(enriched) and stimulated cell populations for use in such cell therapies often require separate selection and stimulation steps, which can prolong the manufacturing process. Furthermore, selection techniques may involve steps that contaminate selected cells with selection-related particles, such as, for example, selection agents such as Fab fragments and competition reagents and/or free binding agents used to facilitate detachment of the cells from the stationary phase, thus requiring additional wash steps and/or media exchange to purify the output composition. The additional processing steps may result in cell stress, potentially affecting downstream cell processing or even cell biology, in addition to requiring considerable time to complete. Improved methods for generating cell populations suitable for use, for example in cell therapy, which minimize cell handling and processing time, are thus needed. Provided are methods, articles of manufacture, and apparatus that meet such needs.

Summary

[0005] Provided are methods of on-column stimulation of T cells, the method including: adding an oligomeric stimulatory reagent capable of delivering a stimulatory signal in T cells to a stationary phase containing a plurality of immobilized T cells, thereby initiating incubation of the stimulatory reagent with one or more T cells, wherein: the stationary phase includes a selection agent that specifically binds to a selection marker on the surface of one or more T cells or subset thereof; the oligomeric stimulatory reagent includes one or more stimulatory agent including (i) a first stimulatory agent that is an anti-CD3 antibody, and (ii) a second stimulatory agent that is an anti-CD28 antibody; and within 24 hours of initiating incubation, collecting one or more of the plurality of T cells from the stationary phase by gravity flow, thereby generating a composition containing stimulated T cells. In the provided method, the selection agent that specifically binds to a selection marker on the surface of one or more T cells or subsets thereof immobilizes the plurality of T cells on the stationary phase.

[0005a] In one aspect, the present disclosure refers to a method of on-column stimulation of T cells, the method comprising: (a) adding an oligomeric stimulatory reagent capable of delivering a stimulatory signal in T cells to a stationary phase of a chromatography column that comprises a plurality of immobilized T cells, thereby initiating incubation of the oligomeric stimulatory reagent with one or more T cells, wherein: the stationary phase comprises a selection agent that specifically binds to a selection marker on the surface of one or more T cells or subset thereof; the oligomeric stimulatory reagent comprises one or more stimulatory agents comprising (i) a first stimulatory agent that is an anti-CD3 antibody, and (ii) a second stimulatory agent that is an anti-CD28 antibody; and (b) within 24 hours of initiating incubation, collecting one or more of the plurality of T cells from the stationary phase by gravity flow, thereby generating a composition comprising stimulated T cells.

[0006] Provided are methods of on-column stimulation of T cells, the method including: incubating an oligomeric stimulatory reagent capable of delivering a stimulatory signal in T cells with a plurality of T cells immobilized on a staitionary phase, thereby initiating incubation of the stimulatory reagent with one or more T cells, wherein: the stationary phase includes a selection agent that specifically binds to a selection marker on the surface of one or more T cells or subset thereof; the oligomeric stimulatory reagent includes one or more stimulatory agent including (i) a

first stimulatory agent that is an anti-CD3 antibody, and (ii) a second stimulatory agent that is an anti-CD28 antibody; and within 24 hours of initiating incubation, collecting one or more of the plurality of T cells from the stationary phase by gravity flow, thereby generating a composition containing stimulated T cells. In the provided method, the selection agent that specifically binds to a selection marker on the surface of one or more T cells or subsets thereof immobilizes the plurality of T cells on the stationary phase.

[0006a] In one aspect, the present disclosure refers to a method of on-column stimulation of T cells, the method comprising: (a) incubating a plurality of T cells immobilized on a stationary phase of a chromatography column with one or more stimulatory agents to deliver a stimulatory signal in one or more T cells of the plurality of T cells, said stationary phase comprising a selection agent that specifically binds to a selection marker on the surface of the one or more T cells, wherein specific binding of the selection agent to the selection marker expressed by the one or more T cells immobilizes the one or more T cells on the stationary phase; and (b) within 24 hours of the initiation of the incubation, collecting the one or more T cells from the stationary phase by gravity flow, thereby generating a composition comprising stimulated T cells.

[0007] Provided are methods of on-column stimulation of T cells, the method including: combining an oligomeric stimulatory reagent capable of delivering a stimulatory signal in T cells with a plurality of T cells immobilized on a staitionary phase, thereby initiating incubation of the stimulatory reagent with one or more T cells, wherein: the stationary phase includes a selection agent that specifically binds to a selection marker on the surface of one or more T cells or subset thereof; the oligomeric stimulatory reagent includes one or more stimulatory agent including (i) a first stimulatory agent that is an anti-CD3 antibody, and (ii) a second stimulatory agent that is an anti-CD28 antibody; and within 24 hours of initiating incubation, collecting one or more of the plurality of T cells from the stationary phase by gravity flow, thereby generating a composition containing stimulated T cells. In the provided method, the selection agent that specifically binds to a selection marker on the surface of one or more T cells or subsets thereof immobilizes the plurality of T cells on the stationary phase.

[0008] Provided are methods for on-column stimulation of T cells, the method including incubating a plurality of T cells immobilized on a stationary phase with one or more stimulatory agent to deliver a stimulatory signal in one or more T cells of the plurality of T cells, said stationary phase including a selection agent that specifically binds to a selection marker on the surface of the one or more T cells, where specific binding of the selection agent to the selection marker expressed by the one or more T cells effects the immobilization of the one or more T cells on the stationary phase; and within 24 hours of the initiation of the incubation, collecting the one or more T cells from the stationary phase by gravity flow without the addition of a competition agent or free binding agent to elute the plurality of T cells from the stationary phase, thereby generating a composition containing stimulated T cells. Provided are methods for on-column stimulation of T cells, the method including incubating a plurality of T cells immobilized on a stationary phase with one or more stimulatory agent to deliver a stimulatory signal in one or

more T cells of the plurality of T cells, said stationary phase including a selection agent that specifically binds to a selection marker on the surface of the one or more T cells, where specific binding of the selection agent to the selection marker expressed by the one or more T cells immobilizes the one or more T cells on the stationary phase; and within 24 hours of the initiation of the incubation, collecting the one or more T cells from the stationary phase by gravity flow, thereby generating a composition containing stimulated T cells. In some examples, the stationary phase contains at least one of the one or more stimulatory agents capable of delivering a stimulatory signal in the one or more T cells. In some examples, the at least one stimulatory agent is a first stimulatory agent, and the stationary phase further includes one or more of a second stimulatory agent capable of enhancing, dampening, or modifying the stimulatory signal of the first stimulatory agent. In some examples, the stimulatory agent is a first stimulatory agent, and wherein prior to the incubating, adding to the stationary phase a stimulatory reagent containing one or more of a second stimulatory agent capable of enhancing, dampening, or modifying the stimulatory signal of the first stimulatory agent. In some examples, the stimulatory agent is a first stimulatory agent, and wherein prior to the incubating, adding to the stationary phase a stimulatory reagent containing one or more of a second stimulatory agent capable of enhancing, dampening, or modifying the stimulatory signal of the first stimulatory agent. In some examples, the one or more stimulatory agents is a first stimulatory agent and a second stimulatory agent, and wherein prior to the incubating, adding to the stationary phase a stimulatory reagent including the second stimulatory agent that is capable of enhancing, dampening, or modifying the stimulatory signal of the first stimulatory agent.In some examples, prior to the incubating, adding a stimulatory reagent to the stationary phase, said stimulatory reagent containing at least one of the one or more stimulatory agent.

[0009] In some examples, the at least one stimulatory agent is a first stimulatory agent and the one or more stimulatory agent further contains one or more of a second stimulatory agent capable of enhancing, dampening, or modifying the stimulatory signal of the first stimulatory agent. In some examples, the at least one of the one or more stimulatory agent, optionally the first stimulatory agent, is capable of delivering a stimulatory signal, wherein the stimulatory signal is through a TCR/CD3 complex in a T cell, a CD3-containing complex in a T cell, and/or

an ITAM-containing molecule in a T cell. In some examples, the at least one of the one or more first stimulatory agent is capable of delivering a stimulatory signal, wherein the stimulatory signal is through a TCR/CD3 complex in a T cell, a CD3-containing complex in a T cell, and/or an ITAM-containing molecule in a T cell. In some examples, the second stimulatory agent is capable of specifically binding to a costimulatory molecule on the one or more T cells. In some examples, the first stimulatory agent delivers a stimulatory signal through a TCR/CD3 complex in a T cell, a CD3-containing complex in a T cell, and/or an ITAM-containing molecule in a T cell and the second stimulatory agent binds to a costimlatory molecule on the T cell.

