10⁶ cells, at or about 10 x 10⁶ cells and at or about 70 x 10⁶ cells, at or about 10 x 10⁶ cells and at or about 50 x 10⁶

Claims

1. A method for enriching T cells, the method comprising:

(a) performing a first selection, the first selection comprising enriching for either of CD57⁻ or CD3⁺ T cells from a biological sample comprising primary human T cells, thereby generating an enriched T cell population; and

(b) performing a second selection on the cells from the enriched T cell population, wherein:

the first selection comprises enriching for CD57⁻ T cells and the second selection comprises enriching for CD3⁺ T cells from the enriched population; or

the first selection comprises enriching for CD3⁺ T cells and the second selection comprises removing CD57⁺ T cells from the enriched T cell population, wherein the method generates a depleted population that comprises fewer CD57⁺ T cells than the biological sample and is enriched for CD3⁺ T cells, and

(c) introducing a heterologous polynucleotide encoding a chimeric antigen receptor into a population of T cells obtained from the depleted population, thereby generating an engineered population of T cells.

2. A method for enriching T cells, the method comprising:

(a) performing a first selection, said first selection comprising removing CD57⁺ T cells from a biological sample comprising primary human T cells, thereby generating a first depleted population, said first depleted population comprising fewer CD57⁺ T cells than the biological sample;

(b) performing a second selection on the cells from the first depleted population, said second selection comprising enriching for one of (i) CD4⁺ T cells and (ii) CD8⁺ T cells from the first depleted population, the enrichment thereby generating a second depleted population enriched for the one of (i) CD4⁺ T cells and (ii) CD8⁺ T cells and a non-selected population;

(c) performing a third selection, said third selection comprising enriching for the other of (i) CD4⁺ cells and (ii) CD8⁺ cells from the non-selected population, the enrichment thereby generating a third depleted population enriched for the other of the (i) CD4⁺ T cells and (ii) CD8⁺ T cells; and

(d) introducing a heterologous polynucleotide encoding a chimeric antigen receptor into a population of T cells obtained from the depleted population, thereby generating an engineered population of T cells.

3. The method of claim 1 or claim 2, wherein the depleted population (optionally the first depleted population, the second depleted population, or the third depleted population) comprises at least one of the following:

- (i) less than at or about 5% CD57+ T cells;
- (ii) a frequency of CD57+ T cells that is less than at or about 35% of the frequency of CD57+ T cells present the biological sample;
- (iii) CD4+ T cells, wherein at least at or about 95% of the CD4+ T cells are CD57-; and
- (iv) CD8+ T cells, wherein at least at or about 95% of the CD8+ T cells are CD57-.

4. The method of any of claims 1-3, wherein the biological sample comprises an apheresis product or a leukapheresis product.

5. The method of any of claims 1-6, wherein at least at or about 95% of the CD3+ T cells of the depleted population comprises CD57-CD3+ T cells.

6. The method of claim 5, wherein:
(a) the depleted population is a first depleted population and the method further comprises selecting for CD4+ T cells from the first depleted population, thereby generating a second depleted population that is an enriched population of CD57-CD4+ T cells and a non-selected population, optionally wherein the method further comprises selecting for CD8+ T cells from the non-selected population, thereby generating a third depleted population that is an enriched population of CD57-CD8+ T cells and a second non-selected population;

(b) the depleted population is a first depleted population and the method further comprises selecting for CD8+ T cells from the first depleted population, thereby generating a second depleted population that is an enriched population of CD57-CD8+ T cells and a non-selected population, optionally wherein the method further comprises selecting for CD4+ T cells

from the non-selected population, thereby generating a third depleted population that is an enriched population of CD57-CD4⁺ T cells and a second non-selected population; or
(c) the depleted population is a first depleted population and the method further comprises selecting for CD3⁺ T cells from the first depleted population, thereby generating a second depleted population that is an enriched population of CD57-CD3⁺ T cells and a non-selected population.

7. The method of any of claims 1-6, wherein:
the frequency of the CD57⁺ T cells in the depleted population (optionally the first depleted population, the second depleted population or the third depleted population) is less than about or about 35%, 30%, 20%, 10%, 5%, 1% or 0.1% of the frequency of CD57⁺ T cells in the biological sample;
the depleted population (optionally the first depleted population, the second depleted population or the third depleted population) comprises less than about or about 3%, less than about or about 2%, less than about or about 1%, less than about or about 0.1% or less than about or about 0.01% CD57⁺ T cells; and/or
the depleted population (optionally the first depleted population, the second depleted population or the third depleted population) is free or is essentially free of CD57⁺ T cells.

