

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.

[0005a] 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 removing CD57+ T cells from a biological sample comprising primary human T cells, thereby generating a depleted population, the depleted population comprising fewer CD57+ T cells than the biological sample; and (b) performing a second selection on the cells from the depleted population, the second selection comprising 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 comprising fewer CD57+ T cells than the biological sample and enriched for CD3+ T cells.

[0006b] In another 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 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, the second selection comprising removing CD57+ T cells from the enriched sample cell population, thereby generating a depleted population, wherein the depleted population comprises fewer CD57+ T cells than the biological sample and/or than the enriched T cell population and is enriched for CD3+ 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-.

[0007a] In one aspect, the present disclosure refers to a method for enriching T cells, the method comprising removing CD57+ T cells from a biological sample comprising an apheresis product or a leukapheresis product comprising primary human T cells, thereby generating a depleted population of T cells, wherein the depleted population comprises fewer CD57+ T cells than the biological sample and wherein the 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 in 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-.

[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;

(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-. In some of any such examples, at least at or about 95% of the CD3+ T cells of the depleted population contains CD57-CD3+ T cells. In some of any such examples, the depleted population is a first depleted population and the method further includes enriching for CD3+ T cells from the first depleted population, thereby generating an enriched population of CD57-CD3+ T cells.

[0009] In some of any such examples, the biological sample comprises an apheresis product or a leukapheresis product.

[0010] Also provided herein are methods for enriching T cells, the methods including performing a first selection, the first selection including removing CD57+ T cells from a biological sample containing an apheresis product or a leukapheresis product comprising primary human T cells, thereby generating a first depleted population, the first depleted population containing fewer CD57+ T cells than the biological sample; and (b) performing a second selection on the cells from the first depleted population, the second selection including enriching for CD3+ T cells from the first depleted population, the enrichment thereby generating an enriched population of CD57-CD3+ T cells.

[0011] Also provided herein are methods for enriching T cells, the methods including (a) performing a first selection, the first selection including enriching for CD3+ T cells from a biological sample containing an apheresis product or a leukapheresis product containing primary human T cells, thereby generating an enriched biological sample; and (b) performing a second selection on the cells from the enriched biological sample, the second selection including removing CD57+ T cells from the enriched biological sample, thereby generating a depleted population that is an enriched population of CD57-CD3+ T cells

[0012] 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: (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/or (iv) CD8+ T cells, wherein at least at or about 95% of the CD8+ T cells are CD57-.

[0013] In some of any such examples, the depleted population contains CD57-CD4+ T cells. In some of any such examples, the depleted population contains CD57- CD8+ T cells. In some of any such examples, the depleted population contains CD57-CD4+ T cells and CD57-CD8+ T cells.

[0014] In some of any such examples, the depleted population is a first depleted population and the method further includes selecting CD4+ T cells from the first depleted population, thereby generating enriched second depleted population enriched in CD57-CD4+ T cells and a non-selected population. In some of any such examples, the method further includes selecting CD8+ T cells from the non-selected population, thereby generated a third depleted population that is an enriched population of CD57-CD8+ T cells and a second non-selected population.

[0015] In some of any such examples, the depleted population is a first depleted population and the method further includes selecting 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. In some of any such examples, the method further includes selecting CD4+ T cells from the non-selected population, thereby generated a third depleted population that is an enriched population of CD57-CD4+ T cells and a second non-selected population.

[0016] In some of any of such examples, 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.

[0017] Also provided are methods for enriching T cells, the method including: (a) performing a first selection, said first selection 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 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) 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; and (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.

[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

third depleted population) is at least at or about 10%, 20%, 30%, 40%, or 50% greater than at or about the frequency of naïve-like cells in the biological sample. In some of any such examples, the frequency of one or more of CD25+ T cells, CD27+ T cells, CD28+ T cells, CCR7+ T cells, or CD45RA+ T cells in the depleted population (optionally the first depleted population, second depleted population or third depleted population) is at least at or about 10%, 20%, 30%, 40%, or 50% greater than at or about the frequency of the respective cells in the biological sample.

[0020] In some of any such examples, the depleted population (optionally the first depleted population, second depleted population or third depleted population) comprises at least at or about 15%, 20%, 25%, 30%, 35%, or 40% CD27+ T cells. In some of any such examples, the depleted population (optionally the first depleted population, second depleted population or third depleted population) comprises at least at or about 10%, 15%, 20%, 25%, 25%, 30%, 35%, or 40% CD28+ T cells. In some of any such examples, the depleted population (optionally the first depleted population, second depleted population or third depleted population) comprises at least at or about 10%, 15%, 20%, 25%, 25%, 30%, 35%, or 40% CD27+CD28+ T cells. In some of any such examples, the depleted population (optionally the first depleted population, second depleted population or third depleted population) comprises at least at or about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 60%, 70% or 80% CD27+CD28+ T cells. In some of any such examples, the depleted population (optionally the first depleted population, second depleted population or third depleted population) comprises at least at or about 70% or 80% CD27+CD28+ T cells. In some of any such examples, the depleted population (optionally the first depleted population, second depleted population or third depleted population) comprises at least at or about 10%, 15%, 20%, or 25% CCR7+ T cells.

[0021] In some of any of the provided examples, the depleted population (optionally the first depleted population, second depleted population or third depleted population) is used as an input composition for subsequent steps in a process for genetically engineering T cells. In some examples, the input composition includes CD57 depleted populations enriched for CD4+ cells (CD57-CD4+) and enriched for CD8+ cells (CD57-CD8+). In some examples, the input composition includes CD57 depleted populations enriched for CD4+ T cells (CD57-CD4+) and enriched for CD8+ T cells (CD57-CD8+). In some examples of any of the

methods, the method includes combining cells of the second depleted population and third depleted population, thereby generating an input composition. In some of any of the provided examples, the method further includes combining the second depleted population and the third depleted population. In some of any of the provided examples, the second depleted population and the third depleted population are combined at a ratio of between at or about 1:3 and at or about 3:1, thereby generating a depleted population comprising the second depleted population and the third depleted population. In some of any of the provided examples, the second depleted population and the third depleted population are combined at a ratio of at or about 1:1, thereby generating a depleted population comprising the second depleted population and the third depleted population.

[0022] In some of any of the provided methods, the method produces a T cell composition containing 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. In some of any of the provided methods, the method produces a T cell composition containing 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-CD8⁺ T cells. In some of any of the provided methods, the method produces a T cell composition containing 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-CD3⁺ T cells.

