

CELL THERAPY FOR TREATING SYSTEMIC AUTOIMMUNE DISEASES

Cross-Reference to Related Applications

[0001] This application claims priority to U.S. Provisional Application No. 63/487,612, filed February 28, 2023, U.S. Provisional Application No. 63/466,671, filed May 15, 2023, U.S. Provisional Application No. 63/522,085, filed June 20, 2023, U.S. Provisional Application No. 63/608,166, filed December 8, 2023, U.S. Provisional Application No. 63/618,271, filed January 5, 2024, U.S. Provisional Application No. 63/624,745, filed January 24, 2024, and U.S. Provisional Application No. 63/553,586, filed February 14, 2024, each entitled “CELL THERAPY FOR TREATING SYSTEMIC AUTOIMMUNE DISEASES” the contents of which are incorporated by reference in their entirety.

Reference to An Electronic Sequence Listing

[0002] The present application is being filed with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled 735042027040SeqList.xml, created on February 23, 2024, which is 200,426 bytes in size. The information in electronic format of the Sequence Listing is incorporated by reference in its entirety.

Field

[0003] The present disclosure relates in some aspects to adoptive cell therapy involving the administration of a dose of T cells expressing a CD19-directed chimeric antigen receptor for treating subjects with a Systemic Autoimmune Disease and related methods, compositions, uses and articles of manufacture.

Background

[0004] Systemic autoimmune disease relates to a wide range of diseases and disorders characterized by dysregulation of the immune system. Among these is Systemic Lupus Erythematosus (SLE), which is an aberrant immune disorder which presents an array of clinical manifestations including most prominently renal involvement, *i.e.*, lupus nephritis. Many patients eventually relapse or become refractory to available therapies, and second-line, third-

line, and particularly fourth-line treatments are limited. Effective therapies for patients with SLE, such as severe refractory SLE, who have failed one or more prior therapy are needed. Provided are methods and uses that meet such needs.

Summary

[0005] Provided herein is a method of treating a subject having a systemic autoimmune disease, the method comprising administering a dose of CD19-directed genetically modified T cells from a composition comprising engineered T cells expressing a chimeric antigen receptor (CAR) to a subject having or suspected of having a severe systemic autoimmune disease, wherein the T cells of the dose are positive for expression of a CAR that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

[0006] Also provided herein is a method of treating a subject having systemic autoimmune disease, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having a moderate systemic autoimmune disease, wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

[0007] In some embodiments, the systemic autoimmune disease is selected from the group consisting of systemic lupus erythematosus (SLE), Sjogren's syndrome, progressive systemic sclerosis (i.e., scleroderma), idiopathic inflammatory myositis (IIM), including dermatomyositis, polymyositis and necrotizing myositis), mixed connective tissue disorder (MCTD), highly active relapsing-remitting multiple sclerosis, primary progressive MS, ANCA-associated vasculitis (AAV), Crohn's disease, myasthenia gravis, Behçet's, rheumatoid arthritis, IgA nephropathy, pemphigus vulgaris, myasthenia gravis, autoimmune hemolytic anemia, immune thrombocytopenia, IgG4-related diseases, membranous nephropathy, cutaneous lupus erythematosus, sarcoidosis, light chain amyloidosis, acute respiratory distress syndrome, atopic eczema, hereditary angioedema, hidradenitis suppurative, inclusion-body myositis, inflammatory bowel disease, mastocytosis, multifocal motor neuropathy, necrotizing myopathy, neuromyelitis optica spectrum disorder, mixed connective tissue disorder, POEMS syndrome, primary biliary cholangitis, psoriasis, rhesus hemolytic disease, Still's disease, type 1 diabetes, urticaria, capillary leakage syndrome, cytokine release syndrome, erythema multiforme, pyoderma gangrenosum, x-linked agammaglobulinemia, antiphospholipid syndrome, and

chronic inflammatory demyelinating polyneuropathy (also known as inflammatory demyelinating polyradiculoneuropathy).

[0008] In some embodiments, the systemic autoimmune disease is rheumatoid arthritis. In some embodiments, the systemic autoimmune disease is myositis. In some embodiments, the systemic autoimmune disease is myasthenia gravis. In some embodiments, the systemic autoimmune disease is bullous pemphigoid. In some embodiments, the systemic autoimmune disease is immune thrombocytopenia. In some embodiments, the systemic autoimmune disease is autoimmune hemolytic anemia. In some embodiments, the systemic autoimmune disease is pemphigus vulgaris. In some embodiments, the systemic autoimmune disease is demyelinating polyradiculoneuropathy. In some embodiments, the systemic autoimmune disease is membranous nephropathy.

[0009] In some embodiments, the systemic autoimmune disease is a refractory disease. In some embodiments, the subject is refractory to treatment with one or more prior therapies for the systemic autoimmune disease. In some embodiments, the subject is refractory to treatment with two or more prior therapies for the systemic autoimmune disease. In some embodiments, the systemic autoimmune disease is a severe disease.

[0010] Also provided herein is a method of treating a subject having severe systemic lupus erythematosus (SLE), the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having severe systemic lupus erythematosus (SLE), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

[0011] Also provided herein is a method for reducing systemic lupus erythematosus (SLE) disease activity, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having severe systemic lupus erythematosus (SLE), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

[0012] In some embodiments, the SLE in the subject has one or more of the following: renal, central nervous system, or hematologic involvement.

[0013] In some embodiments, the subject has at least one organ system categorized by the British Isles Lupus Assessment Group 2004 (“BILAG”) as category A (“BILAG A”) or at least two organ systems categorized as BILAG B.

[0014] In some embodiments, the subject fulfills the 2019 American College of Rheumatology (ACR)/ European League Against Rheumatism (EULAR) classification criteria of SLE and/or the subject has detectable anti-dsDNA, anti-histone, anti-chromatin or anti-Sm antibodies in their blood. In some embodiments, the subject fulfills the 2019 American College of Rheumatology (ACR)/ European League Against Rheumatism (EULAR) classification criteria of SLE. In some embodiments, the subject has detectable anti-dsDNA, anti-histone, anti-chromatin or anti-Sm antibodies in their blood.

[0015] In some embodiments, the subject has lupus nephritis.

[0016] Also provided herein is a method of treating a subject having lupus nephritis, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having lupus nephritis, wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

[0017] In some embodiments, the subject is refractory to treatment with one or more prior therapies for the lupus.

[0018] In some embodiments, the subject achieved an insufficient response to one or more prior therapies for the lupus.

[0019] In some embodiments, the two or more prior therapies for the lupus comprise a glucocorticoid, an antimalarial, an immunosuppressant, an anti-CD20 antibody, or an inhibitor of soluble B lymphocyte stimulator (BLyS).

[0020] In some embodiments, the two or more prior therapies are selected from any two or more of the following: mycophenolate mofetil (MFF), cyclophosphamide (cyc), belimumab, rituximab, anifrolumab, azathioprine, methotrexate cyclosporine (csp) or voclosporin.

[0021] In some embodiments, the subject does not have drug-induced SLE, clinically significant CNS pathology, related systemic autoimmune diseases, and/or SLE overlap syndromes.

[0022] In some embodiments, the subject does not have related systemic autoimmune diseases, including by not limited to multiple sclerosis, psoriasis, and inflammatory bowel disease.

[0023] In some embodiments, the subject does not have SLE overlap syndromes, including by not limited to rheumatoid arthritis, scleroderma, and mixed connective tissue disease. In some embodiments, the subject is at high risk for organ failure.

[0024] In some embodiments, the method reduces the systemic autoimmune disease activity in the subject.

[0025] In some embodiments, reducing cr7 disease activity in the subject comprises a reduced inflammation in the subject.

[0026] In some embodiments, the method reduces SLE disease activity in the subject.

[0027] In some embodiments, reducing SLE disease activity in the subject comprises: a BILAG-Based Composite Lupus Assessment (BICLA) response in the subject, reducing the subject's Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI) score compared to the subject's CLASI score pre-treatment, reducing the subject's tender and swollen joint count compared to the subject's tender and swollen joint count pre-treatment, the subject having a maximum of 1 BILAG-2004 B score following treatment, the subject having a BILAG-2004 score of C or better following treatment, the subject having an improvement in at least one patient reported outcome (PRO) compared to pre-treatment, and/or reducing the subject's SLE flare rate compared to the subject's flare rate pre-treatment.

[0028] In some embodiments, reducing SLE disease activity in a subject involves the subject achieving clinical remission as defined by The Definitions of Remission in Systemic Lupus Erythematosus (DORIS). In some embodiments, reducing SLE involves the subject achieving Lupus Low Disease Activity State (LLDAS).

[0029] In some embodiments, the subject achieves clinical remission of the lupus within 3 months or within 6 months of administering the dose of CD19-directed genetically modified T cells.

[0030] In some embodiments, the clinical remission is maintained for at least about 6 months, at least about 12 months, at least about 24 months, at least about 3 years, at least about 4 years, or at least about 5 years.

[0031] In some embodiments, the subject achieves prolonged remission of the lupus.

[0032] Also provided herein is a method of treating a subject having idiopathic inflammatory myopathy (IIM), the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having idiopathic inflammatory myopathy (IIM), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

[0033] Also provided herein is a method for reducing idiopathic inflammatory myopathy (IIM) disease activity, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having idiopathic inflammatory myopathy (IIM), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

[0034] In some embodiments, the subject is refractory to treatment with one or more prior therapies for the IIM. In some embodiments, the subject achieved an insufficient response to one or more prior therapies for the IIM.

[0035] Also provided herein is a method of treating a subject having systemic sclerosis (SSc), the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having systemic sclerosis (SSc), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

[0036] Also provided herein is a method for reducing systemic sclerosis (SSc) disease activity, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having systemic sclerosis (SSc), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

[0037] In some embodiments, the subject is refractory to treatment with one or more prior therapies for the SSc. In some embodiments, the subject achieved an insufficient response to one or more prior therapies for the SSc.

[0038] Also provided herein is a method of treating a subject having multiple sclerosis (MS), the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having multiple sclerosis (MS), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

[0039] Also provided herein is a method for reducing multiple sclerosis (MS) disease activity, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having multiple sclerosis (MS), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

[0040] In some embodiments, the subject is refractory to treatment with one or more prior therapies for the MS. In some embodiments, the subject achieved an insufficient response to one or more prior therapies for the MS. In some embodiments, the subject has or is suspected of having a relapsing form of MS. In some embodiments, the subject has or is suspected of having a progressive form of MS.

[0041] In some embodiments, the subject has or is suspected of having highly active relapse-remitting MS. In some embodiments, the subject has or is suspected of having primary progressive MS. In some embodiments, the subject has or is suspected of having active secondary progressive MS (aSPMS). In some embodiments, the subject has or is suspected of having inactive secondary progress MS (iSPMS).

[0042] In some embodiments, the subject has an Expanded Disability Status Scale (EDSS) of ≥ 3.0 and ≤ 5.5 or of ≥ 3.0 and ≤ 6.0 . In some embodiments, the subject can complete the 9-Hole Peg Test (9-HPT) for each hand in <240 seconds, and subjects can perform a Timed 25-Foot Walk Test (T25FWT) in <150 seconds. In some embodiments, the subject does not have MS lesions or symptoms that may place them at increased risk of neurotoxicity.

[0043] In some embodiments, the method reduces the autoimmune disease activity in the subject.

[0044] In some embodiments, reducing disease activity in the subject comprises a reduced inflammation in the subject.

[0045] In some embodiments, the reducing the autoimmune disease activity in the subject comprises reducing the subject's IMACS score after treatment compared to the subject's IMACS score before treatment, reducing the subject's skin lesions, muscle fatigue, and/or weakness compared to the subject's skin lesions, muscle fatigue, and/or weakness pre-treatment, or the subject having an improvement in at least one patient reported outcome (PRO) compared to pre-treatment.

[0046] In some embodiments, the reducing the autoimmune disease activity in the subject comprises reducing the subjects modified Rodnan skin score, European Scleroderma Study Group (EScSG) indices, minimum clinically important differences (MCID), patient reported short-form quality of life assessment (SF-36) Physical Component Summary (PCS) and/or Mental Component Summary (MCS) or a combination thereof or improving forced vital capacity.

[0047] In some embodiments, the reducing the autoimmune disease activity in the subject comprises improving the subjects score in any of the following tests; expanded disability status scale (EDSS), disease steps, multiple sclerosis functional composit (MSFC), minimum clinically important differences (MCID), patient reported short-form quality of life assessment (SF-36) Physical Component Summary (PCS) and/or Mental Component Summary (MCS) or a combination thereof.

[0048] Also provided herein is a method of treating a subject having autoimmune vasculitis (AAV), the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having autoimmune vasculitis (AAV), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

[0049] Also provided herein is a method for reducing autoimmune vasculitis (AAV) disease activity, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having autoimmune vasculitis (AAV), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

[0050] Also provided herein is a method of treating a subject having IgA nephropathy, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having IgA nephropathy, wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

[0051] Also provided herein is a method for reducing IgA nephropathy disease activity, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having IgA nephropathy, wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

[0052] Also provided herein is a method of treating a subject having pemphigus vulgaris, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having pemphigus vulgaris, wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

[0053] Also provided herein is a method for reducing pemphigus vulgaris disease activity, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having pemphigus vulgaris, wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

[0054] Also provided herein is a method of treating a subject having myasthenia gravis, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having myasthenia gravis, wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

[0055] Also provided herein is a method for reducing myasthenia gravis disease activity, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having myasthenia gravis, wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

[0056] In some embodiments, the dose is at or about 1×10^6 to 40×10^6 CAR-positive viable T cells.

[0057] In some embodiments, the dose is at or about 1×10^6 to 25×10^6 CAR-positive viable T cells.

[0058] In some embodiments, the dose is at or about 5×10^6 CAR-positive viable T cells.

[0059] In some embodiments, the dose is at or about 10×10^6 CAR-positive viable T cells.

[0060] In some embodiments, the dose is at or about 25×10^6 CAR-positive viable T cells.

[0061] In some embodiments, the dose is at or about 50×10^6 CAR-positive viable T cells.

[0062] In some embodiments, the T cells are autologous to the subject.

[0063] In some embodiments, the method further comprises obtaining a leukapheresis sample from the subject for manufacturing the composition comprising engineered T cells.

[0064] In some embodiments, prior to the administration, the subject has been preconditioned with a lymphodepleting therapy.

[0065] In some embodiments, the method further comprises, immediately prior to the administration of the dose of CD19-directed genetically modified T cells, administering a lymphodepleting therapy to the subject, wherein the lymphodepleting therapy comprises the administration of fludarabine and/or cyclophosphamide.

[0066] In some embodiments, the administration of the dose of CD19-directed genetically modified T cells and/or the lymphodepleting therapy is carried out via outpatient delivery.

[0067] In some embodiments, the lymphodepleting therapy comprises the administration of fludarabine at 30 mg/m² body surface area of the subject, daily, and cyclophosphamide at 300 mg/m² body surface area of the subject, daily, each for 3 days.

[0068] In some embodiments, the dose of CD19-directed genetically modified T cells is administered between at or about 48 hours and at or about 9 days, inclusive, after completion of the lymphodepleting therapy.

[0069] In some embodiments, the dose of CD19-directed genetically modified T cells is administered to the subject by intravenous infusion.

[0070] In some embodiments, the CAR comprises an extracellular antigen-binding domain that binds CD19, a transmembrane domain, and an intracellular signaling domain.

[0071] In some embodiments, the CAR comprises a hinge spacer between the extracellular antigen-binding domain and the transmembrane domain, optionally wherein the hinge spacer is an immunoglobulin hinge or a CD8a hinge.

[0072] In some embodiments, the extracellular antigen-binding domain is an FMC63 monoclonal antibody-derived single chain variable fragment (scFv).

[0073] In some embodiments, the extracellular antigen-binding domain comprises a variable heavy chain set forth in SEQ ID NO:41 and a variable light chain set forth in SEQ ID NO:42.

[0074] In some embodiments, the scFv is set forth as SEQ ID NO: 43.

[0075] In some embodiments, the extracellular antigen-binding domain is an Hu19 single chain variable fragment (scFv).

[0076] In some embodiments, the extracellular antigen-binding domain comprises a variable heavy chain set forth in SEQ ID NO:114 and a variable light chain set forth in SEQ ID NO:112.

[0077] In some embodiments, the extracellular antigen-binding domain comprises in order a variable light chain set forth in SEQ ID NO: 112, a linker peptide set forth in SEQ ID NO: 113, and a variable heavy chain set forth in SEQ ID NO: 114.

[0078] In some embodiments, the CAR is a monospecific CAR directed to CD19.

[0079] In some embodiments, the CAR is a tandem bispecific CAR directed against CD19 and at least one other antigen expressed on B cells. In some embodiments, the other antigen expressed on B cells is selected from the group consisting of CD20, CD19, CD22, ROR1,

BCMA, CD45, CD21, CD5, CD33, Igkappa, Iglambda, CD79a, CD79b or CD30. In some embodiments, the other antigen expressed on B cells is CD20.

[0080] In some embodiments, the extracellular antigen-binding domain comprises a variable heavy chain and a variable light chain derived from a CD20 antibody selected from the group consisting of Leu16, C2B8, 11B8, 8G6-5, 2.1.2 and GA101.

[0081] In some embodiments, the transmembrane domain is a CD28 transmembrane domain.

[0082] In some embodiments, the transmembrane domain is a transmembrane domain from CD28, optionally a transmembrane domain that comprises the sequence of amino acids set forth in SEQ ID NO: 8 or a sequence of amino acids that exhibits at least or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:8.

[0083] In some embodiments, the intracellular signaling domain comprises a 4-1BB costimulatory domain and a CD3zeta activation domain.

[0084] In some embodiments, the CAR comprises, in order from N- to C-terminus, an FMC63 monoclonal antibody-derived single chain variable fragment (scFv), IgG4 hinge region, a CD28 transmembrane domain, a 4-1BB (CD137) costimulatory domain, and a CD3 zeta signaling domain.

[0085] In some embodiments, the 4-1BB costimulatory domain is or comprises the sequence set forth in SEQ ID NO: 12 or a variant thereof having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:12.

[0086] In some embodiments, the CD3zeta signaling domain is or comprises the sequence set forth in SEQ ID NO: 13, 14 or 15 or a sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto.

[0087] In some embodiments, the CAR contains in order from N-terminus to C-terminus: an extracellular antigen-binding domain that is the scFv set forth in SEQ ID NO: 43, the spacer set forth in SEQ ID NO:1, the transmembrane domain set forth in SEQ ID NO:8, the 4-1BB costimulatory signaling domain set forth in SEQ ID NO:12, and the signaling domain of a CD3-zeta (CD3ζ) chain set forth in SEQ ID NO:13.

[0088] In some embodiments, the CAR comprises the amino acid sequence set forth in SEQ ID NO:59 or a sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto.

[0089] In some embodiments, the composition produced by a manufacturing process comprising: (i) stimulating an input composition comprising primary T cells from the subject with an oligomeric stimulatory reagent, thereby generating a stimulated population, wherein the oligomeric stimulatory reagent comprises a plurality of cross-linked tetramers of a streptavidin or streptavidin mutein and wherein the streptavidin or streptavidin mutein are reversibly bound to a first agent comprising an anti-CD3 antibody or antigen binding fragment thereof and a second agent comprising an anti-CD28 antibody or antigen binding fragment thereof; (ii) introducing into T cells of the stimulated population, a heterologous polynucleotide encoding the CAR that targets CD19, thereby generating a population of transformed cells; (iii) incubating the population of transformed cells for up to 96 hours; and (iv) harvesting T cells of the population of transformed cells, thereby producing a composition of CD19-directed genetically modified T cells wherein the harvesting is carried out at a time between 24 and 120 hours, inclusive, after the exposing to the stimulatory reagent is initiated.

[0090] In some embodiments, the anti-CD3 antibody or antigen binding fragment is a Fab and the anti-CD28 antibody or antigen binding fragment is a Fab.

[0091] In some embodiments, the first agent and the second agent each comprise a streptavidin-binding peptide that reversibly binds the first agent and the second agent to the oligomeric particle reagent, optionally wherein the streptavidin-binding peptide comprises the sequence of amino acids set forth in any of SEQ ID NOS:78-82.

[0092] In some embodiments, the streptavidin mutein molecule is a tetramer of a streptavidin mutein comprising amino acid residues Val44-Thr45-Ala46-Arg47 or Ile44-Gly45-Ala46-Arg47, optionally wherein the streptavidin mutein comprises the sequence set forth in any of SEQ ID NOS: 69, 84, 87, 88, 90, 85 or 59.

[0093] In some embodiments, the oligomeric particle reagent comprises between 1,000 and 5,000 streptavidin mutein tetramers, inclusive.

[0094] In some embodiments, the method further comprises, prior to harvesting the cells, adding biotin or a biotin analog after or during the incubation.

[0095] In some embodiments, the harvesting is carried out at a time between 48 and 120 hours, inclusive, after the exposing to the stimulatory reagent is initiated.

[0096] In some embodiments, the dose of autologous CD19-directed genetically modified T cells is cryopreserved prior to administration to the subject.

[0097] In some embodiments, the cryopreserved dose of autologous CD19-directed genetically modified T cells is thawed prior to administration to the subject.

[0098] In some embodiments, the dose of autologous CD19-directed genetically modified T cells is administered to the subject within about two hours of being thawed.

[0099] In some embodiments, the dose of autologous CD19-directed genetically modified T cells is provided in a formulation comprising a cryoprotectant.

[0100] In some embodiments, the formulation comprises dimethylsulfoxide (DMSO).

[0101] In some embodiments, the formulation comprises albumin, optionally human albumin.

[0102] In some embodiments, the dose of T cells comprises CD4⁺ T cells expressing the CAR and CD8⁺ T cells expressing the CAR at a ratio between about 1:5 and about 5:1.

[0103] In some embodiments, the dose of T cells comprises CD4⁺ T cells expressing the CAR and CD8⁺ T cells expressing the CAR at a ratio between about 1:3 and about 3:1.

[0104] In some embodiments, at least or at least about 90% of the cells in the composition are CD3⁺ cells.

[0105] In some embodiments, at least or at least about 91%, at least or at least about 92%, at least or at least about 93%, at least or at least about 94%, at least or at least about 95%, or at least or at least about 96% of the cells in the composition are CD3⁺ cells.

[0106] In some embodiments, at least 25% of the T cells in the composition are CAR⁺ T cells. In some embodiments, at least 30%, at least 35%, at least 40%, at least 45% or at least 50% of the T cells in the composition are CAR⁺ T cells.

[0107] In some embodiments, between at or about 5% and at or about 30% of the CAR⁺ T cells in the composition express a marker of apoptosis, optionally between at or about 10% and at or about 15% of the CAR⁺ T cells in the composition, more optionally wherein the marker of apoptosis is Annexin V or active Caspase 3.

[0108] In some embodiments, less than 10% of the T cells in the composition express a marker of apoptosis. In some embodiments, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, or less than 4% of the T cells in the composition express a marker of apoptosis. In some embodiments, less than 10% of the CAR⁺ T cells in the composition express a marker of apoptosis. In some embodiments, less than 9%, less than 8%, less than 7%, less than

6%, less than 5%, or less than 4% of the CAR+ T cells in the composition express a marker of apoptosis. In some of any embodiments, the the marker of apoptosis is Annexin V or active Caspase 3.