[0010] Provided are methods of on-column stimulation of T cells, the method including adding a sample containing a plurality of T cells to a stationary phase, said stationary phase containing a selection agent that binds to a selection marker on the surface of one or more of the plurality of T cells, thereby immobilizing the one or more of the plurality of T cells on the stationary phase; adding, to the stationary phase, a stimulatory reagent containing one or more stimulatory agent capable of delivering a stimulatory signal in one or more of said plurality of T cells, thereby initiating incubation of the stimulatory reagent with the one or more T cells; and within 24 hours of the initiating incubation, collecting one or more of said plurality of T cells from the stationary phase by gravity flow without the addition of a competition agent or free binding agent to elute the plurality of T cells from the stationary phase, thereby generating a composition containing stimulated T cells.

[0010a] In one aspect, the present disclosure refers to a method of on-column stimulation of T cells, the method comprising: (a) adding a sample comprising a plurality of T cells to a stationary phase of a chromatography column, said stationary phase comprising a selection agent that binds to a selection marker on the surface of one or more of the plurality of T cells, thereby immobilizing the one or more of the plurality of T cells on the stationary phase; (b) adding, to the stationary phase, an oligomeric stimulatory reagent comprising one or more stimulatory agents capable of delivering a stimulatory signal in one or more of said plurality of T cells, thereby initiating incubation of the oligomeric stimulatory reagent with the one or more T cells; and (c) within 24 hours of the initiating incubation, collecting one or more of said plurality of T cells from the stationary phase by gravity flow, thereby generating a composition comprising stimulated T cells.

[0011] Provided are methods of on-column stimulation of T cells, the method including adding a sample containing a plurality of T cells to a stationary phase, said stationary phase containing a selection agent that binds to a selection marker on the surface of one or more of the plurality of T cells, thereby immobilizing the one or more of the plurality of T cells on the stationary phase; adding, to the stationary phase, a stimulatory reagent containing one or more stimulatory agent capable of delivering a stimulatory signal in one or more of said plurality of T cells, thereby initiating incubation of the stimulatory reagent with the one or more T cells; and within 24 hours of the initiating incubation, collecting one or more of said plurality of T cells from the stationary phase by gravity flow, thereby generating a composition containing stimulated T cells.

stimulatory reagent with the one or more T cells; and (3) within 24 hours of the initiating incubation, collecting one or more of said plurality of T cells from the stationary phase by gravity flow, thereby generating a composition containing stimulated T cells.

[0015] Provided are methods of on-column stimulation of T cells, the method including adding an oligomeric stimulatory reagent to a stationary phase containing a plurality of immobilized T cells, thereby initiating incubation of the stimulatory reagent with one or more T cells of the plurality of immobilized T cells, wherein: the stationary phase comprises a selection agent that specifically binds to a selection marker on the surface of one or more T cells ,wherein specific binding of the selection agent to the selection marker expressed by the one or more T cells effects the immobilization of said one or more T cells on the stationary phase; and the oligomeric stimulatory reagent contains (i) a plurality of streptavidin or streptavidin mutein molecules and (ii) one or more stimulatory agent capable of delivering a stimulatory signal in one or more T cells, wherein the size of the oligomeric stimulatory reagent contains i) a radius of greater than 50 nm, ii) a molecular weight of at least 5 x 10^6 g/mol; and/or (iii) at least 100 streptavidin or streptavidin mutein tetramers per oligomeric stimulatory reagent. In some examples, the method further includes within 24 hours of the initiating incubation, collecting one or more of the plurality of T cells from the stationary phase by gravity flow, thereby generating a composition containing stimulated T cells. In some examples, the method further includes within 24 hours of the initiating incubation, collecting one or more of the plurality of T cells from the stationary phase by gravity flow without the addition of a competition agent or free binding agent to elute the plurality of T cells from the stationary phase, thereby generating a composition containing stimulated T cells.

[0015a] In one aspect, the present disclosure refers to a method of on-column stimulation of T cells, the method comprising: (a) adding an oligomeric stimulatory reagent to a stationary phase of a chromatography column that comprises a plurality of immobilized T cells, thereby initiating incubation of the oligomeric stimulatory reagent with one or more T cells of the plurality of immobilized T cells, wherein: the stationary phase comprises a selection agent that specifically binds to a selection marker on the surface of one or more T cells, wherein specific binding of the selection agent to the selection marker expressed by the one or more T cells immobilizes said one or more T cells on the stationary phase; the oligomeric stimulatory reagent comprises (i) a plurality of streptavidin or streptavidin mutein molecules and (ii) one or more stimulatory agents capable of delivering a stimulatory signal in one or more T cells, wherein the size of the oligomeric stimulatory reagent comprises i) a radius of greater than 50 nm; ii) a molecular weight of at least 5 x 10^6 g/mol; and/or (iii) at least 100 streptavidin or streptavidin mutein tetramers per oligomeric stimulatory reagent; and (b) within 24 hours of initiating incubation, collecting one or more of the plurality of T cells from the stationary phase by gravity flow, thereby generating a composition comprising stimulated T cells.

[0016] In some examples, the collecting of the one or more of the plurality of T cells from the stationary phase occurs within about 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, or 2 hours of initiating the incubation. In some examples, the collecting one or more of the plurality of T cells from the stationary phase occurs within about 2 to 24, 3 to 24, 4 to 24, 5, to 24, 6 to 24, 7 to 24, 8 to 24, 9 to 24, 10 to 24, 11 to 24, 12 to 24, 13 to 24, 14 to 24, 15 to 24, 16 to 24, 17 to 24, 18 to 24, 19 to 24, 20 to 24, 21 to 24, 22 to 24, 23 to 24, 2 to 23, 2 to 22, 2 to 21, 2 to 20, 2 to 19, 2 to 18, 2 to 17, 2 to 16, 2 to 15, 2 to 14, 2 to 13, 2 to 12, 2 to 11, 2 to 10, 2 to 9, 2 to 8, 2 to 7, 2 to 6, 2 to 5, 2 to 4, or 2 to 3 hours of initiating the incubation.

In some examples, the collecting one or more of the plurality of T cells from the stationary phase occurs within about 12, 10, 8, 6, 4, or 2 hours of initiating the incubation. In some examples, the collecting one or more of the plurality of T cells from the stationary phase occurs within 6 hours of initiating the incubation. In some examples, the collecting one or more of the plurality of T cells from the stationary phase occurs within 5 hours of initiating the incubation. In some examples, the collecting one or more of the plurality of T cells from the stationary phase occurs within at or about 4.5 hours of initiating the incubation. In some examples, the collecting one or more of the plurality of T cells from the stationary phase occurs within 4 hours of initiating the incubation. In some examples, the collecting one or more of the plurality of T cells from the stationary phase occurs within 3 hours of initiating the incubation.

[0017] In some examples, the initiating incubation with the stimulatory reagent is carried out within or within about 10 minutes, within or within about 20 minutes, within or within about 30 minutes, within or within about 45 minutes, within or within about 60 minutes, within or within about 90 minutes or within or within about 120 minutes after adding or combining the sample containing the plurality of T cells to or with the stationary phase. In some examples, the initiating incubation with the stimulatory reagent is carried out within or within about 20 to 100 minutes after adding or combining the sample containing the plurality of T cells to or with the stationary phase. In some examples, the initiating incubation with the stimulatory reagent is carried out within or within about 30 to 90 minutes after adding or combining the sample containing the plurality of T cells to or with the stationary phase. In some examples, the initiating incubation with the stimulatory reagent is carried out within or within about 30 to 80 minutes after adding or combining the sample containing the plurality of T cells to or with the stationary phase. In some examples, the initiating incubation with the stimulatory reagent is carried out within or within about 30 to 70 minutes after adding or combining the sample containing the plurality of T cells to or with the stationary phase. In some examples, the initiating incubation with the stimulatory reagent is carried out within or within about 30 to 60 minutes after adding or combining the sample containing the plurality of T cells to or with the stationary phase. In some examples, the initiating incubation with the stimulatory reagent is carried out within or within about 30 to 50 minutes after adding or combining the sample

containing the plurality of T cells to or with the stationary phase. In some examples, the initiating incubation with the stimulatory reagent is carried out within or within about 30 to 40 minutes after adding or combining the sample containing the plurality of T cells to or with the stationary phase. In some examples, the initiating incubation with the stimulatory reagent is carried out within or within about 30 minutes after adding or combining the sample containing the plurality of T cells to or with the stationary phase. In some examples, the initiating incubation with the stimulatory reagent is carried out within or within about 40 minutes after adding or combining the sample containing the plurality of T cells to or with the stationary phase. In some examples, the initiating incubation with the stimulatory reagent is carried out within or within about 50 minutes after adding or combining the sample containing the plurality of T cells to or with the stationary phase. In some examples, the initiating incubation with the stimulatory reagent is carried out within or within about 60 minutes after adding or combining the sample containing the plurality of T cells to or with the stationary phase. In some examples, the initiating incubation with the stimulatory reagent is carried out within or within about 70 minutes after adding or combining the sample containing the plurality of T cells to or with the stationary phase. In some examples, the initiating incubation with the stimulatory reagent is carried out within or within about 80 minutes after adding or combining the sample containing the plurality of T cells to or with the stationary phase. In some examples, the initiating incubation with the stimulatory reagent is carried out within or within about 90 minutes after adding or combining the sample containing the plurality of T cells to or with the stationary phase.