[0023] In some of any of the provided examples, the ratio of CD4⁺ to CD8⁺ T cells in the composition is between 3:1 and 1:3, each inclusive. In some of any of the provided examples, the ratio of CD4⁺ to CD8⁺ T cells in the composition is between 2:1 and 1:2, each inclusive. In some of any of the provided examples, the ratio of CD4⁺ to CD8⁺ T cells in the composition is between 1.5:1 and 1:1.5, each inclusive. In some of any of the provided examples, the ratio of CD4⁺ to CD8⁺ T cells in the composition is between 1.2:1 and 1:1.2, each inclusive. . In some of any of the provided examples, the ratio of CD4⁺ and CD8⁺ T cells in the composition is at or about 1:1 CD4⁺. In some of any of the provided examples, the primary T cells are from a human subject. In some of any of the provided examples, the primary T cells are from a subject with a disease or condition. In some of any of the provided examples, the disease or condition is a cancer. In some of any of the provided examples, the primary T cells are from a healthy subject.

[0024] In some of any of the provided examples, the removing of the CD57+ T cells includes immunoaffinity-based selection. In some of any of the provided examples, the immununoaffinity-based selection includes 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. In some of any of the provided examples, the enriching cells comprises immunoaffinity-based selection. In some of any of the provided examples, the first, second and/or third selection enriches for CD4 or CD8 T cells and the immununoaffinity-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. In some of any of the provided examples, 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. In some of any of the provided examples, the antibody is immobilized on a solid surface. In some of any of the provided examples, the solid surface is a magnetic particle. In some of any of the provided examples, the antibody is immobilized on or attached to an affinity chromatography matrix.

[0025] In some of any of the provided examples, the antibody further includes 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. In some of any of the provided examples, the binding reagent is a streptavidin mutein that reversibly binds to the binding partner. In some of any of the provided examples, the streptavidin mutein contains the amino acid sequence Ile44-Gly45-Ala46-Arg47 at sequence positions corresponding to positions 44 to 47 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO:66; or the streptavidin mutein contains the amino acid sequence Val44-Thr45-Ala46-Arg47 at sequence positions corresponding to positions 44 to 47 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO: 66. In some of any of the provided examples, the binding partner is a streptavidin binding peptide. In some of any of the provided examples, the streptavidin-binding peptide is selected from the group consisting of Trp-Ser-His-Pro-Gln-

Phe-Glu-Lys (SEQ ID NO: 69), Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)3-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO:78), SAWSHPQFEKGGGSGGGSGGSAWSHPQFEK (SEQ ID NO:79), 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: 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: 72). In some of any of the provided examples, the streptavidin-binding peptide has the sequence SAWSHPQFEKGGGSGGGSGGSAWSHPQFEK (SEQ ID NO:79). In some of any of the provided examples, the method further includes, 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. In some of any of the provided examples, the competition reagent is biotin or a biotin analog. In some of any of the provided examples, the chromatography matrix is packed in a separation vessel, which is a column. In some of any of the provided examples, the biological sample comprising the primary T cells is a sample formulated with a cyoprectant. In some of any of the provided examples, the apheresis or leukapheresis sample is a sample formulated with a cryoprotectant.

[0026] Also provided 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 the depleted population (optionally the first depleted population, second depleted population or third depleted population) generated by any method described herein.

[0027] Also provided are methods for stimulating T cells, the method including incubating T cells of an input composition under stimulating conditions, wherein the percentage of CD57+ T cells in the input composition is below a threshold percentage of CD57+ T cells, wherein said threshold is no more than at or about 30% CD57+ T cells. In some of any such examples, the threshold percentage is no more than at or about 25%, 20%, 15%, 10%, 5%, or 1% CD57+ T cells.

[0028] In some of any such examples, the input composition contains: (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 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.

[0029a] In one aspect, the present disclosure refers to a method for stimulating T cells, the method comprising incubating T cells of an input composition under stimulating conditions, thereby generating a stimulated population of T cells, wherein the input composition is produced by the method disclosed herein, optionally wherein the input composition comprises 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.

[0029b] In another aspect, the present disclosure refers to a method for stimulating T cells, comprising incubating T cells of an input composition under stimulating conditions, thereby generating a stimulated population of T cells, wherein the input composition comprises 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 at 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

and a second input composition, said first input composition containing T cells from the second depleted population and said second input composition containing T cells from the third depleted population.

[0034] In some of any such examples, the second selection includes enriching for (i) CD4⁺ T cells thereby generating a first enriched population enriched for (i) CD57-CD4⁺ T cells, and wherein the first input composition contains the enriched CD57-CD4⁺ T cells; and the third selection includes enriching for (ii) CD8⁺ T cells thereby generating a second enriched population enriched for (ii) CD57-CD8⁺ T cells, and wherein the second input composition contains enriched CD57-CD8⁺ T cells. In some of any such examples, the second selection includes enriching for (ii) CD8⁺ T cells thereby generating a first enriched population enriched for (ii) CD57-CD8⁺ T cells, and wherein the first input composition contains enriched CD57-CD8⁺ T cells; and the third selection includes enriching for (i) CD4⁺ T cells thereby generating a second enriched population enriched for (i) CD57-CD4⁺ T cells, and wherein the second input composition contains enriched CD57-CD4⁺ T cells. Also provided herein are methods for stimulating T cells, the methods including (a) performing a first selection, the first selection including removing CD57⁺ T cells from a biological sample containing primary human T cells, thereby generating a first depleted population, said 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 CD3⁺ T cells from the first depleted population, the enrichment thereby generating a second depleted population enriched for CD3⁺ T cells; and (c) incubating one or more input compositions under stimulating conditions, the one or more input compositions containing T cells from the second depleted population.

[0035] Also provided herein are methods for stimulating T cells, the methods including (a) performing a first selection on the cells from a biological sample, the first selection including enriching for CD3⁺ T cells from the biological sample, the enrichment thereby generating an enriched biological sample enriched for CD3⁺ T cells; (b) performing a second selection, the second selection including removing CD57⁺ T cells from the enriched biological sample containing primary human T cells, thereby generating a depleted population, the depleted population containing fewer CD57⁺ cells than the enriched biological sample; and (c)

incubating one or more input compositions under stimulating conditions, the one or more input compositions containing T cells from the depleted population.

[0036] In some of any such examples, the input composition comprises less than at or about 5% CD57+ T cells. In some of any such examples, the input composition contains 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 frequency of the CD57+ cells in the input composition is less than at or about 35%, 30%, 25%, 20%, 10%, 5%, 1%, or 0.1% of the frequency of CD57+ T cells present in the biological sample. In some of any such examples, the frequency of the CD57+ T cells in the input composition is less than at or about 35%, 30%, 25%, 20%, 10%, 5%, 1%, or 0.1% of the frequency of CD57+ T cells present in the biological sample. In some of any such examples, the input composition 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.