[0109] In some embodiments, at least 70% of the T cells in the composition are viable T cells. In some embodiments, at least 75% of the T cells in the composition are viable T cells. In some embodiments, at least 80% of the T cells in the composition are viable T cells. In some embodiments, at least 85% of the T cells in the composition are viable T cells. In some embodiments, at least 90% of the T cells in the composition are viable T cells.

[0110] In some of any embodiments, viability is determined by staining for acridine orange (AO) and propidium iodide (PI).

[0111] In some embodiments, at least or at least about 80% of the CAR⁺ T cells in the composition are of a naïve-like or central memory phenotype.

[0112] In some embodiments, the marker expressed on naïve-like or central memory T cell is selected from the group consisting of CD45RA, CD27, CD28, and CCR7.

[0113] In some embodiments, at least 70% of the CAR+ T cells in the composition are CCR7+. In some embodiments, at least 75% of the CAR+ T cells in the composition are CCR7+. In some embodiments, at least 80% of the CAR+ T cells in the composition are CCR7+. In some embodiments, at least 85% of the CAR+ T cells in the composition are CCR7+. In some embodiments, at least 90% of the CAR+ T cells in the composition are CCR7+. In some embodiments, at least 95% of the CAR+ T cells in the composition are CCR7+. In some embodiments, at least 85% of the CD8+CAR+ T cells in the composition are CCR7+ and at least 90% of the CD4+ CAR+ T cells in the composition are CCR7+. In some embodiments, 85% to 98% of the CD8+ CAR+ T cells in the composition are CCR7+ and 94% to 99% of the CD4+ CAR+ T cells in the composition are CCR7+.

[0114] In some embodiments, the at least or at least about 80% of the CAR⁺ T cells in the composition that are of a naïve-like or central memory phenotype have a phenotype selected from CCR7⁺CD45RA⁺, CCR7⁺CD45RA⁻, CD27⁺CCR7⁺, or CD62L⁻CCR7⁺.

[0115] In some embodiments, least 40% of the CAR+ T cells in the composition are CD45RA+CCR7+. In some embodiments, at least 50% of the CAR+ T cells in the composition are CD45RA+CCR7+. In some embodiments, at least 60% of the CAR+ T cells in the composition are CD45RA+CCR7+. In some embodiments, at least 70% of the CAR+ T cells in the composition are CD45RA+CCR7+. In some embodiments, at least 80% of the CAR+ T

cells in the composition are CD45RA+CCR7+. In some embodiments, at least 20% of the CAR+ T cells in the composition are CD45RA-CCR7+. In some embodiments, at least 30% of the CAR+ T cells in the composition are CD45RA-CCR7+. In some embodiments, at least 40% of the CAR+ T cells in the composition are CD45RA-CCR7+. In some embodiments, at least 50% of the CAR+ T cells in the composition are CD45RA-CCR7+. In some embodiments, at least 60% of the CAR+ T cells in the composition are CD45RA-CCR7+.

[0116] In some embodiments, at least about 50% of CD4+CAR+ T cells in the composition are CCR7+CD45RA⁻. In some embodiments, at least about 60% of CD4+CAR+ T cells in the composition are CCR7+CD45RA⁻. In some embodiments, wherein at least about 70% of CD4+CAR+ T cells in the composition are CCR7+CD45RA⁻. In some embodiments, at least about 30% of CD8+CAR+ T cells in the composition are CCR7+CD45RA⁻. In some embodiments, at least about 40% of CD8+CAR+ T cells in the composition are CCR7+CD45RA⁻. In some embodiments, at least about 50% of CD8+CAR+ T cells in the composition are CCR7+CD45RA⁻.

[0117] In some embodiments, greater than or greater than about 50%, about 60%, about 70%, or about 80% of the subjects treated according to the method do not exhibit any grade of cytokine release syndrome (CRS).

[0118] In some embodiments, greater than or greater than about 40%, 50%, or about 60% of the subjects treated according to the method do not exhibit any grade of neurotoxicity.

[0119] In some embodiments, the subject is human.

[0120] In some embodiments, at least 60% of the T cells in the composition are viable, at least 25% of the T cells of the composition are CAR+ T cells; less than 10% of the cells of the composition are positive for an apoptotic marker, optionally wherein the marker of apoptosis is Annexin V or active Caspase 3; at least 85% of the CD8+CAR+ T cells in the composition are CCR7+; and/or at least 90% of the CD4+ CAR+ T cells in the composition are CCR7+.

[0121] In some embodiments, at least 80% of the T cells in the composition are viable, at least 45% of the T cells of the composition are CAR+, less than 4% of the cells of the composition are positive for an apoptotic marker, optionally wherein the marker of apoptosis is Annexin V or active Caspase 3; at least 85% of the CD8+CAR+ T cells in the composition are CCR7+; and/or at least 90% of the CD4+ CAR+ T cells in the composition are CCR7+.

[0122] In some embodiments, at least 60% of the T cells in the composition are viable, at least 25% of the T cells of the composition are CAR+, less than 10% of the cells of the

composition are positive for an apoptotic marker, optionally wherein the marker of apoptosis is Annexin V or active Caspase 3; and/or greater than at or about 40% of the CAR+ T cells in the composition are CCR7+CD45RA+.

[0123] In some embodiments, at least 80% of the T cells in the composition are viable; at least 45% of the T cells of the composition are CAR+; less than 4% of the cells of the composition are positive for an apoptotic marker, optionally wherein the marker of apoptosis is Annexin V or active Caspase 3; and/or at least 40% of the CAR+ T cells in the composition are CCR7+CD45RA+.

[0124] In some embodiments, at least 60% of the T cells in the composition are viable; at least 25% of the T cells of the composition are CAR+; less than 10% of the cells of the composition are positive for an apoptotic marker, optionally wherein the marker of apoptosis is Annexin V or active Caspase 3; and/or greater than 20% of the CAR+ T cells in the composition are CCR7+CD45RA-.

[0125] In some embodiments, at least 80% of the T cells in the composition are viable; at least 45% of the T cells of the composition are CAR+; less than 4% of the cells of the composition are positive for an apoptotic marker, optionally wherein the marker of apoptosis is Annexin V or active Caspase 3; and/or at least 20% of the CAR+ T cells in the composition are CCR7+CD45RA-.

Brief Description of the Drawings

[0126] **FIGS. 1A** and **1B** depict T cell memory subtypes in CAR+ CD4+ and CAR+ CD8+ respectively for the non-expanded and expanded process.

[0127] **FIGS. 2A-2C** depict fold expansion of T cells in a T cell composition produced by the expanded and non-expanded process in a long term stimulation assay after CAR stimulation with an anti-idiotypic antibody as an indication of persistence and expansion potential. After stimulation with an anti-idiotypic antibody of the CAR for 10 days, fold expansion of the T cell compositions from different donors was calculated over time and depicted as fold expansion cell counts (**FIG. 2A**), area under the curve of the fold expansion (**FIG. 2B**) and the fold expansion of CAR T cells produced by the nonexpanded process divided by the donor matched fold expansion of the CAR T cells produced by the expanded process (**FIG. 2C**).

[0128] **FIGS. 3A-3D** depicts the cytokine production of CAR+ CD4+ and CAR+ CD8+ T cells in T cell compositions produced by the expanded and non-expanded process in the long

term stimulation assay after CAR stimulation with an anti-idiotypic antibody for 10 day as described in **FIGS. 2A-2C**. The results show percent of CAR+ CD4+ T cells or CAR+CD8+ T cells positive for IL-2 (**FIG. 3A**), IFN- γ (**FIG. 3B**), TNF α (**FIG. 3C**) or IL-2, IFN γ , and TNF α (**FIG. 3D**).

[0129] **FIGS. 4A** and **4B** depict CD19+ target cell specific lysis by CAR+ T cells produced from both the expanded and non-expanded process over time (**FIG. 4A**) and the area under the curve of the lysis over time (**FIG. 4B**).

[0130] **FIGS. 5A-5F** depict the CAR transgene levels (**FIG. 5A**), serum IgG (**FIG. 5B**), serum IgA (**FIG. 5C**), the number of neutrophils (**FIG. 5D**), the number of total lymphocytes (**FIG. 5E**), and the number of platelets (**FIG. 5F**) in human patients after treatment with 10×10^6 or 25×10^6 anti-CD19 CAR T cell produced by the non-expanded process.

[0131] **FIG. 6A** depicts cumulative population doublings (PDL) of an engineered cell composition at the time of harvest versus at a time after stimulation before transduction in a process for manufacturing anti-CD19 from donor human subjects, including an SLE subject. **FIG. 6B** depicts viability of cells of an engineered cell composition at the time of harvest in a process for manufacturing anti-CD19 from donor human subjects, including an SLE subject.

Detailed Description

[0132] Provided herein are methods and uses of engineered cells (*e.g.*, T cells) and/or compositions thereof, for the treatment of subjects having a disease or condition, which generally is or includes severe or moderate systemic autoimmune diseases. In embodiments of the provided methods, the therapeutic T cell compositions containing the engineered cells are administered to a subject having a severe or moderate systemic autoimmune disease, *e.g.*, via adoptive cell therapy, such as adoptive T cell therapy. In some aspects, the disease or condition is systemic autoimmune disease. In some aspects, the disease or condition is severe or moderate systemic autoimmune disease. Systemic autoimmune diseases are a class of aberrant immune disorders that share similar clinical manifestations and generally are treatable by similar approaches. In addition to Systemic Lupus Erythematosus (SLE), other systemic autoimmune diseases include, for example, Sjogren's syndrome, progressive systemic sclerosis (*i.e.*, scleroderma), idiopathic inflammatory myositis (IIM, including dermatomyositis, polymyositis and necrotizing myositis), mixed connective tissue disorder (MCTD), relapsing-remitting multiple sclerosis, ANCA-associated vasculitis (AAV), Crohn's disease, myasthenia gravis,

Behçet's, rheumatoid arthritis, multiple sclerosis (MS), IgA nephropathy, pemphigus vulgaris, myasthenia gravis, autoimmune hemolytic anemia, immune thrombocytopenia, IgG4-related diseases, membranous nephropathy, cutaneous lupus erythematosus, sarcoidosis, light chain amyloidosis, rheumatoid arthritis, bullous pemphigoid and chronic inflammatory demyelinating polyneuropathy. In particular embodiments of any of the provided methods and uses, the T cells are engineered with a chimeric antigen receptor (CAR) that is directed against cluster of differentiation 19 (CD19).

[0133] In some embodiments, the methods and uses include administering to the subject T cells expressing genetically engineered (recombinant) cell surface receptors in adoptive cell therapy, which generally are chimeric receptors such as chimeric antigen receptors (CARs), recognizing CD19. In some embodiments, CD19 is expressed by cells (*e.g.*, B cells) that play a role in the manifestation of the systemic autoimmune disease. In some embodiments, CD19 is expressed by cells, associated with and/or specific to the manifestation of SLE, IIM, SSc, AAV, systemic sclerosis, highly active relapsing remitting multiple sclerosis (MS), primary progressive MS, IgA nephropathy, pemphigus vulgaris, myasthenia gravis, demyelinating polyradiculoneuropathy, autoimmune hemolytic anemia, immune thrombocytopenia, IgG4-related diseases, membranous nephropathy, Primary Sjorgren's Syndrom, cutaneous lupus erythematosus, sarcoidosis, light chain amyloidosis, rheumatoid arthritis, bullous pemphigoid, acute respiratory distress syndrome, atopic eczema, hereditary angioedema, hidradenitis suppurative, inclusion-body myositis, inflammatory bowel disease, mastocytosis, multifocal motor neuropathy, necrotizing myopathy, neuromyelitis optica spectrum disorder, mixed connective tissue disorder, POEMS syndrome, primary biliary cholangitis, psoriasis, rhesus hemolytic disease, Still's disease, type 1 diabetes, urticaria, capillary leakage syndrome, cytokine release syndrome, erythema multiforme, pyoderma gangrenosum, antiphospholipid syndrome, or x-linked agammaglobulinemia. In some embodiments, CD19 is expressed by cells, associated with and/or specific to the manifestation of SLE, IIM, AAV, systemic sclerosis, highly active relapsing remitting multiple sclerosis (MS), primary progressive MS, IgA nephropathy, pemphigus vulgaris, or myasthenia gravis.

[0134] In particular embodiments, CD19 is expressed by cells, associated with and/or specific to the manifestation of SLE, such as severe refractory SLE. In particular embodiments, CD19 is expressed by cells, associated with and/or specific to the manifestation of idiopathic inflammatory myopathies (IIM). In particular embodiments, CD19 is expressed by cells,

associated with and/or specific to the manifestation of systemic sclerosis (SSc). In particular embodiments, CD19 is expressed by cells, associated with and/or specific to the manifestation of multiple sclerosis (MS). In particular embodiments, CD19 is expressed by cells, associated with and/or specific to the manifestation of rheumatoid arthritis (RA). In particular embodiments, CD19 is expressed by cells, associated with and/or specific to the manifestation of active secondary progressive MS (aSPMS). In particular embodiments, CD19 is expressed by cells, associated with and/or specific to the manifestation of Myositis. In particular embodiments, CD19 is expressed by cells, associated with and/or specific to the manifestation of myasthenia gravis. In particular embodiments, CD19 is expressed by cells, associated with and/or specific to the manifestation of bullous pemphigoid. In particular embodiments, CD19 is expressed by cells, associated with and/or specific to the manifestation of immune thrombocytopenia. In particular embodiments, CD19 is expressed by cells, associated with and/or specific to the manifestation of autoimmune hemolytic anemia. In particular embodiments, CD19 is expressed by cells, associated with and/or specific to the manifestation of pemphigus vulgaris. In particular embodiments, CD19 is expressed by cells, associated with and/or specific to the manifestation of demyelinating polyradiculoneuropathy. In particular embodiments, CD19 is expressed by cells, associated with and/or specific to the manifestation of membranous nephropathy.

[0135] In some embodiments, the systemic autoimmune disease is SLE, IIM, MS, or SSc. In some aspects, the disease or condition is moderate SLE. In some aspects, the disease or condition is severe refractory SLE. In particular, provided herein are methods and uses of engineered cells, (*e.g.*, T cells) and/or compositions thereof, for the treatment of subjects having severe refractory SLE. In embodiments of the provided methods, the therapeutic T cell compositions containing the engineered cells are administered to a subject having severe refractory SLE, *e.g.*, via adoptive cell therapy, such as adoptive T cell therapy. In particular embodiments of such methods and uses, the T cells are engineered with a chimeric antigen receptor (CAR) that is directed against cluster of differentiation 19 (CD19).

[0136] In some aspects, the methods and uses provide for or achieve improved response and/or more durable responses or efficacy and/or a reduced risk of toxicity or other side effects, *e.g.*, in particular groups of subjects treated, as compared to certain alternative methods. In some embodiments, the methods are advantageous by virtue of the administration of specified numbers or relative numbers of the engineered cells, the administration of defined ratios of particular types of the cells, the administration of cells of a particular high percentage of less

differentiated cells (*e.g.*, naïve-like or central memory cells or cells of an early differentiation state, such as CCR7+CD27+ cells), treatment of particular patient populations, such as those having a particular risk profile, staging, and/or prior treatment history, and/or combinations thereof.

[0137] The genetically engineered T cells are generally administered in a composition formulated for administration; the methods generally involve administering one or more doses of the cells to the subject, which dose(s) may include a particular number or relative number of cells or of the engineered cells. In some cases, the CD19-directed CAR+ engineered cells in the composition include a defined ratio or compositions of two or more sub-types within the composition, such as CD4 vs. CD8 T cells.

[0138] In particular embodiments, the compositions of cells for use or administration in the provided methods include primary T cells engineered to express a CD19-directed CAR that (i) contain a low percentage (*e.g.*, less than 40%, less than 30%, less than 20%, or less than 10%) of exhausted cells and/or cells that display markers or phenotypes associated with exhaustion; and/or (ii) contain a relatively high percentage (*e.g.*, greater than 50%, greater than 60%, greater than 70%, greater than 80% or greater than 90%) of memory-like T cells, such as naïve-like T cells, central memory T cells or long-lived memory T cells.

[0139] In provided embodiments, the features of the compositions and provided methods result in improved or enhanced immune activity compared to methods involving administration other CD19-directed CAR T cell therapies that contain a higher percentage of exhausted cells and/or a higher number of cells that display phenotypes associated with exhaustion and/or that contain a lower percentage of certain T cells, such as naïve-like T cells, central memory T cells or long-lived memory T cells. In provided embodiments, the features of the compositions and provided methods result in improved therapeutic efficacy, *e.g.*, increased percentage of patients achieving a complete response (CR), compared to methods involving administration of other CD19-directed CAR T cell therapies that contain a higher percentage of exhausted cells and/or a higher number of cells that display phenotypes associated with exhaustion and/or that contain lower percentage of certain T cells, such as naïve-like T cells, central memory T cells or long-lived memory T cells. In provided methods, the features of the compositions and provided methods result in improved clinical durability of therapeutic response, such as CR, *e.g.*, response that persists after a period of time from initiation of therapy, compared to methods involving administration of other CD19-directed CAR T cell therapies that contain a higher percentage of

exhausted cells and/or a higher number of cells that display phenotypes associated with exhaustion and/or that contain a lower percentage of memory-like T cells, such as naïve-like T cells, central memory T cells or long-lived memory T cells.

[0140] In particular embodiments, the use or administration of the provided CD19-directed CAR T cell compositions in the provided methods can be achieved with doses of cells that are more than 2-fold lower, such as 5-fold or 10-fold, lower than doses of reference CD19-directed CAR T cell compositions (*e.g.*, engineered with the same or similar CAR, such as with the same antigen-binding domain) but in which the reference CD19-directed CAR T cell composition contains a higher percentage of exhausted cells and/or a higher number of cells that display phenotypes associated with exhaustion and/or that contains a lower percentage of memory-like T cells, such as naïve-like T cells, central memory T cells or long-lived memory T cells. In some embodiments, the reference CD19-directed CAR T cell composition is a composition that is produced *ex vivo* by processes that involve steps of cultivating the cells under conditions for expansion, such as resulting in proliferation of cells or population doubling of cells (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more doublings of cells in the population compared to the start of the process) during the process for producing the cells.

[0141] In some embodiments, the CD19-directed CAR T cell compositions for use in the provided methods and uses are produced by a relatively short process that do not include a step for cultivating the cells under conditions for expansion designed for expanding or proliferating the cells. Different processes are available for generating compositions containing genetically engineered T cell populations, including for generating engineered T cells that express a CAR, which typically include a step designed for or for the purpose of cultivating the cells to expand or increase proliferation of the cells. However, in particular aspects, some of these processes may require a long or a relatively long amount of time to generate the engineered cells. In addition, in various aspects, some existing processes may vary in the amount of time required to successfully produce engineered T cells suitable for cell therapy, making it difficult to coordinate that administration of the cell therapy. In certain aspects, some of these processes may produce populations of cells that include a relatively high percentage or amount of exhausted cells, differentiated cells, or cells with a low potency. The provided CD19-directed CAR T cell compositions for use in the provided methods address one or more of these problems.

[0142] In particular embodiments, the provided methods are used in connection with a process for efficiently producing or generating engineered cells that are suitable for use in a cell therapy. In some embodiments, provided compositions containing CD19-directed CAR engineered T cells are produced by a process without the need for any additional steps for expanding the cells, *e.g.*, without an expansion unit operation and/or without steps intended to cause expansion of cells. In aspects of processes for producing CD19-directed CAR T cell composition, the processes include one or more steps for stimulating and genetically engineering (*e.g.*, transforming, transducing or transfecting) T cells to produce a population of engineered T cells that may be collected or formulated for use as a composition for cell therapy. In particular embodiments, the processes include a step of transducing cells with a viral vector (*e.g.*, lentiviral vector) that contains a nucleic acid encoding the CD19-directed CAR. In some aspects, the provided processes result in the stable integration of the heterologous nucleic acid (expressed from the viral vector) into the genome of the cells. In some aspects, the provided processes generate engineered CD19-directed CAR T cells with enhanced potency as compared to engineered T cell compositions produced from alternative processes, such as those that involve expanding the cells.

[0143] In particular aspects, the durations of the processes for producing the provided compositions can be measured from when cells, *e.g.*, T cells of an input cell population or input composition, are first contacted or exposed to stimulating conditions (*e.g.*, as described herein such as in Section II-C), referred to herein as the initiation of the stimulation or stimulating and also referred to herein as the exposing to the stimulatory reagent, *e.g.*, as in when the exposing to the stimulatory reagent is initiated. In some embodiments, the duration of time required to harvest or collect an output population (also referred to herein as an output composition or as a composition of engineered cells, *e.g.*, engineered T cells) containing engineered cells is measured from initiation of the stimulation. In particular embodiments, the duration of the process is, is about, or is less than 120 hours, 108 hours, 96 hours, 84 hours, 72 hours, 60 hours, 48 hours, 36 hours, or 30 hours. In particular embodiments, the duration of the process is, is about, or is less than 5 days, 4 days, 3 days, 2 days, or one day. In particular embodiments, the engineered cells, *e.g.*, the cells of the output composition or population, are more potent, persistent or naïve-like than cells that are engineered with processes that require longer amounts of time. In some aspects, the duration, *e.g.*, the amount of time required to generate or produce an engineered population of T cells, of the provided processes are shorter than those of some

existing processes by, by about, or by at least 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, or more than 7 days. In some embodiments, the duration of the provided process is, is about, or is less than 75%, 60%, 50%, 40%, 30%, 25%, 15%, or 10% of alternative or existing processes.

[0144] In certain embodiments, the provided processes are performed on a population of cells, *e.g.*, CD3+, CD4+, and/or CD8+ T cells, that are isolated, enriched, or selected from a biological sample. In some aspects, the provided methods can produce or generate a composition of engineered T cells from when a biological sample is collected from a subject within a shortened amount of time as compared to other methods or processes. In some embodiments, the provided methods can produce or generate engineered T cells, including any or all times where biological samples, or enriched, isolated, or selected cells are cryopreserved and stored, within or within about 10 days, 9 days, 8 days, 7 days, 6 days, 5 days, 4 days, 3 days, or 2 days, or within or within about 120 hours, 96 hours, 72 hours, or 48 hours, from when a biological sample is collected from a subject to when the engineered T cells are collected, harvested, or formulated (*e.g.*, for cryopreservation or administration).