[0018] In some examples, at least one of the one or more stimulatory agent is capable of delivering a stimulatory signal, wherein the stimulatory signal is through a TCR/CD3 complex in a T cell, a CD3-containing complex in a T cell, and/or an ITAM-containing molecule in a T cell. In some examples, the at least one stimulatory agent is a first stimulatory agent and the stimulatory reagent further contains one or more of a second stimulatory agent capable of enhancing, dampening, or modifying the stimulatory signal of the first stimulatory agent. In some examples, the second stimulatory agent is capable of specifically binding to a costimulatory molecule on the one or more T cells. In some examples, the costimulatory molecule is selected from among CD28, CD90 (Thy-1), CD95 (Apo-/Fas), CD137 (4-1BB),

CD154 (CD40L), ICOS, LAT, CD27, OX40 or HVEM. In some examples, the second stimulatory agent is capable of specifically binding to CD28. In some examples, the first stimulatory agent specifically binds CD3 and the second stimulatory agent specifically binds CD28.

[0019] In some examples, the stimulatory agent is or contains an agent selected from the group consisting of antibody fragments, monovalent antibody fragments, proteinaceous binding molecules with immunoglobulin-like functions, molecules containing Ig domains, cytokines, chemokines, aptamers, MHC molecules, MHC-peptide complexes; receptor ligands; and binding fragments thereof; and/or the stimulatory agent contains an antibody fragment; the stimulatory agent is or contains a Fab fragment; the stimulatory agent is selected from the group of divalent antibody fragments consisting of (Fab)2'-fragments and divalent single-chain Fv (scFv) fragments; the stimulatory agent is a monovalent antibody fragment selected from the group consisting of Fab fragments, Fv fragments, and scFvs; and/or the stimulatory agent is a proteinaceous binding molecule with antibody-like binding properties, selected from the group consisting of aptamers, muteins based on a polypeptide of the lipocalin family, glubodies, proteins based on the ankyrin scaffold, proteins based on the crystalline scaffold, adnectins, and avimers.

[0020] In some examples, the first and second stimulatory agents, independently, are or contain an agent selected from the group consisting of antibody fragments, monovalent antibody fragments, proteinaceous binding molecules with immunoglobulin-like functions, molecules containing Ig domains, cytokines, chemokines, aptamers, MHC molecules, MHC-peptide complexes; receptor ligands; and binding fragments thereof; and/or the first and second stimulatory agents, independently, contain an antibody fragment; the first and second stimulatory agents, independently, are or contain a Fab fragment; the first and second stimulatory agents, independently, are selected from the group of divalent antibody fragments consisting of (Fab)2'-fragments and divalent single-chain Fv (scFv) fragments; the first and second stimulatory agents, independently, are a monovalent antibody fragment selected from the group consisting of Fab fragments, Fv fragments, and scFvs; and/or the first and second stimulatory agents, independently, are a proteinaceous binding molecule with antibody-like binding properties, selected from the group consisting of aptamers, muteins based on a polypeptide of the lipocalin

family, glubodies, proteins based on the ankyrin scaffold, proteins based on the crystalline scaffold, adnectins, and avimers.

[0021] In some examples, the one or more stimulatory agent includes a monovalent antibody fragment. In some examples, the first and second stimulatory agents, independently, comprise a monovalent antibody fragment. In some examples, the first stimulatory agent comprises a monovalent antibody fragment that binds to CD3 and the second stimulatory agent comprises a monovalent antibody fragment that binds to CD28. In some examples, the monovalent antibody fragment is selected from the group consisting of a Fab fragment, an Fv fragment, and a single-chain Fv fragment (scFv). In some examples, the first stimulatory reagent is an anti-CD3 Fab and the second stimulatory agent is an anti-CD28 Fab.

[0022] Provided are methods of on-column stimulation of T cells, the method including adding an oligomeric stimulatory reagent capable of delivering a stimulatory signal in T cells to a stationary phase containing a plurality of immobilized T cells, thereby initiating incubation of the stimulatory reagent with one or more T cells, wherein: the stationary phase contains a selection agent capable of specifically binding to a selection marker on the surface of one or more T cells or subset thereof, wherein specific binding of the selection agent to a selection marker expressed by the one or more T cells or a subset thereof effects the immobilization of said at least a plurality of T cells on the stationary phase, and wherein the selection agent is a Fab fragment capable of specifically binding to a selection marker selected from the group consisting of CD3, CD4, and CD8; the oligomeric stimulatory reagent contains (i) a plurality of streptavidin mutein molecules, (ii) a first stimulatory agent capable of delivering a stimulatory signal in one or more T cells, wherein the first stimulatory agent is a Fab fragment capable of specifically binding to CD3, and (iii) a second stimulatory agent capable of enhancing, dampening, or modifying the stimulatory signal, wherein the second stimulatory agent is a Fab fragment capable of specifically binding to CD28, and wherein the size of the oligomeric stimulatory reagent contains i) a radius of greater than 50 nm, ii) a molecular weight of at least 5×10^6 g/mol; and/or (iii) at least 100 streptavidin or streptavidin mutein tetramers per oligomeric stimulatory reagent; and within 24 hours of initiating incubation, collecting one or more of the plurality of T cells from the stationary phase by gravity flow without the addition of a

competition agent or free binding agent to elute the plurality of T cells from the stationary phase, thereby generating a composition containing stimulated T cells.

[0023] In some examples, the T cells are from a sample that is or includes a whole blood sample, a buffy coat sample, a peripheral blood mononuclear cells (PBMC) sample, an unfractionated T cell sample, a lymphocyte sample, a white blood cell sample, an apheresis product, or a leukapheresis product. In some examples, the sample is an apheresis or leukapheresis product. In some examples, the apheresis or leukapheresis product has been previously cryofrozen.

[0024] In some examples, the incubating with the one or more stimulatory agents releases one or more of the plurality of immobilized T cells from the stationary phase. In some examples, the incubating with the first and second stimulatory agents releases one or more of the plurality of immobilized T cells from the stationary phase.

[0025] In some examples, the stimulatory agent further contains biotin, a biotin analog that reversibly binds to a streptavidin or avidin, a streptavidin-binding peptide selected from the group consisting of Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8), Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_3$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO:15), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_3$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 17), SAWSHPQFEKGGGSGGGSGGSAWSHPQFEK (SEQ ID NO: 16), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 18) and Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$Gly-Gly-Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 19), a calmodulin binding peptide that reversibly binds to calmodulin, a FLAG peptide that reversibly binds to an antibody binding the FLAG peptide, and an oligohistidine tag that reversibly binds to an antibody binding the oligohistidine tag. In some examples, the one or more stimulatory agent further comprises a streptavidin-binding peptide selected from the group consisting of Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8), Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)3-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO:15), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)3-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 17), SAWSHPQFEKGGGSGGGSGGSAWSHPQFEK (SEQ ID NO: 16), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)2-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 18) 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: 19). In some examples, the one or more stimulatory agent further comprises a streptavidin-binding peptide having the sequence SAWSHPQFEKGGGSGGGSGGSAWSHPQFEK (SEQ ID NO: 16).