[0037] In some of any such examples, the input composition contains at least at or about 95% CD57- T cells. In some of any such examples, the input composition contains 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. In some of any such examples, the input composition contains 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-CD8+ T cells. In some of any such examples, the CD57- T cells include CD57-CD4+ T cells and CD57-CD8+ T cells. In some of any such examples, the input composition contains a ratio of between 3:1 and 1:3, 2:1 and 1:2, 1.5:1 and 1:1.5, or 1.2:1 and 1:1.2 CD4+ T cells to CD8+ T cells, inclusive. In some of any such examples, the input composition contains a ratio of or of about 1:1 CD4+ T cells to CD8+ T cells.

[0038] In some of any such examples, the frequency of the naïve-like cells in the input composition is at least at or about 10%, 20%, 30%, 40%, or 50% greater than at or about the frequency of naïve-like cells in the biological sample. In some of any such examples, the frequency of one or more of CD25+ T cells, CD27+ T cells, CD28+ T cells, CCR7+ T cells, or

CD45RA⁺ T cells in the input composition is at least at or about 10%, 20%, 30%, 40%, or 50% greater than at or about the frequency of the respective cells in the biological sample.

[0039] In some of any such examples, the input composition contains at least at or about 15%, 20%, 25%, 30%, 35%, or 40% CD27⁺ T cells. In some of any such examples, the input composition contains at least at or about 10%, 15%, 20%, 25%, 25%, 30%, 35%, or 40% CD28⁺ T cells. In some of any such examples, the input composition contains at least at or about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 60%, 70% or 80% CD27⁺CD28⁺ T cells. In some of any such examples, the input composition contains at least at or about 70% or 80% CD27⁺CD28⁺ T cells. In some of any such examples, the input composition contains at least at or about 10%, 15%, 20%, or 25% CCR7⁺ T cells.

[0040] In some of any such examples, the removing of the CD57⁺ T cells from the biological sample and/or the enriching cells in the first and/or second selection includes immunoaffinity-based selection. In some of any such examples, the immununoaffinity-based selection is effected by contacting cells with an antibody capable of specifically binding to CD57, CD4 or CD8 and recovering cells not bound to the antibody, thereby effecting negative selection, or recovering cells bound to the antibody, thereby effecting positive selection, wherein the recovered cells are depleted for the CD57⁺ cells, and/or enriched for the CD4⁺ cells or the CD8⁺ cells and antibody is immobilized on a magnetic particle. In some of any such examples, the immunoaffinity-based selection is effected by contacting cells with an antibody immobilized on or attached to an affinity chromatography matrix, said antibody capable of specifically binding to CD57, CD4 or CD8 to effect negative selection of CD57⁺ cells or positive selection of CD4⁺ or CD8⁺ cells. In some of any such examples, the immununoaffinity-based selection is effected by contacting cells with an antibody capable of specifically binding to CD57, CD4 or CD8 and recovering cells not bound to the antibody, thereby effecting negative selection, or recovering cells bound to the antibody, thereby effecting positive selection, wherein the recovered cells are depleted for the CD57⁺ T cells, and/or enriched for the CD4⁺ T cells or the CD8⁺ T cells and antibody is immobilized on a magnetic particle. In some of any such examples, the immunoaffinity-based selection is effected by contacting cells with an antibody immobilized on or attached to an affinity chromatography matrix, said antibody capable of specifically binding to CD57, CD4 or CD8 to effect negative selection of CD57⁺ T cells or positive selection of CD4⁺ or CD8⁺ T cells.

[0041] In some of any such examples, the stimulating conditions include the presence of a stimulatory reagent, said stimulatory reagent 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. In some of any such examples, the stimulatory reagent contains (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.

[0042] 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 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. In some of any such examples, 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). In some of any such examples, the primary agent is an anti-CD3 Fab and the secondary agent comprises an anti-CD28 Fab. 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 comprises a bead, optionally a paramagnetic bead. In some of any such examples, the solid support is a paramagnetic bead with surface attached anti-CD3 and anti-CD28 antibodies, and the stimulatory reagent is present at a ratio of less than about or about 3:1 beads to cells.

[0043] In some of any such examples, the stimulating conditions include the presence of stimulatory reagent containing a paramagnetic bead with surface attached anti-CD3 and anti-CD28 antibodies, said stimulatory reagent present at a ratio of less than at or about 3:1 beads to cells. In some of any such examples, said stimulatory reagent present at a ratio of or of about 1:1 beads to cells.

[0044] In some of any such examples, the method further includes separating the stimulatory reagent from the cells, said separating including exposing the cells to a magnetic field.

[0045] In some of any such examples, the primary agent and secondary agent are reversibly bound on the surface of an oligomeric particle reagent comprising a plurality of streptavidin or streptavidin mutein molecules. In some of any such examples, the streptavidin or streptavidin mutein molecules bind to or are capable of binding to biotin, avidin, a biotin analog or mutein, an avidin analog or mutein, and/or a biologically active fragment thereof. In some of any such examples, the streptavidin mutein molecules include the amino acid sequence Val⁴⁴-Thr⁴⁵-Ala⁴⁶-Arg⁴⁷ or Ile⁴⁴-Gly⁴⁵-Ala⁴⁶-Arg⁴⁷ at sequence positions corresponding to positions 44 to 47 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO: 66. In some of any such examples, the streptavidin mutein molecules contain: a) the sequence of amino acids set forth in any of SEQ ID NOS: 66-68, 73, 80-82, or 85-88; b) a sequence of amino acids that exhibit at least at or about 85%, 86%, 87%, 88%, 89%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOs: 70-73, 78, and 85-89 and contain the amino acid sequence corresponding to Val⁴⁴-Thr⁴⁵-Ala⁴⁶-Arg⁴⁷ or Ile⁴⁴-Gly⁴⁵-Ala⁴⁶-Arg⁴⁷ and/or reversibly bind to biotin, a biotin analog or a streptavidin-binding peptide; or c) a functional fragment of a) or b) that reversibly binds to biotin, a biotin analog, or a streptavidin-binding peptide.

[0046] In some of any such examples, the streptavidin mutein molecules contain the sequence of amino acids set forth in SEQ ID NO: 73 or 78. In some of any such examples, the streptavidin mutein molecules contain the sequence of amino acids set forth in SEQ ID NO: 73. In some of any such examples, the streptavidin mutein molecules contain the sequence of amino acids set forth in SEQ ID NO: 78. In some of any such examples, the primary agent and the secondary agent each contain a streptavidin-binding peptide. In some of any such examples, the streptavidin-binding peptide selected from the group consisting of Trp-Ser-His-Pro-Gln-Phe-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: 76), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)₂-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 77) 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). In some of any such examples, the streptavidin-binding peptide

selected from the group consisting of Trp-Ser-His-Pro-Gln-Phe-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: 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). In some of any such examples, the primary agent includes an anti-CD3 Fab and wherein the secondary agent contains an anti-CD28 Fab.