[0145] In particular embodiments, the processes for producing or engineering T cell populations include a step of stimulating the cells, such as prior to transduction with a viral vector. In aspects of the provided processes, stimulation is carried out with an oligomeric stimulatory reagent, such as a streptavidin mutein oligomer, to which is immobilized or attached a stimulatory binding agent(s), *e.g.*, anti-CD3/anti-CD28. Existing reagents for use in stimulating T cells *in vitro*, such as in the absence of exogenous growth factors or low amounts of exogenous growth factors, are known (see *e.g.*, US Patent 6,352,694 B1 and European Patent EP 0 700 430 B1). In general, such reagents may employ beads, *e.g.*, magnetic beads, of greater than 1 μm in diameter to which various binding agents (*e.g.*, anti-CD3 antibody and/or anti-CD28 antibody) are immobilized. However, in some cases, such magnetic beads are, for example, difficult to integrate into methods for stimulating cells under conditions required for clinical trials or therapeutic purposes since it has to be made sure that these magnetic beads are completely removed before administering the expanded T cells to a subject. In some aspects, such removal, such as by exposing the cells to a magnetic field, may decrease the yield of viable cells available for the cell therapy. In certain cases, such reagents, *e.g.*, stimulatory reagents containing magnetic beads, must be incubated with the cells for a minimal amount of time to allow a sufficient amount of detachment of the T cells from the stimulatory reagent.

[0146] The provided processes utilizing oligomeric stimulatory reagents, *e.g.*, streptavidin mutein polymer, overcome such potential limitations. For example, in some embodiments, the provided processes avoid or reduce risk of residual stimulatory reagent, *e.g.*, reagents containing magnetic beads, in the output cells generated or produced by the processes. In some embodiments, this also means that a process that is compliant with GMP standards can be more easily established compared to other methods, such as those where additional measures have to be taken to ensure that the final engineered T cell population is free of beads. In some embodiments, this may be readily accomplished in the present embodiments by the addition of a substance, *e.g.*, a competition reagent, that dissociates the oligomeric stimulatory reagents from the cells, *e.g.*, by simply rinsing or washing the cells, *e.g.*, by centrifugation. Thus, in some aspects, removal or separation of oligomeric stimulatory reagent from cells, such as by the addition of a substance or competition reagent, results in little or no cell loss as compared to removal or separation of bead based stimulatory reagents. In some aspects, the timing of the oligomeric stimulatory reagent removal or separation is not limited or is less limited than the removal or separation of bead based stimulatory reagents. Thus, in some aspects, the oligomeric stimulatory reagent may be removed or separated from the cells at any time or stage during the provided processes.

[0147] In some aspects, the use of oligomeric stimulatory reagents (*e.g.*, anti-CD3/anti-CD28 streptavidin mutein oligomers) can result in an overall reduced stimulatory signal compared to alternative stimulatory reagents, such as anti-CD3/anti-CD28 paramagnetic beads. The provided process, which can involve a weaker or reduced stimulation, can generate engineered CAR+ T cells that are as, or even more, potent, persistent, or efficacious as CAR+ T cells generated by processes that involve stronger stimulatory conditions or higher amounts or concentrations of stimulatory reagent, such as may occur following stimulation with anti-CD3/anti-CD28 paramagnetic beads. In addition, in some embodiments, stimulating cells with a lower amount or relatively low amount of oligomeric stimulatory reagents may increase the potency, efficacy, or persistency of the resulting engineered cell population, as compared to processes using higher amounts of oligomeric stimulatory reagent. Such embodiments contemplate that such effects may persist even at doses sufficiently low enough to reduce the expression of activation markers or the portion of cells positive for the activation markers during and after the process.

[0148] In certain embodiments, the engineered T cells, *e.g.*, output composition or populations of T cells containing T cells expressing a recombinant receptor, such as a chimeric antigen receptor, produced or generated by the provided processes are particularly effective or potent when utilized as cells for a cell therapy. For example, in some aspects, an output composition containing engineered T cells, *e.g.*, CAR+ T cells, that are generated from the provided processes have a much higher degree of potency and/or proliferative capacity than engineered T cells generated or produced by alternative existing processes. In some aspects, an output composition containing engineered T cells, *e.g.*, CAR+ T cells, produced by the provided processes have enhanced immune activity than engineered T cells, *e.g.*, CAR+ T cells, produced by alternative or existing methods.

[0149] In particular embodiments, the processes for producing the provided CD19-directed T cell compositions that do not contain steps where the cells are expanded to a threshold amount or concentration have further advantages. In some aspects, protocols that do not rely on expanding the cells to increase the number or concentration of cells from a starting cell population, *e.g.*, an input population, do not require incubations or cultivations that may vary between cell populations. For example, some embodiments contemplate that cell populations obtained from different subjects, such as subjects having different diseases or disease subtypes, particularly as is the case for patients with SLE, including high-risk, aggressive and/or severe refractory SLE, may divide or expand at different rates. In certain aspects, eliminating potentially variable steps requiring cell expansion allows for the duration of the whole process to be tightly controlled. In certain embodiments, the variability of the process duration is reduced or eliminated which may, in some aspects, allow for improved coordination for appointments and treatment between doctors, patients, and technicians to facilitate autologous cell therapies.

[0150] In some embodiments, the provided methods involve treating a specific group or subset of subjects, *e.g.*, subjects identified as having high-risk disease, *e.g.*, systemic autoimmune disease, such as severe systemic autoimmune disease. In some embodiments, subjects to be treated for the systemic autoimmune disease, such as any described herein, have relapsed or are refractory (R/R) to standard therapy for treating the systemic autoimmune disease and/or have a poor prognosis. In some aspects, the methods treat subjects having a severe disease that has relapsed or is refractory (R/R) to standard therapy. In some embodiments, the provided methods involve treating a specific group or subset of subjects, *e.g.*, subjects identified as having high-risk disease, *e.g.*, SLE, such as severe refractory SLE. In

some aspects, the methods treat subjects having a form of aggressive and/or poor prognosis SLE such as SLE that has relapsed or is refractory (R/R) to standard therapy and/or has a poor prognosis. In some aspects, the methods treat subjects having a severe SLE that has relapsed or is refractory (R/R) to standard therapy.

[0151] In particular aspects, the engineered cells are autologous to the subject and are administered following generation by *ex vivo* processes that are shortened compared to existing methods, that do not include or involve a cultivation step for expanding the cells during the methods of producing the engineered cells, and/or that are able to produce a CAR-engineered T cell composition that is less differentiated permitting administration of lower doses. As a result, the provided methods are advantageous compared to existing methods because they can shorten the time until the engineered T cell therapy is available to the patient, particularly among patients who are in need of treatment, such as subjects that have relapsed to or are refractory to treatment following one or more other prior therapies for treating the disease or condition. In some aspects, the provided methods, compositions, uses and articles of manufacture achieve improved and superior responses to available therapies. In some embodiments, the improved or superior responses are to current standard of care (SOC).

[0152] CD19 is a member of the immunoglobulin superfamily and a component of the B-cell surface signal transduction complex that positively regulates signal transduction through the B-cell receptor. It is expressed by most B-cell malignancies from early development until differentiation into plasma cells (Stamenkovic et al., J Exp Med. 1988; 168(3):1205-10). CD19 is an attractive therapeutic target as CAR-T therapy has unique potential to provide transformational treatment for severe refractory lupus and other related conditions. CD19 CAR T cell therapy offers transformational efficacy and favorable safety profile in severe SLE.

[0153] In particular embodiments, the methods provided herein are based on administration of a CD19-directed CAR T cell therapy in which the CAR contains a CD19-directed scFv antigen binding domain (*e.g.*, from FMC63). The CAR further contains an intracellular signaling domain containing a signaling domain from CD3zeta, and also incorporates a 4-1BB costimulatory domain, which has been associated with lower incidence of cytokine release syndrome (CRS) and neurotoxicity (NE) compared with CD28-containing constructs (Lu et al. J Clin Oncol. 2018;36:3041).

[0154] The provided methods are based on findings that a lower differentiation state of adoptively transferred T cells can influence the ability of these cells to persist and promote

durable immune activity. In some embodiments, the provided CD19-directed CAR+ engineered T cell compositions are produced by a method in which the cells are not cultivated under conditions of expansion, thereby limiting or reducing the number of population doublings of the final engineered output composition and resulting in a less differentiated product. Yet, the provided compositions also are produced via processes that result in stably integrated vector copy number (iVCN) to ensure consistent and reliable expression of the CAR, thereby resulting in a consistent cell product for administration to subjects and low variability among CAR-expressing cells in administered doses. In contrast, most protocols for T cell engineering routinely expand T cells *ex vivo* for 9 to 14 days or more. Provided data exemplified herein support a model in which CAR T cell products with an increased composition of less differentiated memory T cells may exhibit enhanced durable immune activity. These findings reveal that strategies aimed at minimizing effector differentiation in CAR T cell products could result in improved clinical efficacy. Provided herein are embodiments that can meet such aims.

[0155] In particular, results herein demonstrate the advantageous effect that CD19-directed CAR T cells are able to induce an immune reset following targeted cytotoxic killing of CD19-expressing B cells. In some embodiments, as demonstrated in Example 2 in the context of relapsed or refractory (R/R) non-Hodgkins lymphoma (NHL), compositions comprising the anti-CD19 CAR T cells are able to suppress B cell overactivation, resulting in an immune reset and the resoration of homeostatic immune system function. These results thus support use of CD19-directed CAR-expressing T cells to achieve the same effect to reset the immune system in autoimmune diseases by removal of the overactive B cells and to allow for reducing autoimmune disease activity and achieving clinical remission. Although other treatments such as use of HSCT or antibody therapies against B cell surface proteins have sought to deplete B cells or reset the immune system (e.g., Tyndall et al. *Ann Rheum Dis* 2001, 60:702-707; Sullivan et al. *N Engl J Med* 2018, 378:35-47; Wise and Stohl, *Front. Med.*, 2020, &:303), none have been successful to efficiently decrease circulating B cells for reducing disease activity as observed herein by cytotoxic activity of CD19-directed CAR-expressing T cells and/or to do so while also minimizing toxicity to the subject from the therapy.

[0156] In some embodiments, results herein surprisingly demonstrate a reduction in disease activity in subjects with autoimmune or an inflammatory disease, as shown by results of subjects treated that have SLE. The reduction in disease activity was observed with a relatively low dose of CD19-directed CAR T cells of only 10×10^6 viable CAR+ T cells (including CD4+ and

CD8+ CAR+ T cells). This dose is orders of magnitude lower than doses administered for other CD19-directed CAR T cell products. Moreover, the doses for administration herein are generally administered as flat doses (not weight-based doses based on body weight of the subject), which has the added benefit of improving consistency of dosing and reducing risk of toxic side effects that may result from weight-based dosing strategies as a result of administering too many cells in some subjects. Results herein show no severe toxicity was observed demonstrating safety of the provided T cell therapy. Notably, while administering relatively low doses of cells (*e.g.*, CAR-expressing T cells) may decrease the risk of toxic adverse events, relatively low doses of cells manufactured from other methods may not be completely effective for the treatment of a disease or condition. The ability to deliver a CAR-T cell product at a low dose while retaining high disease efficacy is a unique advantage of the provided methods and compositions.

[0157] The provided embodiments also support the successful ability to treat subjects without any further immunosuppression. Typically, successful treatment of autoimmune indications generally requires continued immunosuppression. Yet, as described herein, increased hospitalizations and side effects of medications, such as chronic oral corticosteroids (OCS or glucocorticoids and other immunosuppressive treatments), can add to disease burden in subjects with autoimmune indications, such as SLE. The results herein support that remission of disease activity is possible by a single infusion of a dose of CD19-CAR directed T cells without further administration of an immunosuppressive agent (*e.g.*, corticosteroid such as a glucocorticoid or other immunosuppressive treatment) after the administration of the dose of T cells. In some embodiments, subjects achieve prolonged remission by treatment in accord with the provided methods. In some embodiments, a further treatment for the disease is not necessary and the subject remains in remission following the dose of CD19-CAR directed T cells. For instance, in provided embodiments, after administering the CD19-CAR directed T cells the subject remains in remission and is not administered another treatment (*e.g.*, methotrexate, mycophenolate, cyclophosphamide, tocilizumab, IVIg, rituximab, nintedanib or immunosuppressants).

[0158] The observations herein support treating subjects with high-risk disease with a CD19-directed CAR T cell therapy in accordance with the provided methods. For example, subjects with systemic autoimmune diseases, such as severe or moderate systemic autoimmune diseases are treated by provided methods. In some embodiments, subjects with SLE, including patients with severe SLE or certain high-risk features, such as those with relapsed/refractory

(R/R) severe SLE, can be treated in accordance with the provided methods. In some embodiments, the provided methods can be used to treat subjects that have been heavily pretreated (*e.g.*, with one, two, three, four, or more prior therapies for treating the disease). Any references to methods for treatment of the human or animal body by surgery or therapy herein refer to compounds, compositions, or medicaments for use in said methods.

[0159] All publications, including patent documents, scientific articles, and databases, referred to in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication were individually incorporated by reference. If a definition set forth herein is contrary to or otherwise inconsistent with a definition set forth in the patents, applications, published applications and other publications that are herein incorporated by reference, the definition set forth herein prevails over the definition that is incorporated herein by reference.

[0160] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

I. METHODS AND USES OF CD19-TARGETED CELL THERAPY IN SYSTEMIC AUTOIMMUNE DISEASES

[0161] Provided herein are methods of treatment that involve administering engineered cells or compositions containing engineered cells, such as engineered T cells. In some embodiments, provided herein are methods and use of CD19-directed CAR engineered cells (*e.g.*, T cells) and/or compositions thereof, including methods for the treatment of subjects with systemic autoimmune diseases, including severe or moderate systemic autoimmune diseases that have failed at least two or more prior therapies. In particular embodiments, the method includes administering to the subject a dose of T cells that includes CD4+ and CD8+ T cells, wherein the T cells comprises a chimeric antigen receptor (CAR) that specifically binds to CD19. In particular embodiments, the method includes administering to the subject a dose of T cells that includes CD4+ and CD8+ T cells, wherein the T cells comprises a chimeric antigen receptor (CAR) that specifically binds to CD19.

[0162] In some embodiments, the immune disease include, but are not limited to, Addison's disease, allergies, ankylosing spondylitis, asthma, atherosclerosis, autoimmune diseases of the ear, autoimmune diseases of the eye, autoimmune hepatitis, autoimmune parotitis, colitis, coronary heart disease, diabetes, including Type 1 and/or Type 2 diabetes, epididymitis,

glomerulonephritis, Graves' disease, Guillain-Barre syndrome, Hashimoto's disease, hemolytic anemia, idiopathic thrombocytopenic purpura, inflammatory bowel disease, immune response to recombinant drug products, myasthenia gravis, pemphigus, psoriasis, rheumatic fever, rheumatoid arthritis, sarcoidosis, scleroderma, spondyloarthropathies, thyroiditis, transplant rejection, vasculitis, AIDS, atopic allergy, bronchial asthma, eczema, leprosy, schizophrenia, chronic fatigue syndrome, Alzheimer's disease, Parkinson's disease, myocardial infarction, stroke, autism, epilepsy, Arthus's phenomenon, and anaphylaxis. In some embodiments, the systemic autoimmune diseases include, but are not limited to, systemic lupus erythematosus (SLE) and severe SLE, rheumatoid arthritis (RA), and systemic sclerosis. In some embodiments, the systemic autoimmune diseases may include Systemic Lupus Erythematosus (SLE), Sjogren's' syndrome, progressive systemic sclerosis (i.e., scleroderma), idiopathic inflammatory myositis (IIM, including dermatomyositis, polymyositis and necrotizing myositis), mixed connective tissue disorder (MCTD), relapsing-remitting multiple sclerosis, ANCA-associated vasculitis (AAV), Crohn's disease, myasthenia gravis, Behçet's, rheumatoid arthritis, primary progressive MS, IgA nephropathy, pemphigus vulgaris, myasthenia gravis, autoimmune hemolytic anemia, immune thrombocytopenia, IgG4-related diseases, membranous nephropathy, cutaneous lupus erythematosus, sarcoidosis, light chain amyloidosis, acute respiratory distress syndrome, atopic eczema, hereditary angioedema, hidradenitis suppurative, inclusion-body myositis, inflammatory bowel disease, mastocytosis, multifocal motor neuropathy, necrotizing myopathy, neuromyelitis optica spectrum disorder, mixed connective tissue disorder, POEMS syndrome, primary biliary cholangitis, psoriasis, rhesus hemolytic disease, Still's disease, type 1 diabetes, urticaria, capillary leakage syndrome, cytokine release syndrome, erythema multiforme, pyoderma gangrenosum, x-linked agammaglobulinemia, antiphospholipid syndrome, and chronic inflammatory demyelinating polyneuropathy (also called chronic inflammatory demyelinating polyradiculoneuropathy).

[0163] In some embodiments, the systemic autoimmune disease is SLE, such as a moderate SLE or severe refractory SLE, idiopathic inflammatory myopathy, systemic sclerosis, rheumatoid arthritis (RA) or multiple sclerosis. In some embodiments, the systemic autoimmune disease is SLE, such as a moderate SLE or severe refractory SLE, idiopathic inflammatory myopathy, systemic sclerosis, or multiple sclerosis. Among provided methods are methods of treatment that involve administering engineered cells or compositions containing engineered cells, such as engineered T cells to subjects with SLE, including severe refractory SLE. Also

provided are methods and uses of provided CD19-directed CAR engineered cells (*e.g.*, T cells) and/or compositions thereof, including methods for the treatment of subjects having a SLE, including severe refractory SLE, that involves administration of the engineered cells and/or compositions thereof. In certain embodiments, the subject has severe refractory SLE. In some embodiments, the subject is selected for or identified as having severe refractory SLE, such as by the presence of certain features or clinical manifestations that indicate the presence of severe refractory SLE. Exemplary selection criteria are further described herein. In some embodiments, the methods and use of provided CD19-directed CAR engineered cells (*e.g.*, T cells) and/or compositions thereof, include methods for the treatment of subjects with severe refractory SLE that have failed at least two or more prior therapies. In particular embodiments, the method includes administering to the subject a dose of T cells that includes CD4+ and CD8+ T cells, wherein the T cells comprises a chimeric antigen receptor (CAR) that specifically binds to CD19.

[0164] Also disclosed herein is a method of treating a systemic autoimmune disease, the method comprising administering to a subject having or suspected of having a severe or moderate systemic autoimmune disease, a composition comprising engineered T cells expressing a CAR that targets CD19, produced by a manufacturing process eliciting an output composition which exhibits a predetermined feature wherein iterations of the manufacturing process produce a plurality of the output compositions, optionally from human biological samples encompassing a plurality of different individual subjects, wherein the predetermined feature of the output composition among the plurality of output compositions is selected from the features of the composition disclosed in in Section II-C and Section III, in any combination, including the percentage of CD3+ cells, ratios of CD4+/CD8+ or CD4+CAR+/CD8+CAR+ cells, percentage of cells expressing an apoptosis marker, percentage of less differentiated cells, and iVCN and iVCN/VCN values.

[0165] In some embodiments, the methods and uses include administering to the subject cells expressing genetically engineered (recombinant) cell surface receptors in adoptive cell therapy, which generally are chimeric receptors such as chimeric antigen receptors (CARs), recognizing CD19 expressed by, associated with and/or specific to the cell type from which it is derived. The cells are generally administered in a composition formulated for administration. In some embodiments, cells are collected from the subject prior to treatment for the purpose of engineering the cells with the CD19-directed recombinant receptor (*e.g.*, CAR). In some

embodiments, the cells are collected by leukapheresis. In some embodiments, the cells have been collected by leukapheresis. In some aspects, the cells are engineered by *ex vivo* methods that do not involve cultivating the cells for expansion (hereinafter also called non-expanded process). Exemplary non-expanded processes for engineering the provided CAR-expressing therapeutic compositions are described in Section II-C.

[0166] In some embodiments, the subject has received one or more prior therapies, such as two or more prior therapies, for treating the autoimmune disease. In some embodiments, the subject has received 1 prior therapy for treating the systemic autoimmune disease. In some embodiments, the subject has received 2 prior therapies for treating the systemic autoimmune disease. In some embodiments, the subject has received 3 prior therapies for treating the systemic autoimmune disease.

[0167] In some embodiments, the systemic autoimmune disease is a refractory disease. In some embodiments, the refractory disease is characterized by an absence of response to one or more prior therapy, such as one or more standard therapy. In some embodiments, the refractory disease is characterized by an absence of a complete response to one or more prior therapies, such as to one or more standard therapy. In some embodiments, the subject is refractory to treatment with one or more prior therapy for treating the systemic autoimmune disease. In some embodiments, the subject is refractory to treatment with two or more prior therapies for treating the systemic autoimmune disease.

[0168] In some embodiments, the systemic autoimmune disease is a severe autoimmune disease. In some embodiments, the severe autoimmune disease is one in which the subject has achieved a response to a standard therapy, but the response is inadequate or partial. In some embodiments, the severe autoimmune disease is one in which a response in the subject is only achievable in the subject with a combination of standard therapy drugs.

[0169] In some embodiments, the one or more prior therapies, such as two or more prior therapies, is a standard therapy for treating the autoimmune disease. In some embodiments, the standard therapy is an anti-inflammatory drug, a steroid, such as a corticosteroid, a pain-killing medication (*e.g.*, paracetamol or codeine), or an immunosuppressant drug, or combinations thereof.

[0170] In some embodiments, the subject has not previously received CAR T cell therapy prior to administration of the CD19-directed engineered CAR T cells in accord with the provided methods. In some embodiments, the subject has not received genetically-modified T

cell therapy. In some embodiments, the subject has not received CD19-targeted therapy. Exemplary CD19-targeted therapies include, but are not limited to, anti-CD19 monoclonal antibodies or anti-CD19 bispecific antibodies. In some embodiments, the subject does not have hypersensitivity to fludarabine and/or cyclophosphamide.

[0171] In particular embodiments, prior to administration of the dose of CD19-directed engineered CAR T cells, the subject is administered or has received a lymphodepleting chemotherapy. Lymphodepletion may improve the engraftment and activity of CAR T cells through homeostatic cytokines, reduction of CD4+CD25+ regulatory T cells, increase of SDF-1 within bone marrow microenvironment, and stimulatory effects on antigen presenting cells (Grossman et al., *Nat Rev Immunol.* 2004; 4(5):387-395; Stachel et al., *Pediatr Blood Cancer* 2004; 43(6):644-50; Pinthus et al., *J Clin Invest* 2004; 114(12):1774-81; Turk et al., *J Exp Med* 2004; 200(6):771-82). In addition, LD chemotherapy may further lower the risk and severity of cytokine release syndrome (CRS).

[0172] Thus, in some embodiments, the methods include administering a preconditioning agent, such as a lymphodepleting or chemotherapeutic agent, such as cyclophosphamide, fludarabine, or combinations thereof, to a subject prior to the administration of engineered cells. For example, the subject may be administered a preconditioning agent at least 2 days prior, such as at least 3, 4, 5, 6, 7, 8, or 9 days prior, to the administration of engineered cells. In some embodiments, the subject is administered a preconditioning agent no more than 9 days prior, such as no more than 8, 7, 6, 5, 4, 3, or 2 days prior, to the administration of engineered cells.