[0026] In some examples, the first stimulatory agent and the second stimulatory agent, independently, further contain biotin, a biotin analog that reversibly binds to a streptavidin or avidin, a streptavidin-binding peptide selected from the group consisting of Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8), Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_3$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO:15), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_3$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 17), SAWSHPQFEKGGGSGGGSGGSAWSHPQFEK (SEQ ID NO: 16), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 18) and Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$Gly-Gly-Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 19), a calmodulin binding peptide that reversibly binds to calmodulin, a FLAG peptide that reversibly binds to an antibody binding the FLAG peptide, and an oligohistidine tag that reversibly binds to an antibody binding the oligohistidine tag. In some examples, each of the first and second stimulatory agent further comprises a streptavidin-binding peptide selected from the group consisting of Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8), Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)3-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO:15), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)3-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 17), SAWSHPQFEKGGGSGGGSGGSAWSHPQFEK (SEQ ID NO: 16), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)2-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 18) 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: 19). In some examples, each of the first and second stimulatory agent further comprises a streptavidin-binding peptide having the sequence SAWSHPQFEKGGGSGGGSGGSAWSHPQFEK (SEQ ID NO: 16).

[0027] In some examples, the selection agent is or contains an agent selected from the group consisting of antibody fragments, monovalent antibody fragments, proteinaceous binding molecules with immunoglobulin-like functions, molecules containing Ig domains, cytokines, chemokines, aptamers, MHC molecules, MHC-peptide complexes; receptor ligands; and binding fragments thereof; and/or the selection agent contains an antibody fragment; the selection agent

is or contains a Fab fragment; the selection agent is selected from the group of divalent antibody fragments consisting of (Fab)2'-fragments and divalent single-chain Fv (scFv) fragments; the selection agent is a monovalent antibody fragment selected from the group consisting of Fab fragments, Fv fragments, and scFvs; and/or the selection agent is a proteinaceous binding molecule with antibody-like binding properties, selected from the group consisting of aptamers, muteins based on a polypeptide of the lipocalin family, glubodies, proteins based on the ankyrin scaffold, proteins based on the crystalline scaffold, adnectins, and avimers.

[0028] In some examples, the selection agent further contains biotin, a biotin analog that reversibly binds to a streptavidin or avidin, a streptavidin-binding peptide selected from the group consisting of Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8), Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_3$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO:15), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)3-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 17), SAWSHPQFEKGGGSGGGSGGSAWSHPQFEK (SEQ ID NO:16), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)2-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 18) 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: 19), a calmodulin binding peptide that reversibly binds to calmodulin, a FLAG peptide that reversibly binds to an antibody binding the FLAG peptide, and an oligohistidine tag that reversibly binds to an antibody binding the oligohistidine tag. In some examples, the selection agent further comprises biotin, a biotin analog that reversibly binds to a streptavidin or avidin, a streptavidin-binding peptide selected from the group consisting of Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8), Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_3$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO:15), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_3$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 17), SAWSHPQFEKGGGSGGGSGGSAWSHPQFEK (SEQ ID NO:16), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 18) and Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$Gly-Gly-Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 19). In some examples, the the selection agent further comprises a streptavidin-binding peptide having the sequence SAWSHPQFEKGGGSGGGSGGSAWSHPQFEK (SEQ ID NO:16).

[0029] In some examples, the selection marker is a T cell coreceptor; the selection marker is or contains a member of a T cell antigen receptor complex; the selection marker is or contains a CD3 chain; the selection marker is or contains a CD3 zeta chain; the selection marker is or contains a CD8; the selection marker is or contains a CD4; the selection marker is or contains CD45RA; the selection marker is or contains CD27; the selection marker is or contains CD28; and/or the selection marker is or contains CCR7. In some examples, the selection marker is selected from the group consisting of CD3, CD4, and CD8. In some examples, the selection marker is CD3.

[0030] In some examples, the specific binding between the selection agent and the selection marker does not induce a signal, or does not induce a stimulatory or activating or proliferative signal, to the T cells. In some examples, the selection agent includes a monovalent antibody fragment that binds to CD3, CD8 or CD4. In some examples, the selection agent is an anti-CD3 Fab, an anti-CD8 Fab or an anti-CD4 Fab. In some embodietns, the selection agent is an anti-CD3 Fab. In some examples, the anti-CD3 Fab comprises an OKT3 antibody Fab fragment. In some examples, the anti-CD3 Fab comprises a variable heavy chain having the sequence set forth by SEQ ID NO:31 and a variable light chain having the sequence set forth by SEQ ID NO:32.

[0031] In some examples, stimulatory reagent is soluble. In some examples, the stimulatory reagent is not, and is not bound to or associated with, a solid support, stationary phase, a bead, a microparticle, a magnetic particle, and/or a matrix; and/or the reagent is flexible, does not contain a metal or magnetic core, is comprised entirely or primarily of organic multimer, is not spherical, is not substantially spherical or uniform in shape and/or is not rigid. In some examples, the stimulatory reagent is or contains streptavidin, avidin, a mutein of streptavidin that reversibly binds biotin, a biotin analog or a biologically active fragment thereof; a mutein of avidin or streptavidin that reversibly binds a streptavidin-binding peptide; a reagent that contains at least two chelating groups K, wherein the at least two chelating groups are capable of binding to a transition metal ion; an agent capable of binding to an oligohistidine affinity tag; an agent capable of binding to a glutathione-S-transferase; calmodulin or an analog thereof; an agent capable of binding to calmodulin binding peptide (CBP); an agent capable of binding to a FLAG-peptide; an agent capable of binding to an HA-tag; an agent capable of binding to maltose binding protein (MBP); an agent capable of binding to an HSV epitope; an

agent capable of binding to a myc epitope; or an agent capable of binding to a biotinylated carrier protein.

[0032] In some examples, the stimulatory reagent is or contains a streptavidin mutein or an avidin mutein that reversibly binds to biotin or a biologically active fragment; the stimulatory reagent is or contains a streptavidin mutein or an avidin mutein that reversibly binds to a biotin analog or a biologically active fragment; and/or the stimulatory reagent is or contains a streptavidin mutein or an avidin mutein that reversibly binds to a streptavidin-binding peptide.

[0033] In some examples, the stimulatory reagent is an oligomeric stimulatory reagent containing a plurality of streptavidin or streptavidin mutein molecules, wherein the size of the oligomeric stimulatoryparticle reagent contains i) a radius of greater than 50 nm, ii) a molecular weight of at least 5 x 10^6 g/mol; and/or (iii) at least 100 streptavidin or streptavidin mutein tetramers per oligomeric particle reagent. In some examples, the oligomeric stimulatory reagent is soluble. In some embodments, the oligomeric stimulatory reagent is not, and is not bound to or associated with, a solid support, stationary phase, a bead, a microparticle, a magnetic particle, and/or a matrix; and/or the reagent is flexible, does not contain a metal or magnetic core, is comprised entirely or primarily of organic multimer, and/or is not rigid.

[0034] In some examples, the streptavidin or streptavidin mutein molecules reversibly bind to or are capable of reversibly binding to biotin, a biotin analog or a streptavidin-binding peptide. In some examples, the streptavidin mutein contains the amino acid sequence Va144-Thr45-Ala46-Arg47 or lle44-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:1; or the streptavidin mutein contains the amino acid sequence Va144-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: 1. In some examples, the streptavidin-binding peptide is selected from the group consisting of Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)3-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys ((SEQ ID NO: 17), SAWSHPQFEKGGGSGGGSGGSAWSHPQFEK (SEQ ID NO:16), SAWSHPQFEKGGGSGGGSGGGSWSHPQFEK (SEQ ID NO:15), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)2-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 18) 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: 19).

[0035] In some examples, the oligomeric particle reagent contains a radius of greater than 60 nm, greater than 70 nm, greater than 80 nm, or greater than 90 nm. In some examples, the oligomeric particle reagent contains a radius of between 50 nm and 150 nm, between 75 nm and 125 nm, between 80 nm and 115 nm, or between 90 nm and 110 nm, inclusive; or a radius of 90 nm ±15 nm, or 95 nm ± 20-25nm. In some examples, the radius is a hydrodynamic radius.

[0036] In some examples, the oligomeric particle reagent contains a molecular weight of at least 5 x 10^7 g/mol, or at least 1 x 10^8 g/mol; and/or between 5 x 10^7 g/mol and 5 x 10^8 g/mol, between 1 x 10^8 g/mol and 5 x 10^8 g/mol, or between 1 x 10^8 g/mol and 2 x 10^8 g/mol. In some examples, the oligomeric particle reagent contains at least 500 streptavidin or streptavidin mutein tetramers, at least 1,000 streptavidin or streptavidin mutein tetramers, at least 1,500 streptavidin or streptavidin mutein tetramers, or at least 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. In some examples, the oligomeric stimulatory reagent is added to the stationary phase at a concentration of between about 1 to about 2 µg/1 million cells.