8. The method of any of claims 1-7, wherein the frequency of the naïve-like T cells in the depleted population (optionally the first depleted population, the second depleted population or the third depleted population) is at least about or about 10%, 20%, 30%, 40% or 50% greater than the frequency of naïve-like T cells in the biological sample,
wherein the naïve-like T cells are surface positive for one or more of markers selected from CD45RA, CD27, CD28, and CCR7, further optionally wherein:
the frequency of CD27⁺ T cells in the depleted population (optionally the first depleted population, the second depleted population or the third depleted population) is at least about or about 10%, 20%, 30%, 40% or 50% greater than the frequency of the respective cells in the biological sample;
the frequency of CD28⁺ T cells in the depleted population (optionally the first depleted population, the second depleted population or the third depleted population) is at least about or

about 10%, 20%, 30%, 40% or 50% greater than the frequency of the respective cells in the biological sample; and/or

the frequency of CD27+/CD28+ T cells in the depleted population (optionally the first depleted population, the second depleted population or the third depleted population) is at least about or about 10%, 20%, 30%, 40% or 50% greater than the frequency of the respective cells in the biological sample.

9. The method of any of claims 2-4 and 6-8, further comprising combining the second depleted population and the third depleted population, optionally at a ratio of between at or about 1:3 and at or about 3:1, optionally at or about 1:1, thereby generating a depleted population comprising the second depleted population and the third depleted population.

10. The method of any of claims 1-9, wherein the method produces a T cell composition comprising at least at or about 90%, at least at or about 95%, at least at or about 97%, at least at or about 99% or at least at or about 99.9% CD57-CD4+ T cells;

CD57-CD8+ T cells; or CD57-CD3+ T cells.

11. The method of claim 10, wherein the ratio of CD4+ and CD8+ T cells in the composition is between 3:1 and 1:3, between 2:1 and 1:2, between 1.5:1 and 1:1.5 or between 1.2:1 and 1:1.2, each inclusive, optionally wherein the ratio of CD4+ and CD8+ T cells in the composition is at or about 1:1, further optionally wherein:

the primary T cells are from a subject with a disease or condition, optionally wherein the disease or condition is a cancer; or
the primary T cells are from a healthy subject.

12. The method of any of claims 1-11, wherein the removing of the CD57+ T cells comprises immunoaffinity-based selection, optionally

wherein the immunoaffinity-based selection comprises contacting cells with an antibody capable of specifically binding to CD57 and recovering cells not bound to the antibody, thereby effecting negative selection, wherein the recovered cells are depleted for the CD57+ cells.

13. The method of any of claims 1-12, wherein the enriching cells in the first, second and/or third selection comprises immunoaffinity-based selection.

14. The method of claim 13, wherein the first, second and/or third selection enriches for CD4 or CD8 T cells and the immunoaffinity-based selection is effected by contacting cells with an antibody capable of specifically binding to CD4 or CD8, respectively, and recovering cells bound to the antibody, thereby effecting positive selection, wherein the recovered cells are enriched for the CD4⁺ cells or the CD8⁺ cells.

15. The method of claim 13, wherein the selection enriches for CD3 T cells and the immunoaffinity-based selection is effected by contacting cells with an antibody capable of specifically binding to CD3, and recovering cells bound to the antibody, thereby effecting positive selection, wherein the recovered cells are enriched for the CD3⁺ cells.

16. The method of any one of claims 12-15, wherein the antibody is immobilized on a solid surface, optionally wherein the solid surface is a magnetic particle.

17. The method of any one of claims 12-15, wherein the antibody is immobilized on or attached to an affinity chromatography matrix, optionally

wherein the antibody further comprises one or more binding partners capable of forming a reversible bond with a binding reagent immobilized on the matrix, whereby the antibody is reversibly bound to said chromatography matrix during said contacting, optionally wherein:

the binding reagent is a streptavidin mutein that reversibly binds to the binding partner and/or

the binding partner is a streptavidin binding peptide.

18. The method of claim 17, further comprising, after contacting cells in the sample to the affinity chromatography matrix, applying a competition reagent to disrupt the bond between the binding partner and binding reagent, thereby recovering the cells bound to the antibody, optionally

wherein the competition reagent is biotin or a biotin analog.

19. The method of claim 17 or claim 18, wherein the chromatography matrix is packed in a separation vessel, which is a column.

20. The method of any one of claims 1-19, wherein the stimulating conditions comprise the presence of a stimulatory reagent, said stimulatory reagent being capable of activating one or more intracellular signaling domains of one or more components of a TCR complex and one or more intracellular signaling domains of one or more costimulatory molecules, optionally wherein the stimulatory reagent comprises (i) a primary agent that specifically binds to a member of a TCR complex, optionally that specifically binds to CD3 and (ii) a secondary agent that specifically binds to a T cell costimulatory molecule, optionally wherein the costimulatory molecule is selected from CD28, CD137 (4-1-BB), OX40 or ICOS.