[0173] In some embodiments, the subject is preconditioned with cyclophosphamide at a dose between or between about 20 mg/kg and 100 mg/kg body weight of the subject, such as between or between about 40 mg/kg and 80 mg/kg. In some aspects, the subject is preconditioned or administered with or with about 60 mg/kg of cyclophosphamide. In some embodiments, the cyclophosphamide can be administered in a single dose or can be administered in a plurality of doses, such as given daily, every other day or every three days. In some embodiments, the cyclophosphamide is administered once daily for one or two days. In some embodiments, where the lymphodepleting agent comprises cyclophosphamide, the subject is administered cyclophosphamide at a dose between or between about 100 mg/m² and 500 mg/m² body surface area of the subject, such as between or between about 200 mg/m² and 400 mg/m², or 250 mg/m² and 350 mg/m², inclusive. In some instances, the subject is administered about 100 mg/m² of cyclophosphamide. In some instances, the subject is administered about 150

mg/m² of cyclophosphamide. In some instances, the subject is administered about 200 mg/m² of cyclophosphamide. In some instances, the subject is administered about 250 mg/m² of cyclophosphamide. In some instances, the subject is administered about 300 mg/m² of cyclophosphamide. In some embodiments, the cyclophosphamide can be administered in a single dose or can be administered in a plurality of doses, such as given daily, every other day or every three days. In some embodiments, cyclophosphamide is administered daily, such as for 1-5 days, for example, for 3 to 5 days. In some instances, the subject is administered about 300 mg/m² body surface area of the subject, of cyclophosphamide, daily for 3 days, prior to initiation of the cell therapy. In some embodiments, the subject is administered a total of at or about 300 mg/m², 400 mg/m², 500 mg/m², 600 mg/m², 700 mg/m², 800 mg/m², 900 mg/m², 1000 mg/m², 1200 mg/m², 1500 mg/m², 1800 mg/m², 2000 mg/m², 2500 mg/m², 2700 mg/m², 3000 mg/m², 3300 mg/m², 3600 mg/m², 4000 mg/m² or 5000 mg/m² cyclophosphamide, or a range defined by any of the foregoing, prior to initiation of the cell therapy.

[0174] In some embodiments, where the lymphodepleting agent comprises fludarabine, the subject is administered fludarabine at a dose between at or about 1 mg/m² and at or 100 mg/m², such as between at or about 10 mg/m² and at or about 75 mg/m², at or about 15 mg/m² and at or about 50 mg/m², at or about 20 mg/m² and at or about 40 mg/m², at or about 24 mg/m² and at or about 35 mg/m², inclusive. In some instances, the subject is administered at or at or about 10 mg/m² of fludarabine. In some instances, the subject is administered at or about 15 mg/m² of fludarabine. In some instances, the subject is administered at or about 20 mg/m² of fludarabine. In some instances, the subject is administered at or about 25 mg/m² of fludarabine. In some instances, the subject is administered at or about 30 mg/m² of fludarabine. In some embodiments, the fludarabine can be administered in a single dose or can be administered in a plurality of doses, such as given daily, every other day or every three days. In some embodiments, fludarabine is administered daily, such as for 1-5 days, for example, for 3 to 5 days. In some instances, the subject is administered at or about 30 mg/m² body surface area of the subject, of fludarabine, daily for 3 days, prior to initiation of the cell therapy. In some embodiments, the subject is administered a total of at or about 10 mg/m², 20 mg/m², 25 mg/m², 30 mg/m², 40 mg/m², 50 mg/m², 60 mg/m², 70 mg/m², 80 mg/m², 90 mg/m², 100 mg/m², 120 mg/m², 150 mg/m², 180 mg/m², 200 mg/m², 250 mg/m², 270 mg/m², 300 mg/m², 330 mg/m², 360 mg/m², 400 mg/m² or 500 mg/m² cyclophosphamide, or a range defined by any of the foregoing, prior to initiation of the cell therapy.

[0175] In some embodiments, the lymphodepleting agent comprises a single agent, such as cyclophosphamide or fludarabine. In some embodiments, the subject is administered cyclophosphamide only, without fludarabine or other lymphodepleting agents. In some embodiments, prior to the administration, the subject has received a lymphodepleting therapy comprising the administration of cyclophosphamide at or about 200-400 mg/m² body surface area of the subject, optionally at or about 300 mg/m², daily, for 2-4 days. In some embodiments, the subject is administered fludarabine only, for example, without cyclophosphamide or other lymphodepleting agents. In some embodiments, prior to the administration, the subject has received a lymphodepleting therapy comprising the administration of fludarabine at or about 20-40 mg/m² body surface area of the subject, optionally at or about 30 mg/m², daily, for 2-4 days.

[0176] In some embodiments, the lymphodepleting agent comprises a combination of agents, such as a combination of cyclophosphamide and fludarabine. Thus, the combination of agents may include cyclophosphamide at any dose or administration schedule, such as those described above, and fludarabine at any dose or administration schedule, such as those described above. For example, in some aspects, the subject is administered at or about 60 mg/kg (~2 g/m²) of cyclophosphamide and 3 to 5 doses of 25 mg/m² fludarabine prior to the first or subsequent dose. In some aspects, the subject is administered fludarabine (30 mg/m²/day for 3 days) and cyclophosphamide (300 mg/m²/day for 3 days) (flu/cy) concurrently, intravenously, prior to administration of the cells. In some embodiments, the subject is administered a reduced, delayed or eliminated dose of one or more doses of the lymphodepleting agent(s).

[0177] In some embodiments, the subjects are premedicated, *e.g.*, to minimize the risk of infusion reaction. In some aspects, the premedication includes administering pain reliever and/or an antihistamine. In some embodiments, the premedication includes administering an acetaminophen and/or a diphenhydramine, or another H1-antihistamine. In some embodiments, the patient with acetaminophen (*e.g.*, 650 mg orally) and diphenhydramine (*e.g.*, 25-50 mg, IV or orally), or another H1-antihistamine, at or about 30 to 60 minutes prior to treatment with the cell therapy.

[0178] In embodiments of any of the provided methods, the subject is a human subject.

A. Exemplary Diseases

[0179] In some embodiment, the methods provided herein are used to treat autoimmune diseases caused by, associated with and/or specific to cells expressing CD19, such as, SLE, IIM,

SSc, AAV, systemic sclerosis, highly active relapsing remitting multiple sclerosis (MS), primary progressive MS, IgA nephropathy, pemphigus vulgaris, myasthenia gravis, demyelinating polyradiculoneuropathy, autoimmune hemolytic anemia, immune thrombocytopenia, IgG4-related diseases, membranous nephropathy, Primary Sjorgren's Syndrom, cutaneous lupus erythematosus, sarcoidosis, light chain amyloidosis, rheumatoid arthritis, bullous pemphigoid, acute respiratory distress syndrome, atopic eczema, hereditary angioedema, hidradenitis suppurative, inclusion-body myositis, inflammatory bowel disease, mastocytosis, multifocal motor neuropathy, necrotizing myopathy, neuromyelitis optica spectrum disorder, mixed connective tissue disorder, POEMS syndrome, primary biliary cholangitis, psoriasis, rhesus hemolytic disease, Still's disease, type 1 diabetes, urticaria, capillary leakage syndrome, cytokine release syndrome, erythema multiforme, pyoderma gangrenosum, antiphospholipid syndrome, or x-linked agammaglobulinemia.

[0180] In some embodiments, the methods provided herein are used to treat SLE, IIM, AAV, systemic sclerosis, highly active relapsing remitting multiple sclerosis (MS), primary progressive MS, IgA nephropathy, pemphigus vulgaris, or myasthenia gravis. In some embodiments, the methods provided herein are used to treat SLE. In some embodiments, the methods provided herein are used to treat IIM. In some embodiments, the methods provided herein are used to treat SSc. In some embodiments, the methods provided herein are used to treat MS.

[0181] In some embodiments, the methods provided herein are used to treat rheumatoid arthritis. In some embodiments, the systemic autoimmune disease is rheumatoid arthritis.

[0182] In some embodiments, the methods provided herein are used to treat myositis. In some embodiments, the systemic autoimmune disease is myositis.

[0183] In some embodiments, the methods provided herein are used to treat myasthenia gravis. In some embodiments, the systemic autoimmune disease is myasthenia gravis.

[0184] In some embodiments, the methods provided herein are used to treat bullous pemphigoid. In some embodiments, the systemic autoimmune disease is bullous pemphigoid.

[0185] In some embodiments, the methods provided herein are used to treat immune thrombocytopenia. In some embodiments, the systemic autoimmune disease is immune thrombocytopenia.

[0186] In some embodiments, the methods provided herein are used to treat autoimmune hemolytic anemia. In some embodiments, the systemic autoimmune disease is autoimmune hemolytic anemia.

[0187] In some embodiments, the methods provided herein are used to treat pemphigus vulgaris. In some embodiments, the systemic autoimmune disease is pemphigus vulgaris.

[0188] In some embodiments, the methods provided herein are used to treat demyelinating polyradiculoneuropathy. In some embodiments, the systemic autoimmune disease is demyelinating polyradiculoneuropathy.

[0189] In some embodiments, the methods provided herein are used to treat membranous nephropathy. In some embodiments, the systemic autoimmune disease is membranous nephropathy.

I. Systemic Lupus Erythematosus (SLE)

[0190] Systemic lupus erythematosus is a systemic autoimmune disease resulting from aberrant activity of the immune system, leading to variable clinical symptoms. SLE is characterized by production of autoantibodies directed against nuclear and cytoplasmic antigens, which may affect several different organs, with a plethora of different clinical and immunologic abnormalities, characterized by a relapsing and remitting clinical course. (Yu H, Nagafuchi Y, Fujio K. Clinical and Immunological Biomarkers for Systemic Lupus Erythematosus. Biomolecules. 2021 Jun 22;11(7):928.). SLE presents an array of clinical manifestations, including renal, dermatological, neuropsychiatric, and cardiovascular symptoms. The complexity, heterogeneity and variability of lupus historically led to a focus on the treatment of symptoms and not treatment of disease. The basis of the heterogeneity in lupus disease includes genetics, pathogenetic mechanisms (pathways, autoantibodies), demographics, ethnicity and race, and socioeconomic factors. Thus, leading to various challenges including prognosis, optimizing therapy, efficacy safety, clinical trial designs. (Bazzan M, Vaccarino A, Marletto F. Systemic lupus erythematosus and thrombosis. Thromb J. 2015 Apr 23;13:16. doi: 10.1186/s12959-015-0043-3.).

[0191] In some embodiments, the systemic autoimmune disease is SLE, such as a moderate SLE or severe refractory SLE. Among provided methods are methods of treatment that involve administering engineered cells or compositions comprising engineered cells, such as engineered T cells to subjects with SLE, including severe refractory SLE. Also provided are methods and

uses of provided CD19-directed CAR engineered cells (*e.g.*, T cells) and/or compositions thereof, including methods for the treatment of subjects having a SLE, including severe refractory SLE, that involves administration of the engineered cells and/or compositions thereof. In certain embodiments, the subject has severe refractory SLE. In some embodiments, the subject is selected for or identified as having severe refractory SLE, such as by the presence of certain features or clinical manifestations that indicate the presence of severe refractory SLE. Exemplary selection criteria are further described herein. In some embodiments, the methods and use of provided CD19-directed CAR engineered cells (*e.g.*, T cells) and/or compositions thereof, include methods for the treatment of subjects with severe refractory SLE that have failed at least two or more prior therapies. In particular embodiments, the method includes administering to the subject a dose of T cells that includes CD4+ and CD8+ T cells, wherein the T cells comprises a chimeric antigen receptor (CAR) that specifically binds to CD19.

[0192] Clinical features at the onset and during the evolution of severe SLE include, but are not limited to malar rash, arthritis, nephropathy, photosensitivity, thrombosis, sicca syndrome, serositis, nephropathy, neurologic involvement, oral ulcers, thrombocytopenia, lymphadenopathy, discoid lesions, livedo reticularis, thrombosis, myositis, hemolytic anemia, lung involvement, cutaneous lesions and chorea. (Cervera R, et al. Systemic lupus erythematosus: clinical and immunologic patterns of disease expression in a cohort of 1,000 patients. The European Working Party on Systemic Lupus Erythematosus. Medicine (Baltimore). 1993 Mar;72(2):113-24. PMID: 8479324). These disease manifestations cause a significant burden of illness and can lead to reduced physical function, loss of employment, lower health-related quality of life (QoL) and a lifespan shortened by 10 years. Increased hospitalizations and side effects of medications including chronic oral corticosteroids (OCS or glucocorticoids and other immunosuppressive treatments) add to disease burden in SLE.

[0193] In some cases, subjects develop lupus nephritis. Lupus nephritis (LN) one of a number of proteinuric kidney diseases wherein an inflammation of the kidneys is caused by systemic lupus erythematosus (SLE) whereby up to 60% of SLE patients develop LN. LN is a debilitating and costly disease often leading to renal failure which requires dialysis, or renal transplant and often results in death. Indeed, patients with renal failure have an over 60-fold increased risk of premature death compared to SLE patients in general. A clinical sign of LN is leakage of blood proteins into the urine and the disease can be diagnosed by a number of factors, including urinary protein/creatinine ratio (UPCR) wherein a UPCR of greater than 0.5 mg/mg is

indicative of the condition being in an active state. Further, certain markers in the blood can also be diagnostic—for example, complement 3 (C3), complement 4 (C4) and anti-dsDNA antibodies.

[0194] Treatment of SLE is challenging because of the limited efficacy and poor tolerability of standard therapy. All of the therapies currently used for the treatment of SLE have well known adverse effect profiles and there is a medical need to identify new targeted therapies, particularly agents that may reduce the requirement for corticosteroids and non-specific cytotoxic agents.

[0195] There has been only 1 new treatment (belimumab) for SLE approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) in the approximately 50 years since hydroxychloroquine was approved for use in discoid lupus and SLE. However, belimumab is not approved everywhere, and the uptake has been modest. Many agents currently used to treat SLE, such as azathioprine, cyclophosphamide, and mycophenolate mofetil (MMF)/mycophenolic acid, have not been approved for the disease. Furthermore, these drugs all have well-documented safety issues, and are not effective in all patients, for all manifestations of lupus. Antimalarial agents (*e.g.*, hydroxychloroquine) and corticosteroids may be used to control arthralgia, arthritis, and rashes. Other treatments include nonsteroidal anti-inflammatory drugs (NSAIDs); analgesics for fever, arthralgia, and arthritis; and topical sunscreens to minimize photosensitivity. It is often difficult to taper subjects with moderate or severe disease completely off OCS, which cause long-term morbidity and may contribute to early cardiovascular mortality. Even small daily doses of 5 to 10 mg prednisone used long-term carry increased risks of side effects such as cataracts, osteoporosis, and coronary artery disease.

[0196] Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used for the symptomatic management of arthralgia, mild arthritis, myalgia, serositis and fever in patients with SLE. They do not have any immunosuppressive properties. NSAIDs can only be used for short periods of time and are not suitable for patients with renal involvement, hypertension and established heart disease. NSAIDs can cause fluid retention, renal impairment and interstitial nephritis.

[0197] Mycophenolate mofetil (MMF) is a specific inhibitor of inosine monophosphate dehydrogenase. MMF impairs de novo purine synthesis. Inosine monophosphate dehydrogenase is an essential pathway in activated lymphocytes. MMF thus inhibits both T and B lymphocyte proliferation and reduces antibody synthesis.

[0198] In some cases, Rituximab has been used for treatment subjects with SLE, particularly lupus nephritis. Rituximab is a chimeric anti-CD20 monoclonal antibody. Rituximab is an effective treatment in a number of autoimmune disease, including rheumatoid arthritis and ANCA vasculitis. A small number of uncontrolled trials in lupus nephritis indicate that rituximab could also be potentially effective in patients with lupus nephritis.

[0199] In some embodiments, inhibitors of Type I interferons (IFN) have been used to treat SLE. Type I interferons (IFN) are cytokines that form a crucial link between innate and adaptive immunity and are implicated in SLE by genetic susceptibility data and upregulated interferon-stimulated gene expression in the majority of SLE patients. Sifalimumab is an anti-interferon- α monoclonal antibody. The efficacy and safety of sifalimumab has been seen in some subjects but often the treatment effects are modest. Anifrolumab (MEDI-546) a monoclonal antibody which binds to IFNAR. Anifrolumab reduced disease activity compared to placebo in patients with moderate to severe SLE, however its efficacy has not met all primary endpoints.

[0200] Many subjects with SLE, including severe SLE, exhibit an insufficient response or are refractory to existing treatments, such as treatments with any two or more of the following: MMF, CYC, belimumab, rituximab, anifrolumab, azathioprine, mtx, csp, voclosporin. Severe, refractory SLE patients are often young adults facing lifelong treatment, frequent relapse, and cumulative organ dysfunction over time. Despite advances in SLE therapies, a significant proportion of severe SLE patients do not respond and/or relapse and are at high risk for organ failure or death. There is a huge unmet need for an SLE therapy with a better efficacy and safety profile than currently available therapies, particularly in subjects with severe, refractory SLE.

[0201] In some embodiments, diagnosis of SLE can be made on the basis of criteria defined by the American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR) (Aringer et al. (2019) *Arthritis Rheumatol.* 71:1400-1412). These criteria are anchored on the presence of a positive anti-nuclear antibody test and the presence of clinical features which include discoid rash, oral ulcers, arthritis, serositis, renal disorder, neurologic disorder, hematologic disorder, and immunologic disorder. A mammal (*e.g.*, a human) can be clinically classified with SLE if he or she scores at least 10 points derived from weighted criteria. In some embodiments, diagnosis of SLE can be made based on the presence of detectable SLE-associated antibodies in the blood of the subject. In some embodiments, these antibodies include anti-dsDNA, anti-histone, anti-chromatin and/or anti-Sm antibodies. In some embodiments, the subject has severe SLE characterized by at least one organ system categorized

as BILAG A or at least two organ systems categorized as BILAG B. The term “BILAG” refers to the British Isles Lupus Assessment Group (BILAG) 2004, which is a disease index devised for patients with SLE based on the treating physician's intention to treat (Isenberg et al., 2005). The criteria of “organ system” as used in connection with BILAG refers to the following 9 systems considered in BILAG 2004 index: constitutional, mucocutaneous, central nervous system, musculoskeletal, cardiovascular/respiratory, abdominal, renal and haematological. The BILAG 2004 assessment is composed of 101 questions (and 5 additional items required mainly for calculation of glomerular filtration rate). Each question is answered as: 0=not present; 1=improving; 2=same; 3=worse and 4=new. The index records disease activity occurring over the past 4 weeks as compared with the previous 4 weeks. Based upon the scoring to each of these questions, a pre-defined algorithm, specific for each system, provides a disease activity score ranging from A to E for each system:

[0202] A=12, which is defined as severe disease requiring medium/large doses of corticosteroids (>20 mg prednisolone or equivalent) and/or starting or increasing immunosuppressive drugs, or high-dose anticoagulation (INR>3) (Yee et al., Rheumatology, 2010). In some embodiments, grade A represents very active disease requiring immunosuppressive drugs and/or a prednisone dose of >20 mg/day or equivalent;

[0203] B=8, which is defined as disease activity requiring somewhat lower doses of immunosuppressives, *e.g.*, <20 mg prednisolone, and/or specific drugs, such as anti-malarial, anti-epileptic, anti-depressant and NSAIDs, or topical steroids. In some embodiments, grade B represents moderate disease activity requiring a lower dose of corticosteroids, topical steroids, topical immunosuppressives, antimalarials, or NSAIDs;

[0204] C=1, which is defined as mild persistent disease activity only requiring symptomatic treatment *e.g.*, analgesics or NSAIDs. In some embodiments, grade C indicates mild stable disease;

[0205] D=0, which is defined as the organ or system was once active but is no longer so. In some embodiments, grade D indicates no disease activity but the system has previously been affected; and

[0206] E=0, which is defined as the organ or system was never active. In some embodiments, grade E indicates no current or previous disease activity.

[0207] In some embodiments, the subject has OCS associated organ damage. The OCS may comprise prednisone, prednisolone and/or methylprednisolone. In some embodiments, the subject may be selected for having SLE that is unresponsive to OCS treatment.

[0208] In some embodiments, the subject has an SLEDAI disease activity score of ≥ 10 , which is an indicator for disease severity in SLE.

[0209] In some embodiments, severe disease is based on the presence of major organ involvement (at least one of renal, neurological, cardiovascular, or respiratory system involvement) and requirement treatment with >7.5 mg/day corticosteroids or immunosuppressants.

[0210] In some embodiments, the subject has previously received prior treatment with glucocorticoids, antimalarials, immunosuppressants, anti-CD20 antibody, IFN inhibitor, inhibitor of soluble B lymphocyte stimulator (BLyS). In some embodiments, the immunosuppressant is azathioprine, cyclosporine (csp), cyclophosphamide (cyc), mizoribine, mycophenolate mofetil (MFF), mycophenolic acid, and/or methotrexate (mtx). In some embodiments, the glucocorticoid is an oral corticosteroid such as prednisone, prednisolone and/or methylprednisolone. In some embodiments, the antimalarial is hydroxychloroquine. In some embodiments, the anti-CD20 antibody is Rituximab. In some embodiments, the IFN inhibitor is anifrolumab. In some embodiments, the BLyS inhibitor is belimumab.

[0211] In some embodiments, the subject is refractory to treatment with two or more prior treatments. In some embodiments, the two or more prior treatments (*e.g.*, 2, 3, 4, 5 or more prior treatments) are selected from any two or more of the following: mycophenolic acid or its derivatives, cyclophosphamide (CYC), belimumab, rituximab, anifrolumab, azathioprine, methotrexate (mtx), cisplatin (CSP), obinutuzumab, cyclosporin, tacrolimus and/or voclosporin. In some embodiments, methotrexate and azathioprine count as 1 for the purposes of the number of failed treatments. In some embodiments, the two or more prior treatments (*e.g.*, 2, 3, 4, 5 or more prior treatments) are selected from any two or more of the following: mycophenolate mofetil (MFF), cyclophosphamide (cyc), belimumab, rituximab, anifrolumab, azathioprine, methotrexate cyclosporine (csp) or voclosporin. In some embodiments, the subject has received two or more prior treatments (*e.g.*, 2, 3, 4, 5 or more prior treatments) for lupus that resulted in an insufficient response (*e.g.*, as measured by SLE disease activity). In some embodiments, the subject has an insufficient response to two prior treatments. In some embodiments, the subject has an insufficient response to three prior treatments. In some embodiments, the subject has an

insufficient response to four or more prior treatments. In some embodiments, the subject has failed to attain clinical remission (*e.g.*, after three months of a given treatment) after having been treated with any two or more prior treatments for lupus. In any embodiments, the subject is identified or selected as having an insufficient response to a prior treatment at a time prior to leukapheresis in connection with engineering the CD19-directed CAR T cell composition. Insufficient response to treatments is defined as a lack of response, insufficient response, or a lack of sustained response to appropriate doses. Intolerance is not considered insufficient response.

[0212] In some embodiments, the subject does not have drug-induced SLE. In some embodiments, the subject does not have additional systemic autoimmune diseases, including but not limited to, multiple sclerosis, psoriasis, and/or inflammatory bowel disease. In some embodiments, the subject does not have SLE overlap syndromes, including, but not limited to, rheumatoid arthritis, scleroderma, and/or mixed connective tissue disease. In some embodiments, the subject does not have clinically significant CNS pathology.

a. Response and Efficacy

[0213] In some embodiments, the provided methods and uses involving administration of an anti-CD19 CAR T cell therapy reduce SLE disease activity in the subject.