[0037] In some examples, the selection agent is directly or indirectly bound to the stationary phase. In some examples, the selection agent is bound indirectly to the stationary phase through a selection reagent to which the selection agent reversibly binds. In some examples, the the selection reagent is or contains streptavidin, avidin, a mutein of streptavidin that reversibly binds biotin, a biotin analog or a biologically active fragment thereof; a mutein of avidin or streptavidin that reversibly binds a streptavidin-binding peptide; a reagent that contains at least two chelating groups K, wherein the at least two chelating groups are capable of binding to a transition metal ion; an agent capable of binding to an oligohistidine affinity tag; an agent capable of binding to a glutathione-S-transferase; calmodulin or an analog thereof; an agent capable of binding to calmodulin binding peptide (CBP); an agent capable of binding to a FLAG-peptide; an agent capable of binding to an HA-tag; an agent capable of

binding to maltose binding protein (MBP); an agent capable of binding to an HSV epitope; an agent capable of binding to a myc epitope; or an agent capable of binding to a biotinylated carrier protein. In some examples, the the selection reagent is or contains a streptavidin mutein or an avidin mutein that reversibly binds to biotin or a biologically active fragment; the stimulatory reagent is or contains a streptavidin mutein or an avidin mutein that reversibly binds to a biotin analog or a biologically active fragment; and/or the stimulatory reagent is or contians a streptavidin mutein or an avidin mutein that reversibly binds to a streptavidin-binding peptide. In some examples, the streptavidin or streptavidin mutein molecules reversibly bind to or are capable of reversibly binding to biotin, a biotin analog or a streptavidin-binding peptide.

[0038] In some examples, the streptavidin mutein contains the amino acid sequence Va144-Thr45-Ala46-Arg47 or lle44-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:1; or the streptavidin mutein contains the amino acid sequence Va144-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: 1. In some examples, the streptavidin-binding peptide is selected from the group consisting of Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8), SAWSHPQFEKGGGSGGGSGGSAWSHPQFEK (SEQ ID NO:16), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)$_3$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 17), SAWSHPQFEKGGGSGGGSGGGSWSHPQFEK (SEQ ID NO:15), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)$_2$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 18) and Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)$_2$Gly-Gly-Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 19). In some examples, the streptavidin-binding peptide has the sequence SAWSHPQFEKGGGSGGGSGGSAWSHPQFEK (SEQ ID NO:16).

[0039] In some examples, the methods provided herein are carried out at or at about 37 ºC. In some examples, said collecting includes washing the stationary phase with media, the media not containing a competition agent or free binding agent to elute the T cells from the stationary phase. In some examples, the collecting by gravity flow adding media to the stationary phase, the media not comprising a competition agent or free binding agent to elute the T cells from the stationary phase. In some examples, said composition containing stimulated T cells does not contain a competition agent or free binding agent. In some examples, said

competition agent or free binding agent is or contains biotin or a biotin analog, optionally wherein the biotin analog is D-biotin. In some examples, the competition agent or free binding agent is D-biotin. In some examples, the method includes after said collecting, further incubating the composition containing the stimulated T cells. In some examples, the further incubation is carried out at or about 37 ºC ± 2 ºC; and/or the further incubation is carried out in the presence of a further agent that is capable of delivering a signal to T cells. In some examples, the further agent is contained in the media used for washing the stationary phase. In some examples, the further agent is capable of enhancing or inducing proliferation of T cells, CD4+ T cells and/or CD8+ T cells. In some examples, the further agent is a cytokine selected from among IL-2, IL-15 and IL-7. In some examples, the further incubation is carried out for a time that is 72 hours, no more than 48 hours, no more than 24 hours, or no more than 12 hours.

[0040] In some examples, the method further includes introducing a recombinant nucleic acid molecule into the stimulated T cells of the composition, wherein the nucleic acid molecule encodes a recombinant protein, thereby producing a composition comprising transduced T cells. In some examples, the recombinant protein is an antigen receptor. In some examples, the recombinant protein is a chimeric antigen receptor.

[0041] In some examples, the chimeric antigen receptor (CAR) contains an extracellular antigen-recognition domain that specifically binds to a target antigen and an intracellular signaling domain comprising an ITAM. In some examples, the intracellular signaling domain comprises an intracellular domain of a CD3-zeta (CD3ζ) chain. In some examples, further included is a transmembrane domain linking the extracellular domain and the intracellular signaling domain. In some examples, the transmembrane domain comprises a transmembrane portion of CD28. In some examples, the intracellular signaling domain further contains an intracellular signaling domain of a T cell costimulatory molecule. In some examples, the T cell costimulatory molecule is selected from the group consisting of CD28 and 41BB. In some examples, the nucleic acid further contains a promoter operably linked to the nucleic acid encoding the recombinant antigen receptor.

[0042] In some examples, the introduction of the recombinant nucleic acid is achieved by transduction with a viral particle. In some examples, the viral particle is a retroviral vector particle. In some examples, the viral particle is a lentiviral vector particle.

[0043] In some examples, the method further includesincubating the composition comprising transduced cells under conditions for viral integration, optionally at a temperature of at or about 37º± 2º C. In some examples, the incubating the composition comprising transduced cells is carried out for up to 96 hours subsequent to the introducing. In some examples, the incubating the composition comprising transduced cells is carried out for up to 72 hours subsequent to the introducing. In some examples, the incubating the composition comprising transduced cells is carried out for up to 48 hours subsequent to the introducing. In some examples, the incubating the composition comprising transduced cells is carried out for up to 24 hours subsequent to the introducing. In some examples, the incubatingon the composition comprising transduced cells is carried out for at least 18 hours subsequent to the introducing.

[0044] In some examples, the method further includes cultivating the composition containing transduced cells under conditions for viral integration, thereby producing a composition containing cultivated T cells. In some examples, the method further includes cultivating the composition containing transduced cells under conditions to expand the T cells. In some examples, the cultivating is carried out for a time that is no more than 14 days, no more than 12 days, no more than 10 days, no more than 8 days or no more than 6 days. In some examples, no more than 5 days.

[0045] In some examples, the method further includes harvesting the engineered T cells, thereby producing an output population of engineered T cells. In some examples, the method further includes harvesting the engineered T cells at a time between 48 and 120 hours, inclusive, after the exposing to the stimulatory reagent is initiated. In some examples, the harvesting is carried out within 120 hours after the exposing to the stimulatory agent is initiated. In some examples, the harvesting is carried out within 96 hours after the exposing to the stimulatory agent is initiated. In some examples, the harvesting is carried out within 72 hours after the exposing to the stimulatory agent is initiated. In some examples, the harvesting is carried out within 48 hours after the exposing to the stimulatory agent is initiated.

[0046] In some examples, at the time of harvesting the percentage of naïve-like cells is greater than or greater than about 60% among total T cells in the population, total CD4+ T cells in the population or total CD8+ T cells, or of recombinant protein-expressing cells thereof, in the population. In some examples, the naïve-like T cells comprise CD27+CCR7+ cells.

[0047] In some examples, the introducing is carried out in serum free media. In some examples, the incubating is carried out in serum free media. In some examples, wherein the cultivating is carried out in serum free media. In some examples, the serum free media contains 0.5 mM to 5 mM of a dipeptide form of L-glutamine in a basal media; 0.5 mM to 5 mM L-glutamine; and optionally at least one protein, wherein the media is free of serum. In some examples, the serum free media contains a recombinant cytokine selected from among IL-2, IL-15 and IL-7, optionally recombinant human IL-2, recombinant human IL-15 and/or recombinant human IL-7. In some examples, the serum free media does not contain a recombinant cytokine selected from among IL-2, IL-15 and IL-7, optionally recombinant human IL-2, recombinant human IL-15 and/or recombinant human IL-7.

[0048] In some examples, the method further includes incubating the composition containing transduced cells. In some examples, the incubation is performed for or for about 24 hours ± 6 hours, 48 hours ± 6 hours, or 72 hours ± 6 hours.

[0049] In some examples, the method further includes adding a competition agent or free binding agent to the composition containing the stimulated T cells, thereby disrupting the reversible bond(s). In some examples, the method further includes adding a competition agent or free binding agent to the composition containing the transduced T cells, thereby disrupting the reversible bond(s). In some examples, the method futher includes adding a competition agent or free binding agent to the composition containing the cultivated T cells, thereby disrupting the reversible bond(s). In some examples, the method further includes adding a competition agent or free binding agent to the composition containing the engineered cells, optionally transduced T cells, optionally wherein the agent is added under conditions to dissociate the one or more stimulatory agent from the oligomeric stimulatory reagent in the composition. In some examples, the method further includes adding a competition agent or free binding agent to the composition containing the incubated T cells, optionally under conditions to dissociate the one or more stimulatory agent from the oligomeric stimulatory

reagent in the composition. In some examples, the method further includes adding a competition agent or free binding agent to the composition containing the cultivated T cells, optionally under conditions to dissociate the one or more stimulatory agent from the oligomeric stimulatory reagent in the composition. In some examples, adding the competition agent or free binding agent is carried out prior to the harvesting.