[0047] In some of any such examples, the oligomeric particle reagent contains a radius of greater than at or about 60 nm, greater than at or about 70 nm, greater than at or about 80 nm, or greater than at or about 90 nm. In some of any such examples, the oligomeric particle reagent contains a molecular weight of: at least at or about 5×10^7 g/mol, or at least at or about 1×10^8 g/mol; and/or between 5×10^7 g/mol and 5×10^8 g/mol, between 1×10^8 g/mol and 5×10^8 g/mol, or between 1×10^8 g/mol and 2×10^8 g/mol. In some of any such examples, the oligomeric particle reagent contains at least at or about 500 streptavidin or streptavidin mutein tetramers, at least at or about 1,000 streptavidin or streptavidin mutein tetramers, at least at or about 1,500 streptavidin or streptavidin mutein tetramers, or at least at or about 2,000 streptavidin or streptavidin mutein tetramers; and/or; between 1,000 and 20,000 streptavidin or streptavidin mutein tetramers, between 1,000 and 10,000 streptavidin or streptavidin mutein tetramers, or between 2,000 and 5,000 streptavidin or streptavidin mutein tetramers.

[0048] In some of any such examples, the method further includes separating the stimulatory reagent from the cells, said separating including contacting a substance to the cells, said substance being capable of reversing bonds between the primary and secondary agent and the oligomeric particle reagent.

[0049] In some of any such examples, the substance is a free binding partner and/or is a competition agent. In some of any such examples, the presence of the substance terminates or lessens the signal induced or modulated by the primary and secondary agent in the T cells. In some of any such examples, the substance is or includes a streptavidin-binding peptide, biotin or a biologically active fragment, or a biotin analog or biologically active fragment. In some of any such examples, the substance is or includes a biotin analog. In some of any such examples, the substance is or includes a streptavidin-binding peptide and the streptavidin-binding peptide is selected from the group consisting of Trp-Ser-His-Pro-Gln-Phe-

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 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%.

[0052a] In one aspect, the present disclosure refers to a method of genetically engineering T cells, the method comprising introducing a heterologous polynucleotide encoding a recombinant receptor into a population of T cells from the T cell composition produced by the method disclosed herein, thereby generating an engineered population of T cells.

[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 recombinant 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%

CD57+ T cells; (ii) at least at or about 95% CD57- T cells; (iii) at least at or about 90% CD57-CD4+ T cells; or (iv) at least at or about 90% CD57-CD8+ T cells.

[0055] Also provided are methods for genetically engineering 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 depleted population, said depleted population containing fewer CD57+ T cells than the biological sample; (b) performing a second selection on the cells from the depleted population, said second selection including enriching for one of (i) CD4+ T cells and (ii) CD8+ T cells from the biological sample containing primary human T cells, the enrichment thereby generating a first enriched population enriched for the one of (i) CD57-CD4+ T cells and (ii) CD57-CD8+ T cells and a non-selected population; and (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 second enriched population enriched for the other of the (i) CD57-CD4+ T cells and (ii) CD57-CD8+ T cells; (d) introducing a heterologous polynucleotide encoding a recombinant receptor into T cells from one or both of the first and second enriched populations, thereby generating a first and a second engineered population of T cells.

[0056] Also provided are methods for genetically engineering 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, said depleted population containing fewer CD57+ T cells than the biological sample; performing a second selection on the cells from the depleted population, the second selection including enriching for CD3+ T cells from the biological sample containing primary human T cells, the enrichment thereby generating a first enriched population enriched for CD57-CD3+ T cells; and introducing a heterologous polynucleotide encoding a recombinant receptor into T cells from the first enriched population, thereby generating a first engineered population of T cells. In some of any such examples, prior to the introducing, the first enriched population is incubated under stimulatory conditions.

[0057] Also provided herein are methods for genetically engineering 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, the enrichment thereby generating

an enriched biological sample enriched for CD3⁺ T cells; performing a second selection on the cells from the enriched biological sample, the second selection including removing CD57⁺ T cells from the enriched biological sample containing primary human T cells, thereby generating a depleted population, said depleted population containing fewer CD57⁺ T cells than the enriched biological sample; and introducing a heterologous polynucleotide encoding a recombinant receptor into T cells from the depleted population, thereby generating a first engineered population of T cells. In some of any of such examples, prior to the introducing, the depleted population is incubated under stimulatory conditions.

[0058] In some of any such examples, prior to the introducing, the input composition or the enriched populations are incubated under stimulatory conditions.

[0059] In some of any such examples, the step (d) includes introducing the heterologous polynucleotide into a population of cells containing CD4⁺ T cells and CD8⁺ T cells from the first and second enriched populations. In some of any such examples, step (d) includes separately introducing the heterologous polynucleotide into cells of the first enriched population and cells of the second enriched population.

[0060] In some of any such examples, the second selection includes enriching for (i) CD4⁺ T cells thereby generating a first enriched population enriched for (i) CD57-CD4⁺ T cells, and wherein the first engineered population comprises CD57-CD4⁺ T cells; and the third selection includes enriching for (ii) CD8⁺ T cells thereby generating a second enriched population enriched for (ii) CD57-CD8⁺ T cells, and wherein the second engineered population comprises CD57-CD8⁺ T cells. In some of any such examples, the second selection includes enriching for (ii) CD8⁺ T cells thereby generating a first enriched population enriched for (ii) CD57-CD8⁺ T cells, and wherein the first engineered population comprises CD57-CD8⁺ T cells; and the third selection includes enriching for (i) CD4⁺ T cells thereby generating a second engineered population enriched for (i) CD57-CD4⁺ T cells, and wherein the second input population comprises CD57-CD4⁺ T cells.

[0061] In some of any such examples, prior to the introducing, the cells of the one or both of the first and second enriched populations are incubated under stimulating conditions. In some of any such examples, the introducing includes transduction with a viral vector comprising the heterologous polynucleotide. In some of any such examples, the viral vector

is a gammaretroviral vector or a lentiviral vector. In some of any such examples, the viral vector is a lentiviral vector.

[0062] In some of any such examples, the method further includes incubating the composition containing transduced cells for up to 96 hours subsequent to the introducing. In some of any such examples, the incubating is at a temperature of at or about $37^{\circ}\pm 2^{\circ}$ C. In some of any such examples, the incubating is carried out for up to 72 hours subsequent to the introducing. In some of any such examples, the incubating is carried out for up to 48 hours subsequent to the introducing. In some of any such examples, the incubating is carried out for up to 24 hours subsequent to the introducing. In some of any such examples, the incubating results in integration of the viral vector into the genome of the T cells.