[0214] In some embodiments, the treatment is effective to reduce lupus disease activity. In some embodiments, the lupus disease activity is measured by a disease activity score selected from the group consisting of British Isles Lupus Assessment Group 2004 (BILAG), SLE disease activity index (SLEDAI-2K), SLEDAI-2K Responder Index 50 (SRI-50), composite SLE Responder Index (cSRI), minimum clinically important differences (MCID), patient reported short-form quality of life assessment (SF-36) Physical Component Summary (PCS) and/or Mental Component Summary (MCS), and lupus-specific quality of life form (Lupus-QOL) or a combination thereof.

[0215] In some embodiments, reducing SLE disease activity in the subject may include one or more of the following: a BILAG-Based Composite Lupus Assessment (BICLA) response in the subject, reducing the subject's Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI) score compared to the subject's CLASI score pre-treatment, reducing the subject's tender and swollen joint count compared to the subject's

tender and swollen joint count pre-treatment, the subject having a maximum of 1 BILAG-2004 B score following treatment, the subject having a BILAG-2004 score of C or better following treatment, the subject having an improvement in at least one patient reported outcome (PRO) compared to pre-treatment, or reducing the subject's SLE flare rate compared to the subject's flare rate pre-treatment.

[0216] In some embodiments, the the subject's BILAG score may be measured before and after administration of the CD19-targeted cell therapy. In some embodiments, patient reported outcomes (PROs) are measured in the subject before and after administration of the CD19-targeted cell therapy. The PRO's may include the subject's Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F), Short Form 36 Health Survey version 2 (SF-36-v2), mental component summary (MCS), and/or SF-36, physical component summary (PCS) score.

[0217] In some embodiments, the treatment results in the subject who had at least one organ system categorized as BILAG A or at least two organ systems categorized as BILAG B at baseline, having reduction in the one organ system categorized as BILAG A or in the at least two organ systems categorized as BILAG B by one score in any one organ system, without having any other organ systems deteriorated to BILAG A or B.

[0218] In some embodiments, reducing SLE disease activity in the subject includes a BILAG-Based Composite Lupus Assessment (BICLA) response. In some embodiments, reducing SLE disease activity in the subject includes a BICLA response by at least week 4 of treatment. In some embodiments, reducing SLE disease activity includes a BICLA response by at least week 8 of treatment. In some embodiments, the BICLA response may be sustained in the subject for at least 52 weeks. In some embodiments, the BICLA response includes reduction of the subject's BILAG-2004 A and B domain scores to B/C/D and C/D, respectively.

[0219] In some embodiments, the treatment results in the subject who had at least one organ system categorized as BILAG A or at least two organ systems categorized as BILAG B at baseline, having all organ systems categorized as either BILAG C or BILAG D/E after treatment.

[0220] In some embodiments, the treatment results in a minimum clinically important difference (MCID) of one for SRI-50.

[0221] In some embodiments, the treatment results in the subject who had at least one organ system categorized as BILAG A or at least two organ systems categorized as BILAG B at baseline, having all organ systems categorized as either BILAG C or BILAG D/E after treatment, and no deterioration measured by SLEDAI-2K after treatment. In some embodiments, SLE disease activity index “SLEDAI-2K” (also referred to as “SLEDAI”) is a validated tool developed as a global assessment of disease activity in SLE patients (Gladman et al., 2002). It represents the consensus of a group of experts in the field of lupus research. The SLEDAI-2K assesses 24 descriptors (sixteen clinical manifestations and eight laboratory measures) in 9 organ systems. Descriptors are given different weights, based on clinical importance, with dichotomic score (present/not present within the previous 30 days). A descriptor must be attributed to active SLE or otherwise should not be scored. The SLEDAI-2K is intended to evaluate current lupus activity and not chronic damage. In some embodiments, deterioration in the context of SLEDAI-2K means worsening of disease activity as measured by SLEDAI-2K.

[0222] In some embodiments, disease activity is monitored by SRI-50. “SRI-50” is a SLE disease activity index comprising the same 24 descriptors, covering nine organ systems, which generates a total score and reflects disease activity over the previous 30 days as does SLEDAI-2K (Touma et. al., 2012). Each of the SRI-50 descriptors has a definition to identify 50% or more improvement and generates a score for the corresponding descriptor. Overall, SRI-50 is an index, developed to reflect partial important improvement in disease activity between visits.

[0223] In some embodiments, the treatment results in an SRI (Systemic Lupus Erythematosus Responder Index) of >4 , or SRI(4). A subject achieves SRI(4) if all of the following criteria are met: Reduction from baseline of >4 points in the SLEDAI-2K; No new organ system affected as defined by 1 or more BILAG-2004 A or 2 or more BILAG-2004 B items compared to baseline using BILAG-2004; No worsening from baseline in the subjects’ lupus disease activity defined by an increase >0.30 points on a 3-point PGA VAS.

[0224] In some embodiments, SRI(X) (X=5, 6, 7, or 8) is defined by the proportion of subjects who meet the following criteria: Reduction from baseline of $>X$ points in the SLEDAI-2K; No new organ systems affected as defined by 1 or more BILAG-2004 A or 2 or more BILAG-2004 B items compared to baseline using BILAG-2004; No worsening

from baseline in the subjects' lupus disease activity defined by an increase >0.30 points on a 3-point PGA VAS.

[0225] In some embodiments, disease activity is monitored by a “composite SLE Responder Index” (cSRI), which is a SLE disease index which incorporates two different systems: BILAG and SLEDAI-2K, defined as substantial response as measured by BILAG 2004 and no deterioration as measured by SLEDAI-2K.

[0226] In some embodiments, the treatment results in the subject having equal or greater than 4 point improvement in the SELENA-SLEDAI, wherein the subject having no new organ system categorized as BILAG A or no more than one organ system categorized as BILAG B, and wherein the subject having less than 0.3 point increase in the physician global assessment.

[0227] In some embodiments, disease activity is monitored using CLASI (Cutaneous Lupus Erythematosus Disease Area and Severity Index). CLASI is tool used to measure disease severity and response to treatment. A 4-point or 20% decrease in CLASI activity score is commonly viewed as a cut-off for classifying subjects as responders to treatment. In particular embodiments, treatment using a CD19-targeted cell therapy as provided results in at least 50% reduction of a subject's CLASI score compared to the subject's baseline score. In some embodiments, the CLASI is a validated index used for assessing the cutaneous lesions of SLE and is composed of 2 separate scores: the first summarizes the inflammatory activity of the disease; the second is a measure of the damage done by the disease. The activity score takes into account erythema, scale/hypertrophy, mucous membrane lesions, recent hair loss, and nonscarring alopecia. The damage score represents dyspigmentation, scarring/atrophy/panniculitis, and scarring of the scalp. Subjects are asked if their dyspigmentation lasted 12 months or longer, in which case the dyspigmentation score is doubled. Each of the above parameters is measured in 13 different anatomical locations, included specifically because they are most often involved in cutaneous lupus erythematosus (CLE). The most severe lesion in each area is measured.

[0228] In some embodiments, the treatment results in the subject achieving Lupus Low Disease Activity State (LLDAS). LLDAS is a comparable, validated goal for SLE used to measure low disease activity. LLDAS is defined by (1) SLE Disease Activity Index (SLEDAI)-2K ≤ 4 , with no activity in major organ systems, (2) no new lupus disease activity, (3) a SELENA-SLEDAI physician global assessment (scale 0–3) ≤ 1 , (4) a current

prednisolone (or equivalent) dose ≤ 7.5 mg daily, and (5) well tolerated standard maintenance doses of immunosuppressive drugs and approved biological agents (Franklyn et al., 2015),

[0229] In some embodiments, the treatment results in the subject having significant change in SF-36 PCS and/or MCS relative to baseline. In some embodiments, “patient reported short-form quality of life assessment” (SF-36) is a widely validated generic patient questionnaire shown to be sensitive to change in a variety of chronic diseases: hypertension and cardiovascular disease, diabetes, pulmonary disease, low back pain, rheumatoid arthritis (RA) and osteoarthritis (Ware J E, et al. (1992) Medical Care 30:473-483). The SF-36 is made up of 36 questions representing eight important health concepts, each of which is scored on an individual “domain” scale: Physical Functioning, Role-Physical, Bodily Pain, General Health, Vitality, Social Functioning, Role-Emotional and Mental Health (Ware et al. Medical Care, 1992). These eight scales can be aggregated into two summary measures: the Physical (PCS) and Mental (MCS) Component Summary scores.

[0230] In some embodiments, the treatment results in the subject having significant change in the Health Assessment Questionnaire-Disability Index (HAQ-DI) relative to baseline. In some embodiments, the patient reported quality of life assessment is a widely validated generic patient questionnaire that measures difficulty in performing activities of daily living. The questions are rated on a 0-3 scale, where 0 indicates “without difficulty” and 3 indicates “unable to do” (Allanore et al., 2020).

[0231] In some embodiments, reducing SLE disease activity in the subject results in at least a 50% improvement in the tender joint count and swollen joint count in the subject compared to the tender joint and swollen count in the subject pre-treatment value. In some embodiments, the swollen and tender joint count is based on left and right shoulder, elbow, wrist, metacarpophalangeal (MCP) 1, MCP2, MCP3, MCP4, MCP5, proximal interphalangeal (PIP) 1, PIP2, PIP3, PIP4, PIP5 joints of the upper extremities and left and right knee of the lower extremities. An active joint for the joint count assessment is defined as a joint with tenderness and swelling.

[0232] In some embodiments, reducing SLE disease activity in the subject includes preventing flares in the subject. In some embodiments, a flare may be defined as ≥ 1 new

BILAG-2004 A or ≥ 2 new (worsening) BILAG-2004 B domain scores compared to the subject's scores one month previously.

[0233] In some embodiments, the treatment results in the subject having increased time to first confirmed severe SLE flare or time to first confirmed major SLE flare.

[0234] In some embodiments, the treatment results in increased time to first confirmed severe SLE flare, and wherein a severe SLE flare comprises a subject having any new organ system categorized as BILAG A or having any two new organ systems categorized as BILAG B.

[0235] In some embodiments, the treatment results in increased time to first confirmed major SLE flare defined by the Fortin definition of major flare, which comprises initiation or increase of immunosuppressive or high-dose corticosteroids therapy, hospitalization or death due to SLE.

[0236] In some embodiments, the method of treatment reduces the oral corticosterpoid (OCS) dose administered to the subject compared to the OCS dose administered to the subject pre-treatment. In some embodiments, reducing SLE disease activity in the subject is characterized by a reduced flare rate in the subject compared to the flare rate pre-treatment, wherein the method comprises reducing OCS dose administration to the subject compared to the OCS dose administered to the subject pre-treatment. In some embodiments, OCS comprises prednisone, prednisolone and/or methylprednisolone.

[0237] In some embodiments, the treatment results in the subject having a significant change in cumulative damage index as measured by Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SLICC/ACR DI). In some embodiments, the "Systemic Lupus Erythematosus International Collaborating Clinics/American College of Rheumatology" (SLICC/ACR) is an index for accumulated organ damage (Dayal et al., Lupus 2002). SLE damage is defined as an irreversible change in organ or system that has been present for at least 6 months.

[0238] In some embodiments, the treatment results in the subject having a significant change in daily glucocorticoid dose.

[0239] In some embodiments, the treatment results in the subject having a significant improvement in Lupus-QOL.

[0240] In some embodiments, the treatment results in the subject having significant improvement of global assessment of disease activity based on minimum clinically

important differences (MCID). In some embodiments, MCID refers to patient derived scores that reflect changes in a clinical intervention that are meaningful for the patient.

[0241] In some embodiments, the method reduces the SLE disease activity in the subject as characterized by reducing the anti-dsDNA levels in the subject.

[0242] In some embodiments, a subject who has been treated in accord with the provided methods is evaluated or monitored after treatment for a period of time to determine whether a complete or partial remission has occurred. In some embodiments, the subject is evaluated or monitored to assess whether the remission achieved according to the measurement is being maintained.

[0243] In some embodiments, remission is monitored using the Definitions of Remission in Systemic Lupus Erythematosus (DORIS) (Correction: 2021 DORIS definition of remission in SLE: final recommendations from an international task force *Lupus Science & Medicine* 2022;9:e000538corr1. doi: 10.1136/lupus-2021-000538corr1). In some embodiments, remission defined as a score of 0 on the SLE disease activity index (SLEDAI) and an Evaluator's Global Assessment score of <0.5 (0-3). Subjects may be on stable antimalarials, immunosuppressive drugs, biologics, and/or low-dose glucocorticoids (prednisolone of 5 mg/day or less).

[0244] In some embodiments, the subject has lupus nephritis. In some embodiments, evaluation for effectiveness can be based on the protein/creatinine ratio in urine (UPCR) where a ratio of ≤ 0.5 mg/mg indicates complete response; alternatively, or in addition, an eGFR of ≥ 60 mL/min/1.73 m² or no decrease from baseline and eGFR of $\geq 20\%$ is shown. Other indications of complete response include lack of need for rescue medications such as intravenous steroids, cyclophosphamide or a need for ≤ 10 mg prednisone for more than three consecutive days or more than seven days total. In some embodiments, complete remission (CR) is defined as: Confirmed protein/creatinine ratio of ≤ 0.5 mg/mg, and $\text{eGFR} \geq 60$ mL/min/1.73 m² or no confirmed decrease from baseline in eGFR of $\geq 20\%$. Partial remission is defined as: 50% reduction in UPCR from baseline.

[0245] In some embodiments, the treatment in accord with the provided methods results in clinical remission of SLE in the subject that is maintained for greater than 3 months. In some embodiments, the treatment in accord with the provided methods results in clinical remission of SLE in the subject that is maintained for greater than 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 18

months, 24 months, 3 years, 4 years, 5 years or more. In some embodiments, the treatment in accord with the provided methods results in clinical remission of SLE in the subject that is maintained for greater than 6 months. In some embodiments, the treatment in accord with the provided methods results in clinical remission of SLE in the subject that is maintained for greater than 12 months. In some embodiments, the treatment in accord with the provided methods results in clinical remission of SLE in the subject that is maintained for greater than 24 months. In some embodiments, the treatment in accord with the provided methods results in clinical remission of SLE in the subject that is maintained for greater than 3 years. In some embodiments, the treatment in accord with the provided methods results in clinical remission of SLE in the subject that is maintained for greater than 4 years. In some embodiments, the treatment in accord with the provided methods results in clinical remission of SLE in the subject that is maintained for greater than 5 years.

[0246] In some embodiments, the treatment in accord with the provided methods results in prolonged remission. In some embodiments, prolonged remission is defined as a 5-year consecutive period of no disease activity (SLE disease activity index, SLEDAI = 0) and without treatment (corticosteroids, antimalarials, or immunosuppressants).

[0247] In some cases, the pharmacokinetics of administered cells, *e.g.*, adoptively transferred cells are determined to assess the availability, *e.g.*, bioavailability of the administered cells. Methods for determining the pharmacokinetics of adoptively transferred cells may include drawing peripheral blood from subjects that have been administered engineered cells, and determining the number or ratio of the engineered cells in the peripheral blood. Approaches for selecting and/or isolating cells may include use of chimeric antigen receptor (CAR)-specific antibodies (*e.g.*, Brentjens et al., *Sci. Transl. Med.* 2013 Mar; 5(177): 177ra38) Protein L (Zheng et al., *J. Transl. Med.* 2012 Feb; 10:29), epitope tags, such as Strep-Tag sequences, introduced directly into specific sites in the CAR, whereby binding reagents for Strep-Tag are used to directly assess the CAR (Liu et al. (2016) *Nature Biotechnology*, 34:430; international patent application Pub. No. WO2015095895) and monoclonal antibodies that specifically bind to a CAR polypeptide (see international patent application Pub. No. WO2014190273). Extrinsic marker genes may in some cases be utilized in connection with engineered cell therapies to permit detection or selection of cells and, in some cases, also to promote cell suicide. A truncated

epidermal growth factor receptor (EGFRt) in some cases can be co-expressed with a transgene of interest (a CAR) in transduced cells (see *e.g.*, U.S. Patent No. 8,802,374). EGFRt may contain an epitope recognized by the antibody cetuximab (Erbix®) or other therapeutic anti-EGFR antibody or binding molecule, which can be used to identify or select cells that have been engineered with the EGFRt construct and another recombinant receptor, such as a chimeric antigen receptor (CAR), and/or to eliminate or separate cells expressing the receptor. See U.S. Patent No. 8,802,374 and Liu et al., *Nature Biotech.* 2016 April; 34(4): 430–434.

[0248] In some embodiments, the number of CAR⁺ T cells in a biological sample obtained from the patient, *e.g.*, blood, can be determined at a period of time after administration of the cell therapy, *e.g.*, to determine the pharmacokinetics of the cells. In some embodiments, number of CAR⁺ T cells, optionally CAR⁺ CD8⁺ T cells and/or CAR⁺ CD4⁺ T cells, detectable in the blood of the subject, or in a majority of subjects so treated by the method, is greater than 1 cells per μL , greater than 5 cells per μL or greater than per 10 cells per μL .

2. Idiopathic inflammatory myopathy (IIM)

[0249] Idiopathic inflammatory myopathy (IIM) is a group of chronic autoimmune conditions that primarily affects the proximal muscles. IIM includes dermatomyositis, polymyositis, and other diseases such as immune-mediated necrotizing myopathy (IMNM), with many patients having anti-synthetase syndrome (aSS). aSS is characterized by autoantibodies directed against aminoacyl transfer RNA synthetase that overlap with interstitial lung disease (ILD), myositis, and other conditions. IIM manifestations include skin lesions, muscle fatigue, and weakness, with patients experiencing greatly reduced quality of life and are at risk for a variety of serious long-term complications. For example, 10-25% of IIM cases also have ILD, with 5% of cases being acute. 15-25% of patients with IIM either have malignancies or will have them. One third of patients with IIM will develop myocarditis, with a heightened risk for congestive heart failure. IIM has a 10 year survival rate across various indications of 70%. There are currently only 2 approved drugs for IIM, including IVIg, and Acthar Gel, with no drugs approved for aSS. There is strong evidence of B-cell involvement, with IVIg being approved for dermatomyositis and Rituximab used as an off-label treatment. There is evidence that B-cells play a role in disease pathogenesis, including complete resolution of aSS after anti-CD19 CAR

T-cell therapy in a patient who was refractory to steroids, rituximab, tacrolimus, and cyclophosphamide.

[0250] In some embodiments, the systemic autoimmune disease is Idiopathic inflammatory myopathy (IIM), such as dermatomyositis, polymyositis, and/or immune-mediated necrotizing myopathy. In some embodiments, the patients have anti-synthetase syndrome (aSS). Among provided methods are methods of treatment that involve administering engineered cells or compositions containing engineered cells, such as engineered T cells to subjects with IIM, including dermatomyositis, polymyositis, and/or immune-mediated necrotizing myopathy. Also provided are methods and uses of provided CD19-directed CAR engineered cells (*e.g.*, T cells) and/or compositions thereof, including methods for the treatment of subjects having an IIM, including dermatomyositis, polymyositis, and/or immune-mediated necrotizing myopathy, that involves administration of the engineered cells and/or compositions thereof. In certain embodiments, the subject has dermatomyositis, polymyositis, and/or immune-mediated necrotizing myopathy. In some embodiments, the subject is selected for or identified as having dermatomyositis, polymyositis, and/or immune-mediated necrotizing myopathy, such as by the presence of certain features or clinical manifestations that indicate the presence of dermatomyositis, polymyositis, and/or immune-mediated necrotizing myopathy. In some embodiments, the methods and use of provided CD19-directed CAR engineered cells (*e.g.*, T cells) and/or compositions thereof, include methods for the treatment of subjects with dermatomyositis, polymyositis, and/or immune-mediated necrotizing myopathy that have failed at least two or more prior therapies. In particular embodiments, the method includes administering to the subject a dose of T cells that includes CD4+ and CD8+ T cells, wherein the T cells comprises a chimeric antigen receptor (CAR) that specifically binds to CD19.

[0251] In any of the embodiments herein, at or immediately prior to the time of the administration of the composition comprising engineered T cells, the subject has relapsed following remission after treatment with, or become refractory to one or more prior therapies for IIM.

[0252] In any of the embodiments herein, at or immediately prior to the time of the administration of the composition comprising engineered T cells, the subject has relapsed following remission after treatment with, or become refractory to, one or more prior therapies for IIM. In any of the embodiments herein, at or immediately prior to the time of the administration of the composition comprising engineered T cells, the subject has relapsed

following treatment with, or become refractory to, one or more prior therapies for the IIM. In any of the embodiments herein, the one or more prior therapies for the IIM does not comprise another dose of cells expressing the CAR.

[0253] In any of the embodiments herein, the one or more prior therapies for the IIM may comprise corticosteroids, Octagam (IVIg), Acthar or rituximab. In any of the embodiments herein, the one or more prior therapies for the IIM comprise corticosteroids, Octagam, Acthar, or rituximab. In any of the embodiments herein, CD19-directed CAR engineered cells (*e.g.*, T cells) and/or compositions thereof are used to treat patients with IIM that are refractory to prior therapies.

a. Response and Efficacy

[0254] In some embodiments, the provided methods and uses involving administration of an anti-CD19 CAR T cell therapy reduce IIM disease activity in the subject.

[0255] In some embodiments, the treatment is effective to reduce IIM disease activity. In some embodiments, the IIM disease activity is measured by a disease activity score selected from the International Myositis Assessment and Clinical Studies Group (IMACS), minimum clinically important differences (MCID), patient reported short-form quality of life assessment (SF-36) Physical Component Summary (PCS) and/or Mental Component Summary (MCS), or a combination thereof.

[0256] In some embodiments, reducing IIM disease activity in the subject may include one or more of the following: reducing the subject's IMACS score after treatment compared to the subject's IMACS score before treatment, reducing the subject's skin lesions, muscle fatigue, and/or weakness compared to the subject's skin lesions, muscle fatigue, and/or weakness pre-treatment, or the subject having an improvement in at least one patient reported outcome (PRO) compared to pre-treatment.

[0257] In some embodiments, the subject's IMACS score may be measured before and after administration of the CD19-targeted cell therapy. In some embodiments, patient reported outcomes (PROs) are measured in the subject before and after administration of the CD19-targeted cell therapy. The PRO's may include the subject's Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F), Short Form 36 Health Survey version 2 (SF-36-v2), mental component summary (MCS), and/or SF-36, physical component summary (PCS) score.

[0258] In some embodiments, the treatment results in the subject having significant change in the Health Assessment Questionnaire-Disability Index (HAQ-DI) relative to baseline. In some embodiments, the patient reported quality of life assessment is a widely validated generic patient questionnaire that measures difficulty in performing activities of daily living. The questions are rated on a 0-3 scale, where 0 indicates “without difficulty” and 3 indicates “unable to do” (Allanore et al., 2020).

[0259] In some embodiments, the treatment results in the subject having significant improvement of global assessment of disease activity based on minimum clinically important differences (MCID). In some embodiments, MCID refers to patient derived scores that reflect changes in a clinical intervention that are meaningful for the patient.