[0050] In some examples, the competition agent or free binding agent is not detrimental to the T cells and/or wherein the addition of said substance does not reduce the percentage of surviving T cells to less than 90 %, 80%, 70 %, 60 %, or 50 %, as compared to incubation of the T cells, under comparable or the same conditions, without the competition agent or free binding agent. In some examples, said disruption terminates or lessens the stimulatory signal in the T cells. In some examples, the competition reagent and free binding agent independently contain a molecule from the group consisting of: streptavidin-binding molecules; biotin; D-biotin; biotin analogs; biotin analogs that specifically bind to streptavidin or a streptavidin analog having an amino acid sequence Va144-Thr45-Ala46-Arg47 or lle44-Gly45-Ala46-Arg47 at sequence positions corresponding to positions 44 to 47 of a wild type streptavidin; or the competition reagent and free binding agent independently comprise a metal chelator, which is optionally EDTA or EGTA. In some examples, the competition agent or free binding agent is D-biotin, optionally 1 mM of D-biotin. In some examples, the method further includes washing the cells, optionally wherein the washing reduces or removes the stimulatory reagent and/or the one or more stimulatory agents in the composition. In some examples, the washing is carried out before the harvesting.

[0051] In some examples, the T cells contain antigen-specific T cells or a population thereof, a T helper cell or population thereof, a cytotoxic T cell or population thereof, a memory T cell or population thereof, or a regulatory T cell or population thereof. In some examples, the T cells comprise CD3+ T cells or comprise CD4+ and/or CD8+ T cells.

[0052] In some examples, the method includes selecting a T cell subset from the stimulated T cells of the composition prior to the introducing, wherein the recombinant nucleic acid molecule is introduced into the selected T cell subset. In some examples, the method includes selecting a subset of T cells from the composition containing transduced cells prior to the incubation, wherein the selected subset of T cells are incubated under the conditions for viral

integration. In some examples, method includes selecting a subset of T cells from the composition containing engineered cells prior to the cultivating, wherein the selected subset of T cells is cultivated under the conditions to expand the T cells. In some examples, the method includes selecting a subset of T cells from the composition containing engineered cells prior to the harvesting, wherein the selected subset of T cells is harvested to produce the output population of engineered T cells. In some examples, the subset of T cells are naïve-like T cells or are T cells that are surface positive for a marker expressed on naïve-like T cells are CCR7+CD45RA+, CD27+CCR7+ or CD62L-CCR7+. In some examples, the naïve-like T cells comprise CD27+CCR7+ T cells. In some examples, the naïve-like T cells comprise CCR7+CD45RA+ T cells. In some examples, wherein the subset of T cells expresses the recombinant protein, optionally the chimeric antigen receptor. In some examples, the selecting the subset of T cells is carried out by affinity column chromatography.

[0053] In some examples, the method further includes formulating the harvested cells for cryopreservation and/or administration to a subject, optionally in the presence of a pharmaceutically acceptable excipient. In some examples, the harvested cells are formulated in the presence of a cryoprotectant. In some examples,

[0054] In some examples, the stationary phase is or comprises a chromatography matrix. In some examples, the stationary phase has a binding capacity, optionally a static binding capacity or a dynamic binding capacity, of between about 75 million and about 125 million T cells per mL of stationary phase. In some examples, (a) the stationary phase is about 20 mL; and/or (b) the stationary phase has a binding capacity of 2 billion ± 0.5 billion cells. In some examples, the method includes two stationary phases. In some examples, the two stationary phases are arranged in parallel. In some examples, wherein the two stationary phases are arranged sequentially.

[0055] Provided are articles of manufacture for on-column stimulation of T cells, the article of manufacture containing a first stimulatory agent and a second stimulatory agent capable of specifically binding to a first molecule and a second molecule, respectively, on the surface of a T cell, thereby stimulating the T cell; and a stationary phase comprising a selection agent capable of specifically binding to a selection marker on a T cell, thereby immobilizing the T cell onto the stationary phase. In some examples, the stationary phase further contains the first stimulatory

agent and the second stimulatory agent. In some examples, the first stimulatory agent, the second stimulatory agent, and the selection agent are bound indirectly to the stationary phase through a selection reagent. In some examples, the article further includes a stimulatory reagent, wherein the first and second stimulatory agents are or are capable of being reversibly bound. In some examples, the stimulatory reagent is an oligomeric stimulatory reagent. In some examples, the selection agent is bound indirectly to the stationary phase through a selection reagent.

[0055a] In one aspect, the present disclosure refers to an article of manufacture for on-column stimulation of T cells, the article of manufacture comprising: (a) a first stimulatory agent and a second stimulatory agent capable of specifically binding to a first molecule and a second molecule, respectively, on the surface of a T cell, thereby stimulating the T cell; and (b) a stationary phase comprising a selection agent capable of specifically binding to a selection marker on a T cell, thereby immobilizing the T cell onto the stationary phase.

[0056] In some examples, the stationary phase is or includes a chromatography matrix, and wherein the article of manufacture further contains a container in which all or part of the chromatography matrix is contained. In some examples, article of manufacture includes two stationary phases. In some examples, the two stationary phases are arranged in parallel. In some examples, the wherein the two stationary phases are arranged in sequentially.

[0057] Provided are apparatus including the articles of manufacture and examples thereof. In some examples, the apparatus further includes a fluid inlet, being fluidly connected to one or more component of the apparatus, and/or a fluid outlet, being fluidly connected to one or more component of the apparatus. In some embobiments, the apparatus is in a closed or sterile system. In some examples, the system is a closed and sterile system.

[0058] Provided are apparatus and/or articles of manufacture for use in any of the methods provided herein, including examples thereof, wherein the method is optionally carried out in an automated fashion.

[0058a] In one aspect, the present disclosure refers to an apparatus comprising the article of manufacture disclosed herein, optionally wherein the apparatus is in a closed or sterile system.

[0058b] In one aspect, the present disclosure refers to the apparatus disclosed herein or the article disclosed herein, for use in the method disclosed herein, wherein the method is optionally carried out in an automated fashion.

Brief Description of the Drawings

[0059] **FIG. 1** provides a schematic representation of an exemplary embodiment for stimulating and selecting for target cells, in which the stimulation is carried out by an incubation of the cells, which occurs, at least in part, in the presence of a support, **6**, drawn here as a stationary phase, having immobilized thereon component(s) of a selection reagent **1** for cell selection (Panel A), which has a binding site for a selection agent **2**, which is capable of binding to a molecule (selection marker) **4** present on some or all of the target cells. The selection agent **2** is added to the support with immobilized selection reagent **1**, under conditions whereby the reagent and agent reversibly bind, *e.g.*, via binding sites, generating an oligomeric complex with the agent multimerized thereon (Panel B). The selection agent can include more than one agent.

Claims

WHAT IS CLAIMED:

1. A method of on-column stimulation of T cells, the method comprising:

(a) adding an oligomeric stimulatory reagent capable of delivering a stimulatory signal in T cells to a stationary phase of a chromatography column that comprises a plurality of immobilized T cells, thereby initiating incubation of the oligomeric stimulatory reagent with one or more T cells, wherein:

the stationary phase comprises a selection agent that specifically binds to a selection marker on the surface of one or more T cells or subset thereof;

the oligomeric stimulatory reagent comprises one or more stimulatory agents comprising (i) a first stimulatory agent that is an anti-CD3 antibody, and (ii) a second stimulatory agent that is an anti-CD28 antibody; and

(b) within 24 hours of initiating incubation, collecting one or more of the plurality of T cells from the stationary phase by gravity flow, thereby generating a composition comprising stimulated T cells.

2. A method of on-column stimulation of T cells, the method comprising:

(a) incubating a plurality of T cells immobilized on a stationary phase of a chromatography column with one or more stimulatory agents to deliver a stimulatory signal in one or more T cells of the plurality of T cells, said stationary phase comprising a selection agent that specifically binds to a selection marker on the surface of the one or more T cells, wherein specific binding of the selection agent to the selection marker expressed by the one or more T cells immobilizes the one or more T cells on the stationary phase; and

(b) within 24 hours of the initiation of the incubation, collecting the one or more T cells from the stationary phase by gravity flow, thereby generating a composition comprising stimulated T cells.