[0063] In some of any such examples, the method optionally further includes cultivating cells of the transformed population under conditions to promote proliferation or expansion of the engineered cells, thereby generating an expanded population of cells. In some of any such examples, the cultivating is carried out in the presence of one or more recombinant cytokines. In some examples, the one or more recombinant cytokines is one or more of IL-2, IL-7, and IL-15. In some of any such examples, the proliferation or expansion results in, in about, or in at least at or about a 2-fold, 3-fold, 4-fold, 5-fold, or greater than at or about a 5-fold increase in the number of viable T cells comprising the heterologous polynucleotide.

[0064] Also provided are methods of harvesting or collecting cells, the method including harvesting or collecting a population of cells produced by any method described herein.

[0065] Also provided are methods for genetically engineering T cells, the method including: (a) incubating T cells from the depleted population generated by any method described herein under stimulating conditions, said stimulating conditions including the presence of a stimulatory reagent 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, thereby generating stimulated T cells; and (b) introducing a heterologous polynucleotide into the stimulated T cells, said introducing including transducing the stimulated T cells with a viral vector comprising the heterologous polynucleotide encoding a recombinant receptor, thereby generating an engineered population of T cells.

[0066] Also provided are methods for genetically engineering T cells, the method including: (a) performing a first selection, said first selection including removing CD57⁺ T cells from a

polynucleotide encoding a recombinant receptor into the stimulated T cells of the enriched population, said introducing including transducing the stimulated T cells with a viral vector containing the heterologous polynucleotide, thereby generating an engineered population of T cells from the enriched population.

[0068] Also provided herein are methods of genetically engineering 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, the enrichment thereby generating an enriched biological sample enriched for CD3⁺ T cells; performing a second selection on the cells from the enriched biological sample, the second selection including removing CD57⁺ T cells from the enriched biological sample containing primary human T cells, thereby generating a depleted population, said depleted population containing fewer CD57⁺ T cells than the enriched biological sample; incubating T cells from the depleted population under stimulating conditions, the stimulating conditions comprising the presence of a stimulatory reagent 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, thereby generating stimulated T cells of the depleted population; and (d) introducing a heterologous polynucleotide encoding a recombinant receptor into the stimulated T cells of the depleted population, said introducing including transducing the stimulated T cells with a viral vector containing the heterologous polynucleotide, thereby generating an engineered population of T cells from the depleted population.

[0069] In some of any such examples, the method further includes: (i) cultivating the populations of engineered T cells under conditions to promote proliferation or expansion of the engineered population, thereby generating an expanded population of T cells; and (ii) harvesting or collecting the expanded population.

[0070] In some of any such examples, prior to the incubating, the first enriched population and the second enriched population contain one or more of: (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; or (iii) at least at or about 95% CD57⁻ T cells. In some of any such examples, prior to the incubating, the first enriched population and the second enriched population contain less than at or about 5% CD57⁺ T cells. In some of any such examples, prior to the incubating, the first enriched population and the

second enriched population each contain 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, prior to the incubating, the frequency of the CD57+ cells in the first enriched population and the second enriched population contain 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, prior to the introducing, the first enriched population and the second enriched population are free or are 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.

[0071] In some of any such examples, the cells of the first enriched composition and the second composition are incubated, and/or cultivated together in a single mixed population. In some of any such examples, prior to the introducing, the single mixed population includes a ratio of between 3:1 and 1:3, 2:1 and 1:2, 1.5:1 and 1:1.5, or 1.2:1 and 1:1.2 CD4+ T cells to CD8+ T cells, inclusive. In some of any such examples, prior to the introducing, the single mixed population includes a ratio of or of about 1:1 CD4+ T cells to CD8+ T cells. In some of any such examples, the cells of the first enriched population and the second enriched population are separately incubated, and/or cultivated.

[0072] In some of any such examples, the stimulatory reagent includes a paramagnetic bead with surface attached anti-CD3 and anti-CD28 antibodies. In some of any such examples, the stimulatory reagent includes an oligomeric particle reagent containing a plurality of streptavidin or streptavidin mutein molecules with reversibly bound anti-CD3 and anti-CD28 Fabs.

[0073] In some of any such examples, the removing of the CD57+ T cells from the biological sample and/or the enriching cells in the first and/or second selection includes immunoaffinity-based selection. In some of any such examples, the immununoaffinity-based selection is effected by contacting cells with an antibody capable of specifically binding to CD57, CD4 or CD8 and recovering cells not bound to the antibody, thereby effecting negative selection, or recovering cells bound to the antibody, thereby effecting positive selection, wherein the recovered cells are depleted for the CD57+ cells, and/or enriched for the CD4+ cells or the CD8+ cells and antibody is immobilized on a magnetic particle. In some of any such

examples, the immunoaffinity-based selection is effected by contacting cells with an antibody immobilized on or attached to an affinity chromatography matrix, said antibody capable of specifically binding to CD57, CD4 or CD8 to effect negative selection of CD57+ cells or positive selection of CD4+ or CD8+ cells. In some of any such examples, the immununoaffinity-based selection is effected by contacting cells with an antibody capable of specifically binding to CD57, CD4 or CD8 and recovering cells not bound to the antibody, thereby effecting negative selection, or recovering cells bound to the antibody, thereby effecting positive selection, wherein the recovered cells are depleted for the CD57+ T cells, and/or enriched for the CD4+ T cells or the CD8+ T cells and antibody is immobilized on a magnetic particle. In some of any such examples, the immunoaffinity-based selection is effected by contacting cells with an antibody immobilized on or attached to an affinity chromatography matrix, said antibody capable of specifically binding to CD57, CD4 or CD8 to effect negative selection of CD57+ T cells or positive selection of CD4+ or CD8+ T cells.

[0074] In some of any such examples, the harvesting is performed at or after the time in which the engineered population or the expanded population of T cells include a threshold number of T cells, viable T cells, engineered T cells or viable engineered T cells, or a threshold concentration of T cells, viable T cells, engineered T cells or viable engineered T cells. In some of any such examples, the threshold number or concentration of T cells, viable T cells, engineered T cells or viable engineered T cells is reached within at or about 4, 5, 6 or 7 days after the initiation of stimulation.

[0075] In some of any such examples, among a plurality of populations of engineered T cells or populations of expanded T cells, the threshold number or concentration of T cells, viable T cells, engineered T cells or viable engineered T cells is reached within at or about 5 or 6 days after the initiation of stimulation in at least at or about or at least at or about 70%, 80%, 90% or 95% of the plurality. In some of any such examples, the threshold number or concentration of T cells, viable T cells, engineered T cells or viable engineered T cells is reached within at or about 2, 3, 4 or 5 population doublings after the initiation of stimulation.

[0076] In some of any such examples, the engineered population or the expanded population contains 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 engineered population or the expanded 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.