[0260] In some embodiments, the treatment in accord with the provided methods results in clinical remission of IIM in the subject that is maintained for greater than 3 months. In some embodiments, the treatment in accord with the provided methods results in clinical remission of IIM in the subject that is maintained for greater than 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 18 months, 24 months, 3 years, 4 years, 5 years or more. In some embodiments, the treatment in accord with the provided methods results in clinical remission of IIM in the subject that is maintained for greater than 6 months. In some embodiments, the treatment in accord with the provided methods results in clinical remission of IIM in the subject that is maintained for greater than 12 months. In some embodiments, the treatment in accord with the provided methods results in clinical remission of IIM in the subject that is maintained for greater than 24 months. In some embodiments, the treatment in accord with the provided methods results in clinical remission of IIM in the subject that is maintained for greater than 3 years. In some embodiments, the treatment in accord with the provided methods results in clinical remission of IIM in the subject that is maintained for greater than 4 years. In some embodiments, the treatment in accord with the provided methods results in clinical remission of IIM in the subject that is maintained for greater than 5 years.

[0261] In some embodiments, the treatment in accord with the provided methods results in prolonged remission. In some embodiments, prolonged remission is defined as a 5-year consecutive period of no disease activity and without treatment (corticosteroids, IVIg, rituximab, or immunosuppressants).

[0262] In some embodiment, treatment results in reduced muscle weakness or reduces the progression of muscle weakness. In some embodiment, treatment results in improved muscle

strength. In some embodiment, treatment results in reduced muscle weakness or reduces the progression of muscle weakness in the upper extremities. In some embodiment, treatment results in reduced muscle weakness or reduces the progression of muscle weakness in the lower extremities. In some embodiment, treatment results in reduced muscle weakness or reduces the progression of muscle weakness in the neck flexors. In some embodiment, treatment results in reduced muscle weakness or reduces the progression of muscle weakness in the proximal muscles.

[0263] In some embodiments, treatment results in decreased skin lesions. In some embodiments, treatment results in decreased heliotrope rash presentation. In some embodiments, treatment results in decreased Gottron's papules. In some embodiments, treatment results in decreased Gottron's sign.

[0264] In some embodiments, treatment results in a decrease in dysphagia or esophageal dysmotility. In some embodiments, treatment results in an improvement in swallowing or motility of the esophagus.

[0265] In some embodiments, treatment decreases the presence of anti-Jo-a (anti-histidyl-tRNA synthetase) autoantibody. In some embodiments treatment leads to no detection of anti-Jo-a (anti-histidyl-tRNA synthetase) autoantibody. In some embodiments, treatment leads to a decrease in serum levels of creatine kinase, lactate dehydrogenase, aspartate aminotransferase, and/or alanine aminotransferase.

[0266] In some embodiments, treatment reduces endomysial infiltration of mononuclear cells surrounding, but not invading, myofibres. In some embodiments, treatment reduces perimysial and/or perivascular infiltration of mononuclear cells. In some embodiments, treatment reduces perifascicular atrophy. In some embodiments, treatment reduces rimmed vacuoles in present in muscle biopsies.

[0267] In some cases, the pharmacokinetics of administered cells, *e.g.*, adoptively transferred cells are determined to assess the availability, *e.g.*, bioavailability of the administered cells. Methods for determining the pharmacokinetics of adoptively transferred cells may include drawing peripheral blood from subjects that have been administered engineered cells, and determining the number or ratio of the engineered cells in the peripheral blood. Approaches for selecting and/or isolating cells may include use of chimeric antigen receptor (CAR)-specific antibodies (*e.g.*, Brentjens et al., *Sci. Transl. Med.* 2013 Mar; 5(177): 177ra38) Protein L (Zheng et al., *J. Transl. Med.* 2012 Feb; 10:29), epitope tags, such as Strep-Tag sequences, introduced

directly into specific sites in the CAR, whereby binding reagents for Strep-Tag are used to directly assess the CAR (Liu et al. (2016) *Nature Biotechnology*, 34:430; international patent application Pub. No. WO2015095895) and monoclonal antibodies that specifically bind to a CAR polypeptide (see international patent application Pub. No. WO2014190273). Extrinsic marker genes may in some cases be utilized in connection with engineered cell therapies to permit detection or selection of cells and, in some cases, also to promote cell suicide. A truncated epidermal growth factor receptor (EGFRt) in some cases can be co-expressed with a transgene of interest (a CAR) in transduced cells (see *e.g.*, U.S. Patent No. 8,802,374). EGFRt may contain an epitope recognized by the antibody cetuximab (Erbix®) or other therapeutic anti-EGFR antibody or binding molecule, which can be used to identify or select cells that have been engineered with the EGFRt construct and another recombinant receptor, such as a chimeric antigen receptor (CAR), and/or to eliminate or separate cells expressing the receptor. See U.S. Patent No. 8,802,374 and Liu et al., *Nature Biotech.* 2016 April; 34(4): 430–434).

[0268] In some embodiments, the number of CAR⁺ T cells in a biological sample obtained from the patient, *e.g.*, blood, can be determined at a period of time after administration of the cell therapy, *e.g.*, to determine the pharmacokinetics of the cells. In some embodiments, number of CAR⁺ T cells, optionally CAR⁺ CD8⁺ T cells and/or CAR⁺ CD4⁺ T cells, detectable in the blood of the subject, or in a majority of subjects so treated by the method, is greater than 1 cells per μL , greater than 5 cells per μL or greater than 10 cells per μL .

3. *Systemic sclerosis (SSc)*

[0269] Systemic sclerosis (SSc) is an autoimmune disease that primarily affects the skin and can cause complications in organ systems. The disease is characterized by fibrosis affecting the skin and internal organs with three main varieties of the disease. The first is limited SSc, which accounts for about 60% of cases and is localized to skin effects and is associated with some vascular and lung involvement. The second is diffuse SSc, which is the most severe and accounts for about 35% of cases. It is characterized by broad skin effects with more severe multi-organ involvement, including interstitial lung disease (ILD) and renal failure. The third variety is sine SSc, which is the rarest and accounts for about 5% of cases. It has no skin involvement with varied levels of organ involvement. SSc disease progression can cause fibrosis in the heart, lungs, kidneys, and other organs. Quality of life is severely worsened for patients,

with a 10 year survival rate of about 72%. An estimated 32,000 patients in the United States have diffuse SSc, with about half developing ILD. B cells are believed to play a role in SSc development with off-label use of Rituximab showing some efficacy.

[0270] In some embodiments, the systemic autoimmune disease is SSc, such as limited SSc, diffuse SSc, or sine SSc. Among provided methods are methods of treatment that involve administering engineered cells or compositions containing engineered cells, such as engineered T cells to subjects with SSc, including limited SSc, diffuse SSc, or sine SSc. Also provided are methods and uses of provided CD19-directed CAR engineered cells (*e.g.*, T cells) and/or compositions thereof, including methods for the treatment of subjects having a SSc, including limited SSc, diffuse SSc, or sine SSc, that involves administration of the engineered cells and/or compositions thereof. In certain embodiments, the subject has limited SSc. In some embodiments, the subject is selected for or identified as having limited SSc, diffuse SSc, or sine SSc, such as by the presence of certain features or clinical manifestations that indicate the presence of limited SSc, diffuse SSc, or sine SSc. In some embodiments, the methods and use of provided CD19-directed CAR engineered cells (*e.g.*, T cells) and/or compositions thereof, include methods for the treatment of subjects with SSc that have failed at least two or more prior therapies. In particular embodiments, the method includes administering to the subject a dose of T cells that includes CD4+ and CD8+ T cells, wherein the T cells comprises a chimeric antigen receptor (CAR) that specifically binds to CD19.

[0271] In any of the embodiments herein, at or immediately prior to the time of the administration of the composition comprising engineered T cells, the subject has relapsed following remission after treatment with, or become refractory to one or more prior therapies for SSc.

[0272] In any of the embodiments herein, at or immediately prior to the time of the administration of the composition comprising engineered T cells, the subject has relapsed following remission after treatment with, or become refractory to, one or more prior therapies for SSc. In any of the embodiments herein, at or immediately prior to the time of the administration of the composition comprising engineered T cells, the subject has relapsed following treatment with, or become refractory to, one or more prior therapies for the SSc. In any of the embodiments herein, the one or more prior therapies for the SSc does not comprise another dose of cells expressing the CAR.

[0273] In any of the embodiments herein, the one or more prior therapies for the SSc may comprise mycophenolate and/or methotrexate if the subject does not have ILD. In any of the embodiments herein, the one or more prior therapies for the SSc may comprise mycophenolate, cyclophosphamide, and/or tocilizumab (ACTEMRA) if the subject does have ILD. In any of the embodiments herein, the one or more prior therapies for the SSc may comprise administration of mycophenolate, methotrexate, cyclophosphamide, and/or tocilizumab (ACTEMRA) followed by administration of a B cell depletion therapy such as rituximab and/or a VEGFR inhibitor such as Nintedanib. In any of the embodiments herein, CD19-directed CAR engineered cells (*e.g.*, T cells) and/or compositions thereof are used to treat patients with SSc that are refractory to prior therapies.

a. Response and Efficacy

[0274] In some embodiments, the provided methods and uses involving administration of an anti-CD19 CAR T cell therapy reduce SSc disease activity in the subject.

In some embodiments, the treatment is effective to reduce SSc disease activity. In some embodiments, the SSc disease activity is measured by a disease activity score selected from the modified Rodnan skin score, forced vital capacity, European Scleroderma Study Group (EScSG) indices, minimum clinically important differences (MCID), patient reported short-form quality of life assessment (SF-36) Physical Component Summary (PCS) and/or Mental Component Summary (MCS) or a combination thereof.

[0275] In some embodiments, reducing SSc disease activity in the subject may include one or more of the following: reducing the subject's EScSG indices score after treatment compared to the subject's EScSG indices score before treatment, reducing the subject's skin effects, ILD, and/or pulmonary arterial hypertension compared to the subject's skin effects, ILD, and/or pulmonary arterial hypertension pre-treatment, or the subject having an improvement in at least one patient reported outcome (PRO) compared to pre-treatment.

In some embodiments, the the subject's EScSG indices score may be measured before and after administration of the CD19-targeted cell therapy. In some embodiments, patient reported outcomes (PROs) are measured in the subject before and after administration of the CD19-targeted cell therapy. The PRO's may include the subject's Functional Assessment of Chronic

Illness Therapy-Fatigue (FACIT-F), Short Form 36 Health Survey version 2 (SF-36-v2), mental component summary (MCS), and/or SF-36, physical component summary (PCS) score.

[0276] In some embodiments, the treatment results in the subject having significant change in the Health Assessment Questionnaire-Disability Index (HAQ-DI) relative to baseline. In some embodiments, the patient reported quality of life assessment is a widely validated generic patient questionnaire that measures difficulty in performing activities of daily living. The questions are rated on a 0-3 scale, where 0 indicates “without difficulty” and 3 indicates “unable to do” (Allanore et al., 2020).

[0277] In some embodiments, the treatment results in the subject having significant improvement of global assessment of disease activity based on minimum clinically important differences (MCID). In some embodiments, MCID refers to patient derived scores that reflect changes in a clinical intervention that are meaningful for the patient.

[0278] In some embodiments, the treatment results in a decrease in score for the modified Rodnan skin score. In some embodiments, the treatment results in a decrease in skin thickness. In some embodiments, the treatment results in a decrease in skin thickness in fingers, hands, forearms, upper arms, face, anterior chest, abdomen, thighs, legs, and/or feet. In some embodiments, the treatment reduces the score for the EScSG indices.

[0279] In some embodiments, the treatment in accord with the provided methods results in clinical remission of SSc in the subject that is maintained for greater than 3 months. In some embodiments, the treatment in accord with the provided methods results in clinical remission of SSc in the subject that is maintained for greater than 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 18 months, 24 months, 3 years, 4 years, 5 years or more. In some embodiments, the treatment in accord with the provided methods results in clinical remission of SSc in the subject that is maintained for greater than 6 months. In some embodiments, the treatment in accord with the provided methods results in clinical remission of SSc in the subject that is maintained for greater than 12 months. In some embodiments, the treatment in accord with the provided methods results in clinical remission of SSc in the subject that is maintained for greater than 24 months. In some embodiments, the treatment in accord with the provided methods results in clinical remission of SSc in the subject that is maintained for greater than 3 years. In some embodiments, the treatment in accord with the provided methods results in clinical remission of SSc in the subject that is maintained for greater than 4

years. In some embodiments, the treatment in accord with the provided methods results in clinical remission of SSc in the subject that is maintained for greater than 5 years.

[0280] In some embodiments, the treatment in accord with the provided methods results in prolonged remission. In some embodiments, prolonged remission is defined as a 5-year consecutive period of no disease activity and without treatment (corticosteroids, methotrexate, mycophenolate, cyclophosphamide, tocilizumab, IVIg, rituximab, nintedanib or immunosuppressants).

[0281] In some cases, the pharmacokinetics of administered cells, *e.g.*, adoptively transferred cells are determined to assess the availability, *e.g.*, bioavailability of the administered cells. Methods for determining the pharmacokinetics of adoptively transferred cells may include drawing peripheral blood from subjects that have been administered engineered cells and determining the number or ratio of the engineered cells in the peripheral blood. Approaches for selecting and/or isolating cells may include use of chimeric antigen receptor (CAR)-specific antibodies (*e.g.*, Brentjens et al., *Sci. Transl. Med.* 2013 Mar; 5(177): 177ra38) Protein L (Zheng et al., *J. Transl. Med.* 2012 Feb; 10:29), epitope tags, such as Strep-Tag sequences, introduced directly into specific sites in the CAR, whereby binding reagents for Strep-Tag are used to directly assess the CAR (Liu et al. (2016) *Nature Biotechnology*, 34:430; international patent application Pub. No. WO2015095895) and monoclonal antibodies that specifically bind to a CAR polypeptide (see international patent application Pub. No. WO2014190273). Extrinsic marker genes may in some cases be utilized in connection with engineered cell therapies to permit detection or selection of cells and, in some cases, also to promote cell suicide. A truncated epidermal growth factor receptor (EGFRt) in some cases can be co-expressed with a transgene of interest (a CAR) in transduced cells (see *e.g.*, U.S. Patent No. 8,802,374). EGFRt may contain an epitope recognized by the antibody cetuximab (Erbix®) or other therapeutic anti-EGFR antibody or binding molecule, which can be used to identify or select cells that have been engineered with the EGFRt construct and another recombinant receptor, such as a chimeric antigen receptor (CAR), and/or to eliminate or separate cells expressing the receptor. See U.S. Patent No. 8,802,374 and Liu et al., *Nature Biotech.* 2016 April; 34(4): 430–434).

In some embodiments, the number of CAR⁺ T cells in a biological sample obtained from the patient, *e.g.*, blood, can be determined at a period of time after administration of the cell therapy, *e.g.*, to determine the pharmacokinetics of the cells. In some embodiments, number of CAR⁺ T cells, optionally CAR⁺ CD8⁺ T cells and/or CAR⁺ CD4⁺ T cells, detectable in the blood of the

subject, or in a majority of subjects so treated by the method, is greater than 1 cells per μL , greater than 5 cells per μL or greater than 10 cells per μL .

4. Multiple Sclerosis (MS)

[0282] Multiple Sclerosis (MS) has two main subtypes, relapsing MS and progressive MS. Relapsing MS is associated with an immune-dependent mechanism of damage that is characterized by relapse-remission cycles, while progressive MS is associated with immune-independent mechanisms of damage and is characterized by a steady worsening of symptoms. Early symptoms of MS include fatigue, weakness, and muscle spasms which may progress to advanced or severe disease characterized by vision and bladder problems along with cognitive changes and physical disability. About 33% of patients will be forced to use a wheelchair within 20 years of diagnosis. Patients with MS have about an 80% increased risk of mortality. B cells are believed to play an important role in the pathogenesis of MS, as evidenced by the role of anti-CD20 mAbs used in treatment.

[0283] In some embodiments, the systemic autoimmune disease is MS, such as relapsing MS (RMS) or progressive MS (PMS). In some embodiments, the systemic autoimmune disease is highly active RMS. In some embodiments the MS is clinically isolated syndrome (CIS), relapsing-remitting MS (RRMS), active secondary progressive MS (aSPMS), non-active secondary progressive MS (naSPMS), inactive secondary progressive MS (iSPMS), or primary progressive MS (PPMS). In some embodiments, the MS is aSPMS. In some embodiments, the systemic autoimmune disease is aSPMS. Among provided methods are methods of treatment that involve administering engineered cells or compositions containing engineered cells, such as engineered T cells to subjects with MS, including CIS, RRMS, aSPMS, naSPMS, iSPMS, or PPMS. Also provided are methods and uses of provided CD19-directed CAR engineered cells (*e.g.*, T cells) and/or compositions thereof, including methods for the treatment of subjects having MS, including CIS, RRMS, aSPMS, naSPMS, iSPMS, or PPMS, that involves administration of the engineered cells and/or compositions thereof. In certain embodiments, the subject has CIS, RRMS, aSPMS, naSPMS, iSPMS, or PPMS. In certain embodiments, the subject has RRMS, aSPMS, iSPMS, or PPMS. In some embodiments, the subject is selected for or identified as having RRMS, aSPMS, iSPMS, or PPMS, such as by the presence of certain features or clinical manifestations that indicate the presence of RRMS, aSPMS, iSPMS, or

PPMS. In some embodiments, the methods and use of provided CD19-directed CAR engineered cells (*e.g.*, T cells) and/or compositions thereof, include methods for the treatment of subjects with MS that have failed at least two or more prior therapies. In particular embodiments, the method includes administering to the subject a dose of T cells that includes CD4+ and CD8+ T cells, wherein the T cells comprises a chimeric antigen receptor (CAR) that specifically binds to CD19.

[0284] In any of the embodiments herein, at or immediately prior to the time of the administration of the composition comprising engineered T cells, the subject has relapsed following remission after treatment with, or become refractory to one or more prior therapies for MS.

[0285] In any of the embodiments herein, at or immediately prior to the time of the administration of the composition comprising engineered T cells, the subject has relapsed following remission after treatment with, or become refractory to, one or more prior therapies for MS. In any of the embodiments herein, at or immediately prior to the time of the administration of the composition comprising engineered T cells, the subject has relapsed following treatment with, or become refractory to, one or more prior therapies for the MS. In any of the embodiments herein, the one or more prior therapies for the MS does not comprise another dose of cells expressing the CAR. In any of the embodiments herein, the one or more prior therapies include 2 prior disease modifying therapies (DMT) with one of the prior therapies constituting an anti-CD20 antibody.

[0286] In any of the embodiments herein, the one or more prior therapies for the MS may comprise glucocorticoids, plasma exchange, IVIg, adrenocorticotrophic hormone (ACTH), fingolimod, Siponimod, ozanimod, natalizumab, teriflunomide, ocrelizumab, ofatumumab, alemtuzumab, dimethyl fumarate. In any of the embodiments herein, CD19-directed CAR engineered cells (*e.g.*, T cells) and/or compositions thereof are used to treat patients with MS that are refractory to prior therapies.

[0287] In any of the embodiments herein, at or immediately prior to the time of the administration of the composition comprising engineered T cells, the subject cannot complete a standardized dexterity test. In some embodiments, the subject cannot complete the 9-Hole Peg Test (9-HPT) for each hand in <240 seconds, or subjects that cannot perform a Timed 25-Foot Walk Test (T25FWT) in < 150 seconds.

[0288] The nine-hole pegboard test as described herein involves performing the following task: a subject, who is seated, holds nine dowels (approximately 7 mm in diameter and 32-mm long) in one hand and places them randomly, one by one, with the other hand in a board with nine holes. Timing begins when the first peg is placed in a hole and ends when the last peg is placed. The examiner holds the board steady on the table during the test. The trial is performed with the dominant hand. If the patient drops a peg the examiner stops the timer and the patient starts the test again once from the beginning.

[0289] The "Timed-25 Foot Walk" or "T25FWT" as described herein is a quantitative mobility and leg function performance test based on a timed 25-walk. The patient is directed to one end of a clearly marked 25-foot course and is instructed to walk 25 feet as quickly as possible, but safely. The time is calculated from the initiation of the instruction to start and ends when the patient has reached the 25-foot mark. The task is immediately administered again by having the patient walk back the same distance. Patients may use assistive devices when doing this task. The score for the T25FWT is the average of the two completed trials.

[0290] In some embodiments, subjects also have not had MS lesions or symptoms that may place them at increased risk of neurotoxicity, including, but not limited to, tumefactive lesions (3 cm or greater within 5 years prior to Screening). In some embodiments, subjects have not experienced decreased level of consciousness, and/or presence of active, clinically significant concomitant central nervous system pathology other than MS that may confound the ability to interpret study results or complicate identification or evaluation of neurotoxicity.

a. Response and Efficacy

[0291] In some embodiments, the provided methods and uses involving administration of an anti-CD19 CAR T cell therapy reduce MS disease activity in the subject.

In some embodiments, the treatment is effective to reduce MS disease activity. In some embodiments, the MS disease activity is measured by a disease activity score selected from the expanded disability status scale (EDSS), disease steps, multiple sclerosis functional composite (MSFC), minimum clinically important differences (MCID), patient reported short-form quality of life assessment (SF-36) Physical Component Summary (PCS) and/or Mental Component Summary (MCS) or a combination thereof.

[0292] In some embodiments, reducing MS disease activity in the subject may include one or more of the following: reducing the subject's EDSS indices score after treatment compared to

the subject's EDSS indices score before treatment, improving the subject's energy, pain, fatigue, muscle strength, waking distance, mental health, or visual impairment compared to the subject's energy, pain, fatigue, muscle strength, waking distance, mental health, or visual impairment pre-treatment, or the subject having an improvement in at least one patient reported outcome (PRO) compared to pre-treatment.

[0293] In some embodiments, the the subject's EDSS indices score may be measured before and after administration of the CD19-targeted cell therapy. In some embodiments, patient reported outcomes (PROs) are measured in the subject before and after administration of the CD19-targeted cell therapy. The PRO's may include the subject's Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F), Short Form 36 Health Survey version 2 (SF-36-v2), mental component summary (MCS), and/or SF-36, physical component summary (PCS) score.

[0294] In some embodiments, the treatment results in the subject having significant change in the Health Assessment Questionnaire-Disability Index (HAQ-DI) relative to baseline. In some embodiments, the patient reported quality of life assessment is a widely validated generic patient questionnaire that measures difficulty in performing activities of daily living. The questions are rated on a 0-3 scale, where 0 indicates "without difficulty" and 3 indicates "unable to do" (Allanore et al., 2020).

[0295] In some embodiments, the treatment results in a decrease of the EDSS score of the subject. In some embodiments, the treatment results in a decrease in disease steps score of the subject. In some embodiments, the treatment results in a decrease in the MSFC score of the subject.