3. The method of claim 2, wherein the method comprises prior to the incubating, adding an oligomeric stimulatory reagent to the stationary phase, said oligomeric stimulatory reagent comprising at least one of the one or more stimulatory agents.

4. A method of on-column stimulation of T cells, the method comprising:

(a) adding a sample comprising a plurality of T cells to a stationary phase of a chromatography column, said stationary phase comprising a selection agent that binds to a selection marker on the surface of one or more of the plurality of T cells, thereby immobilizing the one or more of the plurality of T cells on the stationary phase;

(b) adding, to the stationary phase, an oligomeric stimulatory reagent comprising one or more stimulatory agents capable of delivering a stimulatory signal in one or more of said plurality of T cells, thereby initiating incubation of the oligomeric stimulatory reagent with the one or more T cells; and

(c) within 24 hours of the initiating incubation, collecting one or more of said plurality of T cells from the stationary phase by gravity flow, thereby generating a composition comprising stimulated T cells.

5. The method of claim 4, wherein the initiating incubation with the oligomeric stimulatory reagent is carried out within or within about 10 minutes, within or within about 20 minutes, within or within about 30 minutes, within or within about 45 minutes, within or within about 60 minutes, within or within about 90 minutes or within or within about 120 minutes after adding the sample comprising the plurality of T cells to the stationary phase.

6. The method of any of claims 1 and 3-5, wherein the oligomeric stimulatory reagent comprises a plurality of streptavidin or streptavidin mutein molecules, wherein the size of the oligomeric particle reagent comprises i) a radius of greater than 50 nm; ii) a molecular weight of at least 5 x 106 g/mol; and/or (iii) at least 100 streptavidin or streptavidin mutein tetramers per oligomeric stimulatory reagent.

7. A method of on-column stimulation of T cells, the method comprising:

(a) adding an oligomeric stimulatory reagent to a stationary phase of a chromatography column that comprises a plurality of immobilized T cells, thereby initiating incubation of the oligomeric stimulatory reagent with one or more T cells of the plurality of immobilized T cells, wherein:

the stationary phase comprises a selection agent that specifically binds to a selection marker on the surface of one or more T cells, wherein specific binding of the selection agent to the selection marker expressed by the one or more T cells immobilizes said one or more T cells on the stationary phase;

the oligomeric stimulatory reagent comprises (i) a plurality of streptavidin or streptavidin mutein molecules and (ii) one or more stimulatory agents capable of delivering a stimulatory signal in one or more T cells, wherein the size of the oligomeric stimulatory reagent comprises i) a radius of greater than 50 nm; ii) a molecular weight of at least 5 x 10^6 g/mol; and/or (iii) at least 100 streptavidin or streptavidin mutein tetramers per oligomeric stimulatory reagent; and

(b) within 24 hours of initiating incubation, collecting one or more of the plurality of T cells from the stationary phase by gravity flow, thereby generating a composition comprising stimulated T cells.

8. The method of claim 6 or claim 7, wherein the streptavidin or streptavidin mutein molecules reversibly bind to or are capable of reversibly binding to biotin, a biotin analog, or a streptavidin-binding peptide, optionally wherein the streptavidin-binding peptide is selected from the group consisting of Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8), Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)3-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO:15), SAWSHPQFEKGGGSGGGSGGSAWSHPQFEK (SEQ ID NO:16), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)3-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 17), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)2-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 18), 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: 19).

9. The method of any of claims 6-8, wherein:

the streptavidin mutein comprises 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:1; or

the streptavidin mutein comprises 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: 1.

10. The method of any of claims 2-9, wherein at least one of the one or more stimulatory agents is capable of delivering a stimulatory signal, wherein the stimulatory signal is through a TCR/CD3 complex in a T cell, a CD3-containing complex in a T cell, and/or an ITAM-containing molecule in a T cell.

11. The method of claim 10, wherein the at least one stimulatory agent is a first stimulatory agent and the oligomeric stimulatory reagent further comprises one or more of a second stimulatory agent capable of enhancing, dampening, or modifying the stimulatory signal of the first stimulatory agent.

12. The method of claim 11, wherein the second stimulatory agent is capable of specifically binding to a costimulatory molecule on the one or more T cells, optionally wherein the costimulatory molecule is selected from among CD28, CD90 (Thy-1), CD95 (Apo-/Fas), CD137 (4-1BB), CD154 (CD40L), ICOS, LAT, CD27, OX40, and HVEM.

13. The method of claim 11 or claim 12, wherein the first stimulatory agent specifically binds CD3 and the second stimulatory agent specifically binds CD28.

14. The method of any of claims 1-13, wherein

the one or more stimulatory agents and/or the selection agent independently comprises an agent selected from the group consisting of antibody fragments,proteinaceous binding molecules with immunoglobulin-like functions, molecules containing Ig domains, cytokines, chemokines, aptamers, MHC molecules, MHC-peptide complexes, receptor ligands, and binding fragments of any of the foregoing.

15. The method of any of claims 1-14, wherein the one or more stimulatory agents and/or the selection agent independently comprises a monovalent antibody fragment, optionally wherein the monovalent antibody fragment is selected from the group consisting of a Fab fragment, an Fv fragment, and a single-chain Fv fragment (scFv).

16. The method of any of claims 1-15, wherein the one or more stimulatory agents comprise a first stimulatory agent that is an anti-CD3 Fab and a second stimulatory agent that is an anti-CD28 Fab.

17. The method of any of claims 1-16, wherein the collecting one or more of the plurality of T cells from the stationary phase occurs within about 2 to 24, 3 to 24, 4 to 24, 5, to 24, 6 to 24, 7 to 24, 8 to 24, 9 to 24, 10 to 24, 11 to 24, 12 to 24, 13 to 24, 14 to 24, 15 to 24, 16 to 24, 17 to 24, 18 to 24, 19 to 24, 20 to 24, 21 to 24, 22 to 24, 23 to 24, 2 to 23, 2 to 22, 2 to 21, 2 to 20, 2 to 19, 2 to 18, 2 to 17, 2 to 16, 2 to 15, 2 to 14, 2 to 13, 2 to 12, 2 to 11, 2 to 10, 2 to 9, 2 to 8, 2 to 7, 2 to 6, 2 to 5, 2 to 4, or 2 to 3 hours of initiating the incubation.

18. The method of any of claims 1-17, wherein the T cells are from a sample that is or comprises a whole blood sample, a buffy coat sample, a peripheral blood mononuclear cells (PBMC) sample, an unfractionated T cell sample, a lymphocyte sample, a white blood cell sample, an apheresis product, or a leukapheresis product, optionally wherein the sample is an apheresis or leukapheresis product that has been previously cryofrozen.

19. The method of any of claims 1-18, wherein

the one or more stimulatory agents and/or the selection agent further comprises biotin; a biotin analog that reversibly binds to streptavidin or avidin; a streptavidin-binding peptide selected from the group consisting of Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8), Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_3$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO:15), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_3$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 17), SAWSHPQFEKGGGSGGGSGGSAWSHPQFEK (SEQ ID NO: 16), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 18) and Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$Gly-Gly-Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-

Lys (SEQ ID NO: 19); a calmodulin binding peptide that reversibly binds to calmodulin; a FLAG peptide that reversibly binds to an antibody binding the FLAG peptide; or an oligohistidine tag that reversibly binds to an antibody binding the oligohistidine tag.

20. The method of any of claims 1-19, wherein:

the selection marker is a T cell coreceptor;

the selection marker is a member of a T cell antigen receptor complex;

the selection marker is a CD3 chain;

the selection marker is a CD3 zeta chain;

the selection marker is a CD8;

the selection marker is a CD4;

the selection marker is CD45RA;

the selection marker is CD27;

the selection marker is CD28; and/or

the selection marker is CCR7;

optionally wherein the selection marker is selected from the group consisting of CD3, CD4, and CD8.

21. The method of any of claims 1-20, wherein the specific binding between the selection agent and the selection marker does not induce a signal, or does not induce a stimulatory, activating, or proliferative signal, to the T cells.

22. The method of any of claims 1-21, wherein the selection agent is an anti-CD3 Fab, an anti-CD8 Fab, or an anti-CD4 Fab.

23. The method of any of claims 1 and 3-22, wherein:

the oligomeric stimulatory reagent is soluble;

the oligomeric stimulatory reagent is not, and is not bound to or associated with, a solid support, stationary phase, a bead, a microparticle, a magnetic particle, and/or a matrix; and/or

the oligomeric stimulatory reagent is flexible, does not contain a metal or magnetic core, is comprised entirely or primarily of organic multimer, and/or is not rigid.