[0077] In some of any such examples, the frequency of the naïve-like cells in the engineered population or the expanded population is at least at or about 10%, 20%, 30%, 40%, or 50% of the cells in the population. In some of any such examples, the frequency of one or more of CD25+ T cells, CD27+ T cells, CD28+ T cells, CCR7+ T cells, or CD45RA+ T cells in the engineered population or the expanded population is at least at or about 10%, 20%, 30%, 40%, or 50% greater than at or about the frequency of the respective cells in the population. In some of any such examples, the engineered population or the expanded population contains at least at or about 15%, 20%, 25%, 30%, 35%, or 40% CD27+ T cells. In some of any such examples, the engineered population or the expanded population contains at least at or about 10%, 15%, 20%, 25%, 30%, 35%, or 40% CD28+ T cells. In some of any such examples, the engineered population or the expanded population contains at least at or about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 60%, 70% or 80% CD27+CD28+ T cells.

[0078] In some of any such examples, the engineered population or the expanded population contains at least at or about 70% or 80% CD27+CD28+ T cells. In some of any such examples, the engineered population or the expanded population contains at least at or about 10%, 15%, 20%, or 25% CCR7+ T cells.

[0079] In some of any such examples, harvesting the cells includes removing cellular debris by rinsing or washing the cells. In some of any such examples, the harvesting or collecting further includes formulating the cells for cryopreservation or administration to a subject. In some of any such examples, the harvested or collected cells are formulated in the presence of a pharmaceutically acceptable excipient. In some of any such examples, the harvested or collected cells are formulated for cryopreservation in the presence of a cryoprotectant. In some of any such examples, the cryoprotectant comprises DMSO. In some of any such examples, the harvested or collected cells are formulated in a container, optionally a vial or a bag.

[0080] In some of any such examples, the separation occurs prior to the harvesting. In some of any such examples, the separation occurs prior to or during the cultivation. In some of any such examples, the separation occurs subsequent to the introducing.

[0081] In some of any such examples, the recombinant 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. In some of any such examples, the disease, disorder or condition is an infectious disease or disorder, an autoimmune disease, an inflammatory disease, or a tumor or a cancer. In some of any such examples, the target antigen is a tumor antigen. In some of any such examples, the target antigen is selected from among $\alpha v \beta 6$ integrin ($\alpha v \beta 6$ integrin), B cell maturation antigen (BCMA), B7-H3, B7-H6, carbonic anhydrase 9 (CA9, also known as CAIX or G250), a cancer-testis antigen, cancer/testis antigen 1B (CTAG, also known as NY-ESO-1 and LAGE-2), carcinoembryonic antigen (CEA), a cyclin, cyclin A2, C-C Motif Chemokine Ligand 1 (CCL-1), CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD133, CD138, CD171, chondroitin sulfate proteoglycan 4 (CSPG4), epidermal growth factor protein (EGFR), type III epidermal growth factor receptor mutation (EGFR vIII), epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), ephrinB2, ephrin receptor A2 (EPHa2), estrogen receptor, Fc receptor like 5 (FCRL5; also known as Fc receptor homolog 5 or FCRH5), fetal acetylcholine receptor (fetal AChR), a folate binding protein (FBP), folate receptor alpha, ganglioside GD2, O-acetylated GD2 (OGD2), ganglioside GD3, glycoprotein 100 (gp100), glypican-3 (GPC3), G Protein Coupled Receptor 5D (GPRC5D), Her2/neu (receptor tyrosine kinase erb-B2), Her3 (erb-B3), Her4 (erb-B4), erbB dimers, Human high molecular weight-melanoma-associated antigen (HMW-MAA), hepatitis B surface antigen, Human leukocyte antigen A1 (HLA-A1), Human leukocyte antigen A2 (HLA-A2), IL-22 receptor alpha (IL-22R α), IL-13 receptor alpha 2 (IL-13R α 2), kinase insert domain receptor (kdr), kappa light chain, L1 cell adhesion molecule (L1-CAM), CE7 epitope of L1-CAM, Leucine Rich Repeat Containing 8 Family Member A (LRRC8A), Lewis Y, Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, MAGE-A10, mesothelin (MSLN), c-Met, murine cytomegalovirus (CMV), mucin 1 (MUC1), MUC16, natural killer group 2 member D (NKG2D) ligands, melan A (MART-1), neural cell adhesion molecule (NCAM), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME), progesterone receptor, a prostate specific antigen, prostate stem cell antigen (PSCA), prostate specific membrane antigen

(PSMA), Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1), survivin, Trophoblast glycoprotein (TPBG also known as 5T4), tumor-associated glycoprotein 72 (TAG72), Tyrosinase related protein 1 (TRP1, also known as TYRP1 or gp75), Tyrosinase related protein 2 (TRP2, also known as dopachrome tautomerase, dopachrome delta-isomerase or DCT), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor receptor 2 (VEGFR2), Wilms Tumor 1 (WT-1), a pathogen-specific or pathogen-expressed antigen, or an antigen associated with a universal tag, and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV or other pathogens.

[0082] In some of any such examples, the recombinant protein is or includes a functional non-TCR antigen receptor or a TCR or antigen-binding fragment thereof. In some of any such examples, the recombinant protein is a chimeric antigen receptor (CAR). In some of any such examples, the recombinant receptor is an anti-BCMA CAR. In some of any such examples, the recombinant protein is an anti-CD19 CAR. In some of any such examples, the chimeric antigen receptor includes an extracellular domain containing an antigen-binding domain, a spacer and/or a hinge region, a transmembrane domain, and an intracellular signaling domain containing a costimulatory signaling region. In some of any such examples, the extracellular domain including an antigen-binding domain includes an scFv.

[0083] In some of any such examples, the intracellular signaling domain is or includes a primary signaling domain, a signaling domain that is capable of inducing a primary activation signal in a T cell, a signaling domain of a T cell receptor (TCR) component, and/or a signaling domain including an immunoreceptor tyrosine-based activation motif (ITAM). In some of any such examples, the intracellular signaling domain is or includes an intracellular signaling domain of a CD3 chain, optionally a CD3-zeta (CD3 ζ) chain, or a signaling portion thereof. In some of any such examples, the costimulatory signaling region includes an intracellular signaling domain of a CD28, a 4-1BB or an ICOS or a signaling portion thereof.

[0084] Also provided are methods for identifying a population of cells capable of expansion, the method including measuring the frequency of CD57⁺ cells in the population, wherein the population of cells is identified as capable of expansion if frequency of CD57⁺ cells are below a threshold frequency. Also provided are methods for identifying a population of cells capable of expansion, the method including measuring the frequency of CD57⁺ T cells in the population,

wherein the population of cells is identified as capable of expansion if frequency of CD57+ T cells are below a threshold frequency.