21. The method of claim 20, wherein at least one of the primary and secondary agents comprises an antibody or an antigen-binding fragment thereof, optionally wherein the primary agent is an anti-CD3 antibody or an antigen-binding fragment thereof and the secondary agent is an anti-CD28 antibody or an antigen-binding fragment thereof.

22. The method of claim 21, wherein the antigen binding fragment is a monovalent antibody fragment selected from the group consisting of a Fab fragment, an Fv fragment, and a single-chain Fv fragment (scFv), optionally wherein the primary agent is an anti-CD3 Fab and the secondary agent comprises an anti-CD28 Fab.

23. The method of any of claims 20-22, wherein the primary agent and the secondary agent are reversibly bound on the surface of an oligomeric particle reagent comprising a plurality of streptavidin molecules or streptavidin mutein molecules, optionally wherein the streptavidin molecules or the streptavidin mutein molecules bind to or are capable of binding to biotin, avidin, a biotin analog or a biotin mutein, an avidin analog or an avidin mutein and/or a biologically active fragment thereof.

24. A method of genetically engineering T cells, the method comprising introducing a heterologous polynucleotide encoding a chimeric antigen receptor into a population of T cells from the stimulated T cell population of any of claims 20-23, thereby generating an engineered population of T cells.

25. The method of claim 24, wherein the introducing comprises transduction with a viral vector comprising the heterologous polynucleotide, optionally wherein the viral vector is a gamma retroviral vector or a lentiviral vector.

26. The method of claim 24 or 25, wherein the chimeric antigen receptor is capable of binding to a target antigen that is associated with, specific to and/or expressed on a cell or tissue of a disease, disorder or condition, optionally wherein the disease, disorder or condition is an infectious disease or disorder, an autoimmune disease, an inflammatory disease or a tumor or a cancer and/or the target antigen is a tumor antigen.

27. The method of claim 26, wherein the chimeric antigen receptor comprises an extracellular domain comprising an antigen-binding domain, optionally a single-chain variable fragment (scFv), a spacer and/or a hinge region, a transmembrane domain and an intracellular signaling domain comprising a costimulatory signaling region.

28. The method of claim 27, wherein:
the intracellular signaling domain is or comprises an intracellular signaling domain of a CD3 chain, optionally a CD3-zeta (CD3 ζ) chain or a signaling portion thereof; and/or
the costimulatory signaling region comprises an intracellular signaling domain of a CD28, a 4-1BB or an ICOS or a signaling portion thereof.

29. A composition of cells, produced by the method of any of claims 1-19.

30. A composition comprising an engineered population of T cells produced by the method of any of claims 24-28.

31. A therapeutic T cell composition comprising CD4⁺ T cells expressing a chimeric antigen receptor and CD8⁺ T cells expressing a chimeric antigen receptor, wherein at least 80% or of the total receptor⁺/CD8⁺ cells in the composition are CD57⁻ and at least 80% of the total receptor⁺/CD4⁺ cells in the composition are CD57⁻, optionally

wherein at least or at least about 80%, at least or at least about 85%, at least or at least about 90%, at least or at least about 95%, at least or at least about 96%, at least or at least about 97%, at least or at least about 98%, at least or at least about 99%, about 100%, or 100% of the cells in the composition are CD4⁺ T cells and CD8⁺ T cells.

32. A therapeutic T cell composition comprising CD3⁺ T cells expressing a chimeric antigen receptor, wherein at least 80% or of the total receptor⁺/CD3⁺ cells in the composition are CD57⁻, optionally

wherein at least or at least about 80%, at least or at least about 85%, at least or at least about 90%, at least or at least about 95%, at least or at least about 96%, at least or at least about 97%, at least or at least about 98%, at least or at least about 99%, about 100%, or 100% of the cells in the composition are CD3⁺ T cells.

33. A population of engineered T cells produced by the method of any of claims 24-28 or the composition of any of claims 29-32 for use in the treatment of a cancer.

34. Use of a population of engineered T cells produced by the method of any of claims 24-28 or the composition of any of claims 29-32 for the manufacture of a medicament for the treatment of a cancer.

35. An article of manufacture, comprising:

(i) one or more reagents for immunoaffinity-based selection of cells specific for CD57 and one or more of CD3, CD4 and/or CD8; and

(ii) instructions for use of the one or more reagents for performing the methods of any of claims 1-28.

36. An article of manufacture, comprising:

(i) one or more reagents for immunoaffinity-based selection of cells specific for CD57 and one or more of CD3, CD4 and/or CD8;

(ii) one or more stimulatory reagents capable of activating one or more intracellular signaling domains of one or more components of a TCR complex and one or more intracellular signaling domains of one or more costimulatory molecules; and

(iii) instructions for use of the one or more reagents for performing the methods of any of claims 1-28.

37. An article of manufacture, comprising:

(i) the composition of any one of claims 29-32; and

(ii) instructions for administering the composition to a subject.