[0296] In some embodiments, the treatment results in the subject having significant improvement of global assessment of disease activity based on minimum clinically important differences (MCID). In some embodiments, MCID refers to patient derived scores that reflect changes in a clinical intervention that are meaningful for the patient. In some embodiments, the walking speed of the subject is increased after treatment. In some embodiments, treatment results in increased dexterity of the subject, such as in the arm or hand. In some embodiments, treatment results an improvement of cognitive functions, such as math calculations, or measured by the paced auditory serial additions test. In some embodiments, treatment results in an increase in energy of the subject. In some embodiments treatment results in a decrease in pain of

the subject. In some embodiments, treatment results in a decrease in visual impairment. In some embodiments, treatment results in improved bladder or bowel control.

[0297] In some embodiments, the treatment in accord with the provided methods results in clinical remission of MS in the subject that is maintained for greater than 3 months. In some embodiments, the treatment in accord with the provided methods results in clinical remission of MS in the subject that is maintained for greater than 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 18 months, 24 months, 3 years, 4 years, 5 years or more. In some embodiments, the treatment in accord with the provided methods results in clinical remission of MS in the subject that is maintained for greater than 6 months. In some embodiments, the treatment in accord with the provided methods results in clinical remission of MS in the subject that is maintained for greater than 12 months. In some embodiments, the treatment in accord with the provided methods results in clinical remission of MS in the subject that is maintained for greater than 24 months. In some embodiments, the treatment in accord with the provided methods results in clinical remission of MS in the subject that is maintained for greater than 3 years. In some embodiments, the treatment in accord with the provided methods results in clinical remission of MS in the subject that is maintained for greater than 4 years. In some embodiments, the treatment in accord with the provided methods results in clinical remission of MS in the subject that is maintained for greater than 5 years.

[0298] In some embodiments, the treatment in accord with the provided methods results in prolonged remission. In some embodiments, prolonged remission is defined as a 5-year consecutive period of no disease activity and without treatment (fingolimod, Siponimod, ozanimod, natalizumab, dimethyl fumarate, teriflunomide, ocrelizumab, ofatumumab, alemtuzumab, anti-CD20 antibodies, or immunosuppressants).

[0299] In some cases, the pharmacokinetics of administered cells, *e.g.*, adoptively transferred cells are determined to assess the availability, *e.g.*, bioavailability of the administered cells. Methods for determining the pharmacokinetics of adoptively transferred cells may include drawing peripheral blood from subjects that have been administered engineered cells, and determining the number or ratio of the engineered cells in the peripheral blood. Approaches for selecting and/or isolating cells may include use of chimeric antigen receptor (CAR)-specific antibodies (*e.g.*, Brentjens et al., *Sci. Transl. Med.* 2013 Mar; 5(177): 177ra38) Protein L (Zheng et al., *J. Transl. Med.* 2012 Feb; 10:29), epitope tags, such as Strep-Tag sequences, introduced directly into specific sites in the CAR, whereby binding reagents for Strep-Tag are used to

directly assess the CAR (Liu et al. (2016) Nature Biotechnology, 34:430; international patent application Pub. No. WO2015095895) and monoclonal antibodies that specifically bind to a CAR polypeptide (see international patent application Pub. No. WO2014190273). Extrinsic marker genes may in some cases be utilized in connection with engineered cell therapies to permit detection or selection of cells and, in some cases, also to promote cell suicide. A truncated epidermal growth factor receptor (EGFRt) in some cases can be co-expressed with a transgene of interest (a CAR) in transduced cells (see *e.g.*, U.S. Patent No. 8,802,374). EGFRt may contain an epitope recognized by the antibody cetuximab (Erbix®) or other therapeutic anti-EGFR antibody or binding molecule, which can be used to identify or select cells that have been engineered with the EGFRt construct and another recombinant receptor, such as a chimeric antigen receptor (CAR), and/or to eliminate or separate cells expressing the receptor. See U.S. Patent No. 8,802,374 and Liu et al., Nature Biotech. 2016 April; 34(4): 430–434).

[0300] In some embodiments, the number of CAR⁺ T cells in a biological sample obtained from the patient, *e.g.*, blood, can be determined at a period of time after administration of the cell therapy, *e.g.*, to determine the pharmacokinetics of the cells. In some embodiments, number of CAR⁺ T cells, optionally CAR⁺ CD8⁺ T cells and/or CAR⁺ CD4⁺ T cells, detectable in the blood of the subject, or in a majority of subjects so treated by the method, is greater than 1 cells per μL , greater than 5 cells per μL or greater than 10 cells per μL .

5. Rheumatoid arthritis (RA)

[0301] In some embodiments, the systemic autoimmune disease is Rheumatoid arthritis (RA). Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease that affects 1% of the population. Disease progression is characterized by a destructive inflammation of the joints, which can lead to progressive disability and a reduced life expectancy. The synovial membrane in RA is infiltrated by activated immune cells, most abundantly macrophages and T cells, resulting in the chronic production of proinflammatory cytokines and matrix metalloproteinases, leading to inflammation and cartilage and bone degradation (Choy EH and Panayi GS., N Engl Jmed. 2001; 344:907- 916).

[0302] A patient to be treated may have RA as determined according to the 1987 ACR criteria. The patient may test positive for rheumatoid factor (RF) and/or anti-cyclic citrullinated peptide (CCP) IgG antibodies prior to treatment. RF positive and anti-CCP antibody positive

status confirm diagnosis of RA. The patient may have had RA for a duration of at least 5 years or at least 7 years, for example between 5 and 10 years.

[0303] In some embodiments, the methods and use of provided CD19-directed CAR engineered cells (*e.g.*, T cells) and/or compositions thereof, include methods for the treatment of subjects with MS that have failed at least two or more prior therapies. In particular embodiments, the method includes administering to the subject a dose of T cells that includes CD4+ and CD8+ T cells, wherein the T cells comprises a chimeric antigen receptor (CAR) that specifically binds to CD19.

[0304] In any of the embodiments herein, at or immediately prior to the time of the administration of the composition comprising engineered T cells, the subject has relapsed following remission after treatment with, or become refractory to one or more prior therapies for RA.

[0305] In any of the embodiments herein, at or immediately prior to the time of the administration of the composition comprising engineered T cells, the subject has relapsed following remission after treatment with, or become refractory to, one or more prior therapies for RA. In any of the embodiments herein, at or immediately prior to the time of the administration of the composition comprising engineered T cells, the subject has relapsed following treatment with, or become refractory to, one or more prior therapies for the RA. In any of the embodiments herein, the one or more prior therapies for the RA does not comprise another dose of cells expressing the CAR.

[0306] In some embodiments, the provided methods and uses involving administration of an anti-CD19 CAR T cell therapy reduce RA disease activity in the subject. In some embodiments, a reduction in RA disease activity is evident where there is a clinical benefit to the subject after administration of anti-CD19 CAR T cell therapy.

[0307] One measure of how well RA is being controlled is the Disease Activity Score (DAS) (Fransen & van Riel Clin Exp Rheumatol 23:S93-S99 2005). The DAS is calculated by a medical practitioner based on various validated measures of disease activity, including physical symptoms of RA. A reduction in DAS reflects a reduction in disease severity. A DAS of less than 2.6 indicates disease remission. DAS between 2.6 and 3.2 indicates low disease activity. A DAS greater than 3.2 indicates increased disease activity and at this level a patient's therapy could be reviewed to determine whether a change in therapy is warranted. DAS greater than 5.1 indicates severe disease activity. Variations in calculating DAS can include assessing different

numbers of joints in the patient and monitoring different blood components. DAS28 is the Disease Activity Score in which 28 joints in the body are assessed to determine the number of tender joints and the number of swollen joints (Prevoo et al. *Arthritis Rheum* 38:44-48 1995). When the DAS28 calculation includes a measurement of C-reactive protein (CRP) rather than erythrocyte sedimentation rate (ESR), it is referred to as DAS28-CRP (Smolen et al. *Rheumatology* 42:244-257 2003; Wells G, et al. *Annals of the Rheumatic Diseases* 68: 954-960 2009). CRP is believed to be a more direct measure of inflammation than ESR, and is more sensitive to short term changes (Kushner, *Arthritis Rheum* 34: 1065-68 1991). CRP production is associated with radiological progression in RA (van Leeuwen MA, et al. *Br J Rheumatol* 32(suppl 3):9-13 1993) and is considered at least as valid as ESR to measure RA disease activity (Mallya RK, et al. *J Rheumatol* 9:224-8 1982; Wolfe F. *J Rheumatol* 24: 1477-85 1997).

[0308] The American College of Rheumatology (ACR) proposed a set of criteria for classifying RA. The commonly used criteria are the ACR 1987 revised criteria (Arnett et al. *Arthritis Rheum.* 31:315-324 1988). Diagnosis of RA according to the ACR criteria requires a patient to satisfy a minimum number of listed criteria, such as tender or swollen joint counts, stiffness, pain, radiographic indications and measurement of serum rheumatoid factor. ACR 20, ACR 50 and ACR 70 are commonly used measures to express efficacy of RA therapy, particularly in clinical trials. ACR 20 represents a 20% improvement in the measured ACR criteria. Analogously, ACR 50 represents a 50% improvement in the measured ACR criteria, and ACR 70 represents a represents a 70% improvement in the measured ACR criteria. An individual, patient-reported measure of disability in RA patients is the Health Assessment Questionnaire Disability Index (HAQ-DI). HAQ-DI scores represent physical function in terms of the patient's reported ability to perform everyday tasks, including the level of difficulty they experience in carrying out the activity. By recording patients' ability to perform everyday activities, the HAQ-DI score can be used as one measure of their quality of life.

[0309] The clinical benefit can comprise remission of RA. Typically, remission is defined by a DAS28-CRP of less than 2.6.

[0310] The clinical benefit can be an improvement of at least 20%, at least 50% or at least 70% treatment efficacy as determined by the 1987 ACR criteria, i.e. the clinical benefit can be achieving ACR 20, ACR 50 or ACR 70, respectively.

[0311] A form of clinical benefit that is of particular value to RA patients is an improvement in their ability to perform everyday activities. Methods of the disclosure can comprise

improvement in the patient's self-assessed disability measured by the Health Assessment Questionnaire, known as HAQ-DI. Methods comprising providing clinical benefit to an RA patient, wherein the clinical benefit comprises improving physical function of an RA patient as determined by HAQ-DI, and compositions and kits for use in such methods, are all aspects of the disclosure. Clinical benefit can comprise improving physical function of an RA patient as determined by HAQ-DI. In certain embodiments, a statistically significant improvement in HAQ-DI is achieved within twelve, ten, eight or six weeks of starting treatment according to the disclosure, or within four weeks, or within two weeks. The improvement can be at least a 0.25 improvement in HAQ-DI, *i.e.* a reduction of 0.25 or more in the patient's HAQ-DI score. In certain embodiments, the improvement is at least a 0.30, 0.40 or 0.45 improvement in HAQ-DI score. Improvement is generally measured with reference to the patient's baseline average HAQ-DI score prior to treatment with an inhibitor according to the disclosure.

[0312] Patients can be monitored during and/or following a course of treatment with anti-CD19 CAR T cell therapy, to assess the level of clinical benefit, for example by measuring DAS28-CRP and/or determining clinical benefit according to the ACR criteria and/or measuring HAQ-DI. The method can comprise determining that the clinical benefit is achieved, *e.g.* that the specified reduction in DAS28-CRP, and/or achievement of ACR 20, ACR 50 or ACR 70 is met, and/or that the HAQ-DI score is improved, as discussed elsewhere herein.

B. Dosing

[0313] In some embodiments, a dose of engineered cells is administered to subjects in accordance with the provided methods, and/or with the provided articles of manufacture or compositions. In some embodiments, the size or timing of the doses is determined as a function of the particular disease or condition in the subject. In some cases, the size or timing of the doses for a particular disease in view of the provided description may be empirically determined.

[0314] In some of any of the provided embodiments, the dose of T cells, such as engineered T cells expressing a recombinant receptor, includes is enriched for, or comprises a cell composition or a cell population that is enriched for, CD3+ T cells, CD4+ T cells, CD8+ T cells or CD4+ T cells and CD8+ T cells. In some of any such embodiments, greater than at or about 70%, 75%, 80%, 85%, 90%, 95% or 98% of the cells in the dose of T cells are CD3+ T cells, CD4+ T cells, CD8+ T cells or CD4+ T cells and CD8+ T cells. In some of any such embodiments, greater than at or about 70%, 75%, 80%, 85%, 90%, 95% or 98% of the cells in

the dose of T cells are CD3+ T cells. In some of any of the provided embodiments, the dose of T cells comprises both CD4+ cells and CD8+ cells. In some of any such embodiments, greater than at or about 70%, 75%, 80%, 85%, 90%, 95% or 98% of the cells in the dose of T cells are CD4+ T cells and CD8+ T cells.

[0315] In some embodiments, the dose of cells comprises between at or about 0.1×10^5 of the CD19-directed CAR engineered cells per kilogram body weight of the subject (cells/kg) and at or about 2×10^6 cells/kg, such as between at or about 0.1×10^5 cells/kg and at or about 0.5×10^5 cells/kg, between at or about 0.5×10^5 cells/kg and at or about 1×10^5 cells/kg, between at or about 1×10^5 cells/kg and at or about 1.5×10^5 cells/kg, between at or about 1.5×10^5 cells/kg and at or about 2×10^5 cells/kg, between at or about 2×10^5 cells/kg and at or about 2.5×10^5 cells/kg, between at or about 2.5×10^5 cells/kg and at or about 3×10^5 cells/kg, between at or about 3×10^5 cells/kg and at or about 3.5×10^5 cells/kg, between at or about 3.5×10^5 cells/kg and at or about 4×10^5 cells/kg, between at or about 4×10^5 cells/kg and at or about 4.5×10^5 cells/kg, between at or about 4.5×10^5 cells/kg and at or about 5×10^5 cells/kg, between at or about 5×10^5 cells/kg and at or about 5.5×10^5 cells/kg, between at or about 5.5×10^5 cells/kg and at or about 6×10^5 cells/kg, between at or about 6×10^5 cells/kg and at or about 6.5×10^5 cells/kg, between at or about 6.5×10^5 cells/kg and at or about 7×10^5 cells/kg, between at or about 7×10^5 cells/kg and at or about 7.5×10^5 cells/kg, between at or about 7.5×10^5 cells/kg and at or about 8×10^5 cells/kg, or between at or about 8×10^5 of the cells/kg and at or about 10×10^5 of the cells/kg. In some embodiments, the dose of cells comprises no more than 2×10^5 of the CD19-directed CAR engineered cells per kilogram body weight of the subject (cells/kg), such as no more than at or about 3×10^5 cells/kg, no more than at or about 4×10^5 cells/kg, no more than at or about 5×10^5 cells/kg, no more than at or about 6×10^5 cells/kg, no more than at or about 7×10^5 cells/kg, no more than at or about 8×10^5 cells/kg, no more than at or about 9×10^5 cells/kg, no more than at or about 1×10^6 cells/kg, or no more than at or about 2×10^6 cells/kg. In some embodiments, the dose of cells comprises at least or at least about or at or about 0.1×10^5 of the CD19-directed CAR engineered cells per kilogram body weight of the subject (cells/kg), such as at least or at least about or at or about 0.2×10^5 cells/kg, at least or at least about or at or about 0.3×10^5 cells/kg, at least or at least about or at or about 0.4×10^5 cells/kg, at least or at least about or at or about 0.5×10^5 cells/kg, at least or at least about or at or about 0.6×10^5 cells/kg, at least or at least about or at or about 0.7×10^5 cells/kg, at least or at least about or at or about 0.8×10^5 cells/kg, at least or at least about or at or about 0.9×10^5

cells/kg, at least or at least about or at or about 0.1×10^6 cells/kg, or at least or at least about or at or about 0.2×10^6 cells/kg. In some embodiments, the number of cells is the number of such cells that are viable cells, *e.g.*, viable T cells such as viable CD3+ cells expressing the CD19-directed CAR.

[0316] In certain embodiments, the cells, or individual populations of sub-types of cells, are administered to the subject at a range of at or about 0.1 million to at or about 100 billion cells and/or that amount of cells per kilogram of body weight of the subject, such as, *e.g.*, at or about 0.1 million to at or about 50 billion cells (*e.g.*, at or about 5 million cells, at or about 25 million cells, at or about 500 million cells, at or about 1 billion cells, at or about 5 billion cells, at or about 20 billion cells, at or about 30 billion cells, at or about 40 billion cells, or a range defined by any two of the foregoing values), at or about 1 million to at or about 50 billion cells (*e.g.*, at or about 5 million cells, at or about 25 million cells, at or about 500 million cells, at or about 1 billion cells, at or about 5 billion cells, at or about 20 billion cells, at or about 30 billion cells, at or about 40 billion cells, or a range defined by any two of the foregoing values), such as at or about 10 million to at or about 100 billion cells (*e.g.*, at or about 20 million cells, at or about 30 million cells, at or about 40 million cells, at or about 60 million cells, at or about 70 million cells, at or about 80 million cells, at or about 90 million cells, at or about 10 billion cells, at or about 25 billion cells, at or about 50 billion cells, at or about 75 billion cells, at or about 90 billion cells, or a range defined by any two of the foregoing values), and in some cases at or about 100 million cells to at or about 50 billion cells (*e.g.*, at or about 120 million cells, at or about 250 million cells, at or about 350 million cells, at or about 650 million cells, at or about 800 million cells, at or about 900 million cells, at or about 3 billion cells, at or about 30 billion cells, at or about 45 billion cells) or any value in between these ranges and/or per kilogram of body weight of the subject. Dosages may vary depending on attributes particular to the disease or disorder and/or patient and/or other treatments. In some embodiments, such values refer to numbers of recombinant receptor-expressing cells; in other embodiments, they refer to number of T cells or total cells in the composition administered. In some embodiments, the number of cells is the number of such cells that are viable cells.

[0317] In some embodiments, the dose of cells is a flat dose of cells or fixed dose of cells such that the dose of cells is not tied to or based on the body surface area or weight of a subject. In some embodiments, administration of a higher number of cytotoxic cells based on weight of a subject may contribute to increased risk of toxicity, such as neurotoxicity, in the subject.

[0318] In some embodiments, the dose of genetically engineered cells comprises from at or about 1×10^5 to at or about 1×10^8 total T cells expressing the CD19-directed CAR, from at or about 1×10^5 to at or about 1.0×10^7 total T cells expressing the CD19-directed CAR, from at or about 1×10^5 to at or about 1.0×10^6 total T cells expressing the CD19-directed CAR, from at or about 1×10^6 to at or about 1.0×10^8 total T cells expressing the CD19-directed CAR, from at or about 1×10^6 to at or about 1.0×10^7 total T cells expressing the CD19-directed CAR, from at or about 5×10^6 to at or about 1.0×10^8 total T cells expressing the CD19-directed CAR, from at or about 5×10^6 to at or about 1.0×10^7 total T cells expressing the CD19-directed CAR, from at or about 10×10^6 to at or about 1.0×10^8 total T cells expressing the CD19-directed CAR. In some embodiments, the number of cells is the number of such cells that are viable cells, such as viable T cells.

[0319] In some embodiments, the dose of genetically engineered cells comprises from at or about 1×10^5 to at or about 1×10^8 total viable T cells expressing the CD19-directed CAR, from at or about 1×10^5 to at or about 1.0×10^7 total viable T cells expressing the CD19-directed CAR, from at or about 1×10^5 to at or about 1.0×10^6 total viable T cells expressing the CD19-directed CAR, from at or about 1×10^6 to at or about 1.0×10^8 total viable T cells expressing the CD19-directed CAR, from at or about 1×10^6 to at or about 1.0×10^7 total viable T cells expressing the CD19-directed CAR, from at or about 5×10^6 to at or about 1.0×10^8 total viable T cells expressing the CD19-directed CAR, from at or about 5×10^6 to at or about 1.0×10^7 total viable T cells expressing the CD19-directed CAR, from at or about 10×10^6 to at or about 1.0×10^8 total viable T cells expressing the CD19-directed CAR.

[0320] In some embodiments, the dose of cells is a relatively low dose. In some embodiments, anti-CD19 CAR T cell compositions for use in the provided embodiments include cells with a less differentiated phenotype, with a majority of the cells having a naïve-like or central memory cell phenotype. Furthermore, in provided embodiments, compositions include populations of T cells in which greater than 25% of the T cells (e.g., CD3+ T cells) express the CAR, such as greater than 30%, 35%, 40%, 45% or 50% of the T cells (e.g., CD3+ T cells) express the CAR. In some embodiments, compositions include populations of T cells in which greater than 50% of the T cells (e.g., CD3+ T cells) express the CAR, such as greater than 60%, greater than 70% or greater than 80% of the T cells composition express the CAR. Without wishing to be bound by theory, compositions with features as provided herein ensure the cells exhibit higher potency and greater capacity to persist in the subject, while minimizing or

reducing potential toxicity of the CAR-expressing T cells. In some embodiments, anti-CD19 CAR T cells of the dose exhibit higher potency, persistency and/or less toxicity than cells of alternative compositions that include a higher percentage of cells that are more differentiated (*e.g.*, have a higher percentage of effector T cells). In some embodiments, anti-CD19 CAR T cells of the dose exhibit higher potency, persistency and/or less toxicity than cells of alternative compositions that include a lower percentage of cells that express the CAR. In some embodiments, the dose of genetically engineered cells can be administered in an amount that is less than 10×10^7 total T cells expressing the CD19-directed CAR.

[0321] In some embodiments, the dose of genetically engineered cells can be administered in an amount that is less than 9×10^7 total T cells expressing the CD19-directed CAR. In some embodiments, the dose of genetically engineered cells can be administered in an amount that is less than 8×10^7 total viable T cells expressing the CD19-directed CAR. In some embodiments, the dose of genetically engineered cells can be administered in an amount that is less than 7.5×10^7 total viable T cells expressing the CD19-directed CAR. In some embodiments, the dose of genetically engineered cells can be administered in an amount that is less than 7.0×10^7 total viable T cells expressing the CD19-directed CAR. In some embodiments, the dose of genetically engineered cells can be administered in an amount that is less than 6.0×10^7 total viable T cells expressing the CD19-directed CAR. In some embodiments, the dose of genetically engineered cells is from at or about 1×10^6 to at or about 50×10^6 total viable T cells expressing the CD19-directed CAR, from at or about 1×10^6 to at or about 40×10^6 total viable T cells expressing the CD19-directed CAR, at or about 1×10^6 to at or about 30×10^6 total viable T cells expressing the CD19-directed CAR, at or about 1×10^6 to at or about 20×10^6 total viable T cells expressing the CD19-directed CAR, at or about 1×10^6 to at or about 10×10^6 total viable T cells expressing the CD19-directed CAR, at or about 1×10^6 to at or about 5×10^6 total viable T cells expressing the CD19-directed CAR, at or about 1×10^6 to at or about 2.5×10^6 total viable T cells expressing the CD19-directed CAR, from at or about 2.5×10^6 to at or about 50×10^6 total viable T cells expressing the CD19-directed CAR, from at or about 2.5×10^6 to at or about 40×10^6 total viable T cells expressing the CD19-directed CAR, at or about 2.5×10^6 to at or about 30×10^6 total viable T cells expressing the CD19-directed CAR, at or about 2.5×10^6 to at or about 20×10^6 total viable T cells expressing the CD19-directed CAR, at or about 2.5×10^6 to at or about 10×10^6 total viable T cells expressing the CD19-directed CAR, at or about 2.5×10^6 to at or about 5×10^6 total viable T cells expressing the CD19-directed CAR, from at

or about 2.5×10^6 to at or about 50×10^6 total viable T cells expressing the CD19-directed CAR, from at or about 5×10^6 to at or about 50×10^6 total viable T cells expressing the CD19-directed CAR, from at or about 5×10^6 to at or about 40×10^6 total viable T cells expressing the CD19-directed CAR, at or about 5×10^6 to at or about 30×10^6 total viable T cells expressing the CD19-directed CAR, at or about 5×10^6 to at or about 20×10^6 total viable T cells expressing the CD19-directed CAR, at or about 5×10^6 to at or about 10×10^6 total viable T cells expressing the CD19-directed CAR, from at or about 10×10^6 to at or about 50×10^6 total viable T cells expressing the CD19-directed CAR, from at or about 10×10^6 to at or about 40×10^6 total viable T cells expressing the CD19-directed CAR, at or about 10×10^6 to at or about 30×10^6 total viable T cells expressing the CD19-directed CAR, at or about 10×10^6 to at or about 20×10^6 total viable T cells expressing the CD19-directed CAR, from at or about 20×10^6 to at or about 50×10^6 total viable T cells expressing the CD19-directed CAR, from at or about 20×10^6 to at or about 40×10^6 total viable T cells expressing the CD19-directed CAR, at or about 20×10^6 to at or about 30×10^6 total viable T cells expressing the CD19-directed CAR, from at or about 30×10^6 to at or about 50×10^6 total viable T cells expressing the CD19-directed CAR, from at or about 30×10^6 to at or about 40×10^6 total viable T cells expressing the CD19-directed CAR, or from at or about 40×10^6 to at or about 50×10^6 total viable T cells expressing the CD19-directed CAR.