[0085] In some of any such examples, the threshold frequency is a percentage that is less than at or about 35%, 30%, 20%, 10%, 5%, 1%, or 0.1%. In some of any such examples, a population that is capable of expansion expands at least at or about 2-fold, 4-fold, 8-fold, or 16-fold within 4, 5, 6, 7 or 8 days of cultivation under conditions that promote proliferation or expansion.

[0086] Also provided are methods for determining the capacity of expansion of a population of T cells, the method including measuring a value of a trait associated with CD57 expression in a population of T cells, wherein the if a population of T cells is determined as capable of expansion if the value of the trait is less than at or about a threshold value of the trait.

[0087] In some of any such examples, the threshold the threshold value: i) is at, at about, or within 25%, within 20%, within 15%, within 10%, or within 5% below a mean or median measurement of the trait associated with CD57 expression, and/or is below one standard deviation less than at or about the mean or median measurement, in a plurality of reference T cell populations; ii) is below a lowest measurement of the trait associated with CD57 expression, optionally within 50%, within 25%, within 20%, within 15%, within 10%, or within 5% below the lowest measurement, in a population from among a plurality of reference T cell populations; iii) is below a mean or median measurement of the trait associated with CD57 expression calculated from among more than 65%, 75%, 80%, 85% of samples from a plurality of reference T cell compositions; wherein the plurality of reference T cell populations are a plurality of populations that did not expand when cultivated under conditions that promote proliferation or expansion of T cells, optionally wherein the cells did not expand by at least at or about 2-fold, 4-fold, 8-fold, or 16-fold within 4, 5, 6, 7 or 8 days of cultivation.

[0088] In some of any such examples, the trait is a level or amount of CD57 polypeptide expressed in the total T cells, CD4+ T cells, or CD8+ T cells. In some of any such examples, the trait is a frequency, percentage, or amount of CD57+ T cells, CD57+CD4+ T cells, or CD57+CD8+ T cells present in the cell population. In some of any such examples, the trait is a level or amount of CD57 mRNA present in the T cells in the cell population. In some of any such examples, the trait is a level or amount of chromatin accessibility of the gene encoding CD57 (B3GAT1).

[0089] In some of any such examples, wherein the method further includes measuring a second value of second a trait associated with the expression of one or more second gene products in a population of T cells, wherein the population is capable of expanding if the value of the trait is less than at or about the threshold value of the trait and if the second value of the second trait is greater than at or about a second threshold of the second trait.

[0090] In some of any such examples, the second gene product a marker associated with a naïve-like T cell. In some of any such examples, the one or more second gene product is selected from CD27, CD28, CCR7, or CD45RA. In some of any such examples, the one or more second gene product is CD27 and CD28.

[0091] In some of any such examples, second threshold value: i) is at, at about, or within 25%, within 20%, within 15%, within 10%, or within 5% above a mean or median measurement of the trait associated with expression of the second gene, and/or is above one standard deviation greater than at or about the mean or median measurement, in the plurality of second reference T cell compositions; ii) is above a highest measurement of the second trait associated with expression of the second gene, optionally within 50%, within 25%, within 20%, within 15%, within 10%, or within 5% above the highest measurement, in a composition from among the plurality of reference T cell compositions; iii) is below a mean or median measurement of the trait associated with expression of the second gene calculated from among more than 65%, 75%, 80%, 85% of samples from the plurality of reference T cell compositions.

[0092] In some of any such examples, the second trait is: (i) a level or amount of a polypeptide encoded by the second gene present in the total T cells, CD4⁺ T cells, or CD8⁺ T cells; (ii) is a level or amount of a polypeptide encoded by the second gene present on the surface of the total T cells, CD4⁺ T cells, or CD8⁺ T cells; (iii) is a trait that is a frequency, percentage, or amount of T cells, CD4⁺ T cells, or CD8⁺ T cells present positive for expression of the second gene; (iv) is a level or amount of mRNA of the second gene present in the T cells; or (v) is a level or amount of accessibility of the second gene.

[0093] Also provided are methods for genetically engineering T cells, the method including: (a) incubating T cells from a population of T cells identified or determined by any methods described herein under stimulating conditions, said stimulating conditions including the presence of a stimulatory reagent 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, thereby generating stimulated T cells; and (b) introducing a heterologous polynucleotide into the stimulated T cells, said introducing including transducing the stimulated T cells with a viral vector including the heterologous polynucleotide encoding a recombinant receptor, thereby generating an engineered population of T cells.

[0094] Also provided herein are compositions of cells, including any of those produced by the methods described herein. Also provided herein are compositions of cells including CD57-CD4⁺ cells. Also provided herein are compositions of cells including CD57-CD8⁺ cells. Also provided herein are compositions of cells including CD57-CD3⁺ cells.

[0094a] In one aspect, the present disclosure refers to a composition of cells, produced by the method disclosed herein.

[0094b] In another aspect, the present disclosure refers to a composition comprising an engineered population of T cells produced by the method disclosed herein.

[0095] Also provided are compositions of cells, including CD57- T cells of a deleted population produced by any method described herein.

[0096] Also provided are compositions of enriched CD57-CD4⁺ T cells, including CD57-CD4⁺ T cells of the enriched population of CD57-CD4⁺ T cells produced by any method described herein.

[0097] Also provided are compositions of enriched CD57-CD8⁺ T cells, including CD57-CD8⁺ T cells of the enriched population of CD57-CD8⁺ T cells produced by any method described herein.

[0098] Also provided are compositions of enriched CD57-CD3⁺ T cells, including CD57-CD3⁺ T cells of the enriched population of CD57-CD3⁺ T cells produced by any method described herein.

[0099] Also provided are compositions of cells, including the population of stimulated population of T cells produced by any method described herein.

[0100] Also provided are compositions including cells of an engineered population of T cells produced by any method described herein.

[0101] Also provided are compositions including cells of an expanded population of T cells produced by any method described herein.

[0102] Also provided are compositions including a population of T cells identified or determined as being capable of expansion by any method described herein.

[0103] Also provided are methods of treating a subject having or suspected of having a disease, disorder, or condition, the method including administering to the subject a dose of T cells from a population engineered T cells produced by any method described herein or an expanded population of T cells produced by any method described herein.