24. The method of any of claims 1 and 3-23, wherein the oligomeric stimulatory reagent comprises:

a radius of between 50 nm and 150 nm, between 75 nm and 125 nm, between 80 nm and 115 nm, or between 90 nm and 110 nm, inclusive;

a molecular weight of between 5 x 107 g/mol and 5 x 108 g/mol, between 1 x 108 g/mol and 5 x 108 g/mol, or between 1 x 108 g/mol and 2 x 108 g/mol; 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.

25. The method of any of claims 1 and 3-24, wherein the oligomeric stimulatory reagent is added to the stationary phase at a concentration of between about 1 μg/1 million cells to about 2 μg/1 million cells.

26. The method of any of claims 1-25, wherein the selection agent is bound indirectly to the stationary phase through a selection reagent to which the selection agent reversibly binds, optionally wherein the selection reagent comprises streptavidin; avidin; a mutein of streptavidin that reversibly binds biotin, a biotin analog, or a biologically active fragment thereof; a mutein of avidin or streptavidin that reversibly binds a streptavidin-binding peptide; a reagent that comprises at least two chelating groups K, wherein the at least two chelating groups are capable of binding to a transition metal ion; an agent capable of binding to an oligohistidine affinity tag; an agent capable of binding to a glutathione-S-transferase; calmodulin or an analog thereof; an agent capable of binding to calmodulin binding peptide (CBP); an agent capable of binding to a FLAG-peptide; an agent capable of binding to an HA-tag; an agent capable of binding to maltose binding protein (MBP); an agent capable of binding to an HSV epitope; an agent capable of binding to a myc epitope; or an agent capable of binding to a biotinylated carrier protein.

27. The method of any of claims 1-26, wherein:

the incubating with the one or more stimulatory agents releases one or more of the plurality of immobilized T cells from the stationary phase; and/or

said collecting by gravity flow comprises adding media to the stationary phase, the media not comprising a competition agent or free binding agent to elute the T cells from the stationary phase, optionally wherein the media does not comprise biotin or a biotin analog, optionally does not comprise D-biotin.

28. The method of any of claims 1-27, further comprising introducing a recombinant nucleic acid molecule into the stimulated T cells of the composition, wherein the nucleic acid molecule encodes a recombinant protein, optionally a chimeric antigen receptor, thereby producing a composition comprising engineered T cells, optionally transduced T cells.

29. The method of claim 28, wherein the introduction of the recombinant nucleic acid is achieved by transduction with a viral particle, optionally wherein the viral particle is a retroviral vector particle, optionally a lentiviral particle.

30. The method of claim 28 or claim 29, further comprising incubating the composition comprising engineered T cells under conditions for viral integration, optionally at a temperature of at or about 37º± 2º C.

31. The method of claim 30, wherein the incubating the composition comprising engineered T cells is carried out for at least 18 hours and for up to 96, 72, 48, or 24 hours subsequent to the introducing.

32. The method of any of claims 28-31, further comprising cultivating the composition comprising engineered T cells under conditions to expand the T cells.

33. The method of claim 32, wherein the cultivating is carried out for a time that is no more than 14 days, no more than 12 days, no more than 10 days, no more than 8 days, no more than 6 days, or no more than 5 days.

34. The method of any of claims 28-33, further comprising harvesting the engineered T cells, thereby producing an output population of engineered T cells.

35. The method of claim 34, wherein the harvesting of the engineered T cells is at a time between 48 and 120 hours, inclusive, after the exposing to the oligomeric stimulatory reagent is initiated.

36. The method of claim 34 or claim 35, wherein at the time of harvesting, the percentage of naïve-like cells is greater than or greater than about 60% among total T cells in the population, total CD4+ T cells in the population, total CD8+ T cells in the population, or of recombinant protein-expressing cells thereof in the population, optionally wherein the naïve-like T cells comprise CD27+CCR7+ cells.

37. The method of any of claims 34-36, further comprising formulating the harvested cells for cryopreservation and/or administration to a subject, optionally in the presence of a pharmaceutically acceptable excipient or a cryoprotectant.

38. The method of any of claims 28-37, wherein the introducing, incubating, and/or cultivating is carried out in serum free media.

39. The method of claim 38, wherein the serum free media does not comprise recombinant human IL-2, recombinant human IL-15, or recombinant human IL-7.

40. The method of any of claims 28-39, further comprising adding a competition agent or free binding agent to the composition comprising the engineered T cells, the incubated T cells, and/or the cultivated T cells, wherein the competition agent or free binding agent is added prior to the harvesting and optionally wherein the agent is added under conditions to dissociate the one or more stimulatory agents from the oligomeric stimulatory reagent in the composition.

41. The method of claim 40, wherein:

the competition agent or free binding agent is not detrimental to the T cells; the addition of the competition agent or free binding agent does not reduce the percentage of surviving T cells to less than 90 %, 80%, 70 %, 60 %, or 50 %, as compared to incubation of the T cells, under comparable or the same conditions without the competition agent or free binding agent; and/or

said dissociation terminates or lessens the stimulatory signal in the T cells.

42. The method of claim 40 or claim 41, wherein

the competition reagent or free binding agent independently comprises a molecule from the group consisting of streptavidin-binding molecules; biotin, optionally 1 mM of D-biotin; biotin analogs, optionally biotin analogs that specifically bind to streptavidin or a streptavidin mutein having an amino acid sequence Val^{44}-Thr^{45}-Ala^{46}-Arg^{47} or Ile^{44}-Gly^{45}-Ala^{46}-Arg^{47} at sequence positions corresponding to positions 44 to 47 of a wild type streptavidin; or

a metal chelator, optionally EDTA or EGTA.

43. The method of any of claims 28-42, further comprising washing the cells, optionally wherein the washing reduces or removes the oligomeric stimulatory reagent and/or the one or more stimulatory agents in the composition.

44. The method of any of claims 1-43, wherein: the T cells comprises an antigen-specific T cell or population thereof, a helper T cell or population thereof, a cytotoxic T cell or population thereof, a memory T cell or population thereof, or a regulatory T cell or population thereof; and/or

the T cells comprise CD3+ T cells or comprise CD4+ and/or CD8+ T cells.

45. The method of any of claims 1-44, wherein the method comprises: selecting a T cell subset from the stimulated T cells of the composition prior to the introducing of any of claims 28-44, wherein the recombinant nucleic acid molecule is introduced into the selected T cell subset;

selecting a subset of T cells from the composition comprising engineered T cells prior to the incubation of any of claims 30-44, wherein the selected subset of T cells are incubated under conditions for viral integration;

selecting a subset of T cells from the composition comprising engineered T cells prior to the cultivating of any of claims 32-44, wherein the selected subset of T cells is cultivated under the conditions to expand the T cells; and/or

selecting a subset of T cells from the composition comprising engineered T cells prior to the harvesting of any of claims 34-44, wherein the selected subset of T cells is harvested to produce the output population of engineered T cells;

optionally wherein the selecting is carried out by affinity column chromatography.

46. The method of claim 45, wherein the subset of T cells are naïve-like T cells; are T cells that are surface positive for a marker expressed on naïve-like T cells; are CCR7+CD45RA+, CD27+CCR7+, or CD62L-CCR7+; and/or express the recombinant protein.

47. The method of any of claims 1-46, wherein:

the stationary phase is or comprises a chromatography matrix;

the stationary phase is a non-magnetic material or non-magnetizable material;

the stationary phase has a binding capacity of between about 75 million and about 125 million T cells per mL of stationary phase, optionally a binding capacity of 2 billion ± 0.5 billion cells; and/or

the stationary phase is about 20 mL.

48. The method of claim 47, wherein the chromatography matrix is for column chromatography.

49. An article of manufacture for on-column stimulation of T cells, the article of manufacture comprising:

(a) a first stimulatory agent and a second stimulatory agent capable of specifically binding to a first molecule and a second molecule, respectively, on the surface of a T cell, thereby stimulating the T cell; and

(b) a stationary phase comprising a selection agent capable of specifically binding to a selection marker on a T cell, thereby immobilizing the T cell onto the stationary phase.

50. An apparatus comprising the article of manufacture of claim 49, optionally wherein the apparatus is in a closed or sterile system.

51. The apparatus of claim 50 or the article of claim 49, for use in the method of any of claims 1-48, wherein the method is optionally carried out in an automated fashion.