[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 disease, disorder or condition in a subject, optionally wherein the disease, disorder or condition is 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 disease, disorder or condition in a subject, optionally wherein the disease, disorder or condition is 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 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⁻, 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 recombinant 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 10×10^6 cells and at or about 200×10^6 cells, optionally wherein the number of viable T cells in the composition is between at or about 10×10^6 cells and at or about 100×10^6 cells, at or about 10×10^6 cells and at or about 70×10^6 cells, at or about 10×10^6 cells and at or about 50×10^6

cells, at or about 50×10^6 cells and at or about 200×10^6 cells, at or about 50×10^6 cells and at or about 100×10^6 cells, at or about 50×10^6 cells and at or about 70×10^6 cells, at or about 70×10^6 cells and at or about 200×10^6 cells, at or about 70×10^6 cells and at or about 100×10^6 cells, or at or about 100×10^6 cells and at or about 200×10^6 cells, each inclusive. In some of any such examples, the volume of the composition is between 1.0 mL and 10 mL, inclusive, optionally at or about 2 mL, at or about 3 mL, at or about 4 mL, at or about 5 mL, at or about 6 mL, at or about 7 mL, at or about 8 mL, at or about 9 mL, or at or about 10 mL, or any value between any of the foregoing.

Brief Description of the Drawings

[0114] **FIGS. 1A-1D** show the total cell number (**FIG. 1A**), fold expansion (**FIG. 1B**), percent viability (**FIG. 1C**) and percent Ki-67+ cells (marker associated with cell cycle entry; **FIG. 1D**) among CD8+ T cells obtained from seven different donors (Donors A-G), over approximately 240 hours after the start of stimulation in an exemplary manufacturing process for primary human T cells, or a similar process without transduction of the cells.

[0115] **FIG. 2A** shows a flow cytometry analysis for CD57 and Ki-67 expression at various time points over approximately 216 hours after the start of stimulation in an exemplary process for primary human T cells. **FIG. 2B** shows the percentage of CD57+ cells in the cell population during stimulation and cultivation, in three different donors (Donors A-C). **FIG. 2C** shows the percentage of Ki-67+ cells among CD57+ cells or CD57- cells in the cell population during stimulation and cultivation. **FIG. 2D** shows the percentage of CD57+ cells in the CD4+ cell population during stimulation and cultivation, in three different donors (Donors A-C). **FIG. 2E** shows CD57 and Ki67 expression on cells from donor cell compositions, over time, following stimulation. The cells of one donor cell composition required 8 days to reach harvest criterion (“8d to harvest”), while the cells of the other donor cell composition required only 7 days to reach harvest criterion (“7d to harvest”). **FIG. 2F** shows CD45RA and CD27 expression on cells from the donor cell compositions described in **FIG. 2E**.

[0116] **FIG. 3A** shows a flow cytometry analysis for CD69 and CD25 expression (markers associated with activation) among CD57+ cells and CD57- cells in the cell population during stimulation and cultivation in an exemplary process for primary human T cells. **FIGS. 3B** and

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 comprising fewer CD57+ T cells than the biological sample and enriched for CD3+ T cells.

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

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

(b) performing a second selection on the cells from the depleted population, the second selection comprising enriching for CD3+ T cells from the depleted population, the enrichment thereby generating an enriched population of CD57-CD3+ T cells.

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

(a) performing a first selection, the first selection comprising enriching for 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, the second selection comprising removing CD57+ T cells from the enriched sample cell population, thereby generating a depleted population, wherein the depleted population comprises fewer

CD57+ T cells than the biological sample and/or than the enriched T cell population and is enriched for CD3+ T cells.

4. 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; and

(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.

5. The method of any of claims 1-4, 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 in 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-.

6. The method of any of claims 1-5, wherein the biological sample comprises an apheresis product or a leukapheresis product.

7. A method for enriching T cells, the method comprising removing CD57+ T cells from a biological sample comprising an apheresis product or a leukapheresis product comprising primary human T cells, thereby generating a depleted population of T cells, wherein the depleted population comprises fewer CD57+ T cells than the biological sample and wherein the 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 in 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-.

8. The method of any of claims 1-7, wherein at least at or about 95% of the CD3+ T cells of the depleted population comprises CD57-CD3+ T cells.

9. The method of claim 7 or claim 8, 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.

10. The method of any of claims 1-9, 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.

11. The method of any of claims 1-10, 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, optionally 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.

12. The method of any of claims 4-6 and 9-11, 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.

13. The method of any of claims 1-12, 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.

14. The method of claim 13, 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.

15. The method of any of claims 1-14, 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.

16. The method of any of claims 1-15, wherein the enriching cells, optionally in the first, second and/or third selection, comprises immunoaffinity-based selection, optionally

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, further optionally 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.

17. The method of claim 15 or claim 16, wherein the antibody is immobilized on a solid surface, optionally wherein the solid surface is a magnetic particle.

18. The method of claim 15 or claim 16, 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.

19. The method of claim 18, 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.

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

21. A method for stimulating T cells, the method comprising incubating T cells of an input composition under stimulating conditions, thereby generating a stimulated population of T cells, wherein the input composition is produced by the method of any of claims 1-20, optionally wherein the input composition comprises 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 at or about 90% CD57-CD3+ T cells.

22. A method for stimulating T cells, comprising incubating T cells of an input composition under stimulating conditions, thereby generating a stimulated population of T cells, wherein the input composition comprises 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 at or about 90% CD57-CD3+ T cells.

23. The method of claim 21 or claim 22, 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.

24. The method of claim 23, 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.

25. The method of claim 24, 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.

26. The method of any of claims 23-25, 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.

27. A method of genetically engineering T cells, the method comprising 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 claims 1-20, thereby generating an engineered population of T cells.

28. A method of genetically engineering T cells, the method comprising introducing a heterologous polynucleotide encoding a recombinant receptor into a population of T cells from the stimulated T cell population of any of claims 21-26, thereby generating an engineered population of T cells.

29. The method of claim 27 or claim 28, 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.

30. The method of any of claims 27-29, wherein the recombinant 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.

31. The method of claim 30, wherein the recombinant receptor is a chimeric antigen receptor (CAR).

32. The method of claim 30 or claim 31, wherein the recombinant 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.

33. The method of claim 32, 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.

34. A composition of cells, produced by the method of any of claims 1-20.

35. A composition comprising an engineered population of T cells produced by the method of any of claims 27-33.

36. A therapeutic T cell composition comprising 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⁻, 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.

37. A therapeutic T cell composition comprising CD3⁺ T cells expressing a recombinant 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.

38. The therapeutic composition of claim 36 or claim 37, wherein the recombinant protein is a chimeric antigen receptor (CAR).

39. A population of engineered T cells produced by the method of any of claims 27-33 or the composition of any of claims 34-38 for use in the treatment of a disease, disorder or condition in a subject, optionally wherein the disease, disorder or condition is a cancer.

40. Use of a population of engineered T cells produced by the method of any of claims 27-33 or the composition of any of claims 34-38 for the manufacture of a medicament for the treatment of a disease, disorder or condition in a subject, optionally wherein the disease, disorder or condition is a cancer.

41. 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 1-33.

42. 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 1-33.

43. An article of manufacture, comprising:

- (i) the composition of any one of claims 34-38; and
- (ii) instructions for administering the composition to a subject.