[0322] In some embodiments, the dose of genetically engineered cells is from at or at or about 0.1×10^6 total T cells expressing the CD19-directed CAR, at or about 0.2×10^6 total viable T cells expressing the CD19-directed CAR, at or about 0.25×10^6 total viable T cells expressing the CD19-directed CAR, at or about 0.5×10^6 total viable T cells expressing the CD19-directed CAR, at or about 0.75×10^6 total T cells expressing the CD19-directed CAR, at or about 2×10^6 total viable T cells expressing the CD19-directed CAR, at or about 3×10^6 total viable T cells expressing the CD19-directed CAR, at or about 4×10^6 total viable T cells expressing the CD19-directed CAR, at or about 6×10^6 total viable T cells expressing the CD19-directed CAR, at or about 7×10^6 total viable T cells expressing the CD19-directed CAR, at or about 8×10^6 total viable T cells expressing the CD19-directed CAR, at or about 9×10^6 total viable T cells expressing the CD19-directed CAR, at or about 10×10^6 total viable T cells expressing the CD19-directed CAR, at or about 11×10^6 total viable T cells expressing the CD19-directed CAR, at or about 12×10^6 total viable T cells expressing the CD19-directed CAR, at or about 13×10^6 total viable T cells expressing the CD19-directed CAR, at or about 14

x 10^6 total viable T cells expressing the CD19-directed CAR, at or about 15×10^6 total viable T cells expressing the CD19-directed CAR, at or about 16×10^6 total viable T cells expressing the CD19-directed CAR, at or about 17×10^6 total viable T cells expressing the CD19-directed CAR, at or about 18×10^6 total viable T cells expressing the CD19-directed CAR, at or about 19×10^6 total viable T cells expressing the CD19-directed CAR, at or about 25×10^6 total viable T cells expressing the CD19-directed CAR, at or about 35×10^6 total viable T cells expressing the CD19-directed CAR, at or about 45×10^6 total viable T cells expressing the CD19-directed CAR, at or about 60×10^6 total viable T cells expressing the CD19-directed CAR, at or about 70×10^6 total T cells expressing the CD19-directed CAR, at or about 75×10^6 total T cells expressing the CD19-directed CAR, at or about 80×10^6 total viable T cells expressing the CD19-directed CAR, at or about 90×10^6 , 100×10^6 total viable T cells expressing the CD19-directed CAR.

[0323] In some embodiments, the dose of genetically engineered cells is from at or about 5×10^6 to at or about 50×10^6 total viable T cells expressing the CD19-directed CAR. In some embodiments, the dose of genetically engineered cells is from at or about 10×10^6 to at or about 50×10^6 total viable T cells expressing the CD19-directed CAR.

[0324] In some embodiments, the dose of genetically engineered cells is about 5×10^6 total viable T cells expressing the CD19-directed CAR. In some embodiments, a single dose of about 5×10^6 T cells expressing the CD19-directed CAR is administered to the subject.

[0325] In some embodiments, the dose of genetically engineered cells is about 10×10^6 total viable T cells expressing the CD19-directed CAR. In some embodiments, a single dose of about 10×10^6 T cells expressing the CD19-directed CAR is administered to the subject.

[0326] In some embodiments, the dose of genetically engineered cells is about 15×10^6 total viable T cells expressing the CD19-directed CAR. In some embodiments, a single dose of about 15×10^6 T cells expressing the CD19-directed CAR is administered to the subject.

[0327] In some embodiments, the dose of genetically engineered cells is about 20×10^6 total viable T cells expressing the CD19-directed CAR. In some embodiments, a single dose of about 20×10^6 T cells expressing the CD19-directed CAR is administered to the subject.

[0328] In some embodiments, the dose of genetically engineered cells is about 25×10^6 total viable T cells expressing the CD19-directed CAR. In some embodiments, a single dose of about 25×10^6 T cells expressing the CD19-directed CAR is administered to the subject.

[0329] In some embodiments, the dose of genetically engineered cells is about 30×10^6 total viable T cells expressing the CD19-directed CAR. In some embodiments, a single dose of about 30×10^6 T cells expressing the CD19-directed CAR is administered to the subject.

[0330] In some embodiments, the dose of genetically engineered cells is about 40×10^6 total viable T cells expressing the CD19-directed CAR. In some embodiments, a single dose of about 40×10^6 T cells expressing the CD19-directed CAR is administered to the subject.

[0331] In some embodiments, the dose of genetically engineered cells is about 50×10^6 total viable T cells expressing the CD19-directed CAR. In some embodiments, a single dose of about 50×10^6 T cells expressing the CD19-directed CAR is administered to the subject.

[0332] In some embodiments, the number is with reference to the total number of CD3⁺, CD8⁺, or CD4⁺ and CD8⁺, in some cases also recombinant receptor-expressing (*e.g.*, CAR⁺) cells. In some embodiments, the number of cells is the number of such cells that are viable cells.

[0333] In some embodiments, the T cells of the dose include CD4⁺ T cells, CD8⁺ T cells or CD4⁺ and CD8⁺ T cells.

[0334] In some embodiments, the T cells of the dose include CD4⁺ T cells, CD8⁺ T cells or CD4⁺ and CD8⁺ T cells.

[0335] In some embodiments, the dose of cells, *e.g.*, recombinant receptor-expressing T cells, is administered to the subject as a single dose or is administered only one time within a period of two weeks, one month, three months, six months, one year or more.

[0336] In the context of adoptive cell therapy, administration of a given “dose” encompasses administration of the given amount or number of cells as a single composition and/or single uninterrupted administration, *e.g.*, as a single injection or continuous infusion, and also encompasses administration of the given amount or number of cells as a split dose or as a plurality of compositions, provided in multiple individual compositions or infusions, over a specified period of time, such as over no more than 3 days. Thus, in some contexts, the dose is a single or continuous administration of the specified number of cells, given or initiated at a single point in time. In some contexts, however, the dose is administered in multiple injections or infusions over a period of no more than three days, such as once a day for three days or for two days or by multiple infusions over a single day period.

[0337] In particular embodiments, the numbers and/or concentrations of cells refer to the number of recombinant receptor (*e.g.*, CAR)-expressing cells. In other embodiments, the

numbers and/or concentrations of cells refer to the number or concentration of T cells administered.

[0338] In some embodiments, the subject receives multiple doses, *e.g.*, two or more doses or multiple consecutive doses, of the cells. In some embodiments, two doses are administered to a subject. In some embodiments, the subject receives the consecutive dose, *e.g.*, second dose, is administered approximately 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 days after the first dose. In some embodiments, multiple consecutive doses are administered following the first dose, such that an additional dose or doses are administered following administration of the consecutive dose. In some aspects, the number of cells administered to the subject in the additional dose is the same as or similar to the first dose and/or consecutive dose. In some embodiments, the additional dose or doses are larger than prior doses.

[0339] In some aspects, the size of the dose is determined based on one or more criteria such as response of the subject to prior treatment and/or likelihood or incidence of the subject developing toxic outcomes, *e.g.*, CRS, macrophage activation syndrome, neurotoxicity, and/or a host immune response against the cells and/or recombinant receptors being administered.

[0340] In some aspects, the time between the administration of the first dose and the administration of the consecutive dose is about 9 to about 35 days, about 14 to about 28 days, or 15 to 27 days. In some embodiments, the administration of the consecutive dose is at a time point more than about 14 days after and less than about 28 days after the administration of the first dose. In some aspects, the time between the first and consecutive dose is about 21 days. In some embodiments, an additional dose or doses, *e.g.*, consecutive doses, are administered following administration of the consecutive dose. In some aspects, the additional consecutive dose or doses are administered at least about 14 and less than about 28 days following administration of a prior dose. In some embodiments, the additional dose is administered less than about 14 days following the prior dose, for example, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 days after the prior dose. In some embodiments, no dose is administered less than about 14 days following the prior dose and/or no dose is administered more than about 28 days after the prior dose.

[0341] In some embodiments, the dose of cells is generally large enough to be effective in reducing disease burden.

[0342] In particular embodiments, the numbers and/or concentrations of cells refer to the number of recombinant receptor (*e.g.*, CAR)-expressing cells. In other embodiments, the

numbers and/or concentrations of cells refer to the number or concentration of all cells, T cells, or peripheral blood mononuclear cells (PBMCs) administered.

[0343] In some embodiments, the methods also include administering one or more additional doses of cells expressing a chimeric antigen receptor (CAR) and/or lymphodepleting therapy, and/or one or more steps of the methods are repeated. In some embodiments, the one or more additional dose is the same as the initial dose. In some embodiments, the one or more additional dose is different from the initial dose, *e.g.*, higher, such as at or about 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold or 10-fold or more higher than the initial dose, or lower, such as *e.g.*, higher, such as 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold or 10-fold or more lower than the initial dose. In some embodiments, administration of one or more additional doses is determined based on response of the subject to the initial treatment or any prior treatment and/or likelihood or incidence of the subject developing toxic outcomes, *e.g.*, CRS, macrophage activation syndrome, neurotoxicity, and/or a host immune response against the cells and/or recombinant receptors being administered.

C. Toxicity

[0344] In some embodiments, the provided methods are designed to or include features that result in a lower rate and/or lower degree of treatment-emergent or serious adverse events (AEs), AEs of special interest, laboratory abnormalities, or the development of Dose Limiting Toxicities (DLT). Adverse events are defined by the Common Terminology Criteria for Adverse Events (CTCAE). AEs range on a scale from 1 to 5, 1 being mild and 5 being death. In some embodiments, the provided methods are designed to or include features that result in a lower rate and/or lower degree of the development of DLT. In some embodiments, a DLT is characterized by hematological, non-hematological and/or organ-specific adverse events or side effects relative to baseline.

[0345] In some embodiments, the provided methods are designed to or include features that result in a lower rate and/or lower degree of toxicity, toxic outcome or symptom, toxicity-promoting profile, factor, or property, such as a symptom or outcome associated with or indicative of cytokine release syndrome (CRS) or neurotoxicity (NT), for example, compared to administration of an alternative cell therapy, such as an alternative CAR⁺ T cell composition and/or an alternative dosing of cells, *e.g.*, a dosing of cells that is not administered at a defined ratio. Cytokine release syndrome (CRS) and neurotoxicity can be graded according to the

American Society for Transplantation and Cellular Therapy (ASTCT) Consensus Grading System (see *e.g.*, Lee et al. Biol Blood Marrow Transplant. 2019 Apr;25(4):625-38)).

[0346] In some aspects, although the lower differentiation state of the engineered T cells administered as part of the methods provided herein (*e.g.*, the higher proportion of engineered T cells having a naïve-like or central memory phenotype, such as a phenotype selected from CCR7⁺CD45RA⁺, CD27⁺CCR7⁺, or CD62L⁺CCR7⁺) are expected to be more active than cells that are more differentiated, findings indicate that safety of the cell therapy can be successfully managed. In some aspects, providing a lower dose of the composition, *e.g.*, compared to a cell composition produced by a process in which the cells are more differentiated, such as a process that includes expansion of the cells, achieves robust efficacy and high safety. In some aspects, it is found that even higher doses of cells of the provided anti-CD19 CAR compositions can be administered while maintaining a lower degree of toxicity, such as a severe cytokine release syndrome (CRS) or severe neurotoxicity. Thus, the provided methods in some embodiments include the administration of higher doses of engineered T cells (*e.g.*, greater than 50×10^6 CAR-expressing T cells, such as at or about 100×10^6 CAR-expressing T cells), compared to methods that include the administration of an alternative cell therapy, such as an alternative CAR⁺ T cell composition with engineered T cells that are more differentiated than those administered herein.

[0347] In some embodiments, the provided methods do not result in a high rate or likelihood of toxicity or toxic outcomes, or reduces the rate or likelihood of toxicity or toxic outcomes, such as neurotoxicity (NT), cytokine release syndrome (CRS), such as compared to certain other cell therapies. In some embodiments, the methods do not result in, or do not increase the risk of, severe NT (sNT), severe CRS (sCRS), macrophage activation syndrome, fever of at least at or about 38 degrees Celsius for three or more days and a plasma level of CRP of at least at or about 20 mg/dL. In some embodiments, greater than or greater than about 30%, 35%, 40%, 50%, 55%, 60% or more of the subjects treated according to the provided methods do not exhibit any grade of CRS or any grade of neurotoxicity. In some embodiments, no more than 50% of subjects treated (*e.g.*, at least 60%, at least 70%, at least 80%, at least 90% or more of the subjects treated) exhibit a cytokine release syndrome (CRS) higher than grade 2 and/or a neurotoxicity higher than grade 2. In some embodiments, at least 50% of subjects treated according to the method (*e.g.*, at least 60%, at least 70%, at least 80%, at least 90% or more of the subjects treated) do not exhibit a severe toxic outcome (*e.g.*, severe CRS or severe

neurotoxicity), such as do not exhibit grade 3 or higher neurotoxicity and/or does not exhibit severe CRS, or does not do so within a certain period of time following the treatment, such as within a week, two weeks, or one month of the administration of the cells. In some embodiments, parameters assessed to determine certain toxicities include, but are not limited to adverse events (AEs), dose-limiting toxicities (DLTs), CRS and NT.

[0348] Administration of adoptive T cell therapy, such as treatment with T cells expressing chimeric antigen receptors, can induce toxic effects or outcomes such as cytokine release syndrome and neurotoxicity. In some examples, such effects or outcomes parallel high levels of circulating cytokines, which may underlie the observed toxicity.

[0349] In some aspects, the toxic outcome is or is associated with or indicative of cytokine release syndrome (CRS) or severe CRS (sCRS). CRS, *e.g.*, sCRS, can occur in some cases following adoptive T cell therapy and administration to subjects of other biological products. See Davila et al., *Sci Transl Med* 6, 224ra25 (2014); Brentjens et al., *Sci. Transl. Med.* 5, 177ra38 (2013); Grupp et al., *N. Engl. J. Med.* 368, 1509–1518 (2013); and Kochenderfer et al., *Blood* 119, 2709–2720 (2012); Xu et al., *Cancer Letters* 343 (2014) 172-78.

[0350] Typically, CRS is caused by an exaggerated systemic immune response mediated by, for example, T cells, B cells, NK cells, monocytes, and/or macrophages. Such cells may release a large amount of inflammatory mediators such as cytokines and chemokines. Cytokines may trigger an acute inflammatory response and/or induce endothelial organ damage, which may result in microvascular leakage, heart failure, or death. Severe, life-threatening CRS can lead to pulmonary infiltration and lung injury, renal failure, or disseminated intravascular coagulation. Other severe, life-threatening toxicities can include cardiac toxicity, respiratory distress, neurologic toxicity and/or hepatic failure. In some aspects, fever, especially high fever ($\geq 38.5^{\circ}\text{C}$ or $\geq 101.3^{\circ}\text{F}$), is associated with CRS or risk thereof. In some cases, features or symptoms of CRS mimic infection. In some embodiments, infection is also considered in subjects presenting with CRS symptoms, and monitoring by cultures and empiric antibiotic therapy can be administered. Other symptoms associated with CRS can include cardiac dysfunction, adult respiratory distress syndrome, renal and/or hepatic failure, coagulopathies, disseminated intravascular coagulation, and capillary leak syndrome.

[0351] CRS may be treated using anti-inflammatory therapy such as an anti-IL-6 therapy, *e.g.*, anti-IL-6 antibody, *e.g.*, tocilizumab, or antibiotics or other agents as described. Outcomes, signs and symptoms of CRS are known and include those described herein. In some

embodiments, where a particular dosage regimen or administration affects or does not affect a given CRS-associated outcome, sign, or symptom, particular outcomes, signs, and symptoms and/or quantities or degrees thereof may be specified.

[0352] In the context of administering CAR-expressing cells, CRS typically occurs 6-20 days after infusion of cells that express a CAR. See, Xu et al., Cancer Letters 343 (2014) 172-78. In some cases, CRS occurs less than 6 days or more than 20 days after CAR T cell infusion. The incidence and timing of CRS may be related to baseline cytokine levels at the time of infusion. Commonly, CRS involves elevated serum levels of interferon (IFN)- γ , tumor necrosis factor (TNF)- α , and/or interleukin (IL)-2. Other cytokines that may be rapidly induced in CRS are IL-1 β , IL-6, IL-8, and IL-10.

[0353] Exemplary outcomes associated with CRS include fever, rigors, chills, hypotension, dyspnea, acute respiratory distress syndrome (ARDS), encephalopathy, ALT/AST elevation, renal failure, cardiac disorders, hypoxia, neurologic disturbances, and death. Neurological complications include delirium, seizure-like activity, confusion, word-finding difficulty, aphasia, and/or becoming obtunded. Other CRS-related outcomes include fatigue, nausea, headache, seizure, tachycardia, myalgias, rash, acute vascular leak syndrome, liver function impairment, and renal failure. In some aspects, CRS is associated with an increase in one or more factors such as serum-ferritin, d-dimer, aminotransferases, lactate dehydrogenase and triglycerides, or with hypofibrinogenemia or hepatosplenomegaly. Other exemplary signs or symptoms associated with CRS include hemodynamic instability, febrile neutropenia, increase in serum C-reactive protein (CRP), changes in coagulation parameters (for example, international normalized ratio (INR), prothrombin time (PTI) and/or fibrinogen), changes in cardiac and other organ function, and/or absolute neutrophil count (ANC).

[0354] In some embodiments, outcomes associated with CRS include one or more of: persistent fever, *e.g.*, fever of a specified temperature, *e.g.*, greater than at or about 38 degrees Celsius, for two or more, *e.g.*, three or more, *e.g.*, four or more days or for at least three consecutive days; fever greater than at or about 38 degrees Celsius; elevation of cytokines, such as a max fold change, *e.g.*, of at least at or about 75, compared to pre-treatment levels of at least two cytokines (*e.g.*, at least two of the group consisting of interferon gamma (IFN γ), GM-CSF, IL-6, IL-10, Flt-3L, fractalkine, and IL-5, and/or tumor necrosis factor alpha (TNF α)), or a max fold change, *e.g.*, of at least at or about 250 of at least one of such cytokines; and/or at least one clinical sign of toxicity, such as hypotension (*e.g.*, as measured by at least one intravenous

vasoactive pressor); hypoxia (*e.g.*, plasma oxygen (PO₂) levels of less than at or about 90%); and/or one or more neurologic disorders (including mental status changes, obtundation, and seizures). In some embodiments, neurotoxicity (NT) can be observed concurrently with CRS.

[0355] Exemplary CRS-related outcomes include increased or high serum levels of one or more factors, including cytokines and chemokines and other factors associated with CRS. Exemplary outcomes further include increases in synthesis or secretion of one or more of such factors. Such synthesis or secretion can be by the T cell or a cell that interacts with the T cell, such as an innate immune cell or B cell.

[0356] In some embodiments, the CRS-associated serum factors or CRS-related outcomes include inflammatory cytokines and/or chemokines, including interferon gamma (IFN- γ), IL-7, IL-12, sIL-2Ra, granulocyte macrophage colony stimulating factor (GM-CSF), macrophage inflammatory protein (MIP)-1, tumor necrosis factor alpha (TNF α), IL-6, and IL-10, IL-1 β , IL-8, IL-2, MIP-1, Flt-3L, fractalkine, and/or IL-5. In some embodiments, the factor or outcome includes C reactive protein (CRP). In addition to being an early and easily measurable risk factor for CRS, CRP also is a marker for cell expansion. In some embodiments, subjects that are measured to have high levels of CRP, such as ≥ 15 mg/dL, have CRS. In some embodiments, subjects that are measured to have high levels of CRP do not have CRS. In some embodiments, a measure of CRS includes a measure of CRP and another factor indicative of CRS.

[0357] In some embodiments, one or more inflammatory cytokines or chemokines are monitored before, during, or after CAR treatment. In some aspects, the one or more cytokines or chemokines include IFN- γ , TNF- α , IL-2, IL-1 β , IL-6, IL-7, IL-8, IL-10, IL-12, sIL-2Ra, granulocyte macrophage colony stimulating factor (GM-CSF), or macrophage inflammatory protein (MIP). In some embodiments, IFN- γ , TNF- α , and IL-6 are monitored.

[0358] CRS criteria that appear to correlate with the onset of CRS to predict which patients are more likely to be at risk for developing sCRS have been developed (see Davilla et al. Science translational medicine. 2014;6(224):224ra25). Factors include fevers, hypoxia, hypotension, neurologic changes, elevated serum levels of inflammatory cytokines, such as a set of seven cytokines (IFN γ , IL-5, IL-6, IL-10, Flt-3L, fractalkine, and GM-CSF). Other guidelines on the diagnosis and management of CRS are known (see *e.g.*, Lee et al, Blood. 2014;124(2):188-95; Lee et al., Biol Blood Marrow Transplant 2019; 25(4):625-38). In some embodiments, the criteria reflective of CRS grade are those detailed in **Table 1** below.

Table 1: Exemplary Grading Criteria for CRS	
Grade	Description of Symptoms
1 Mild	Not life-threatening, require only symptomatic treatment such as antipyretics and anti-emetics (<i>e.g.</i> , fever, nausea, fatigue, headache, myalgias, malaise)
2 Moderate	Require and respond to moderate intervention: ☐ Oxygen requirement < 40%, or ☐ Hypotension responsive to fluids or low dose of a single vasopressor, or ☐ Grade 2 organ toxicity (by CTCAE v4.0)
3 Severe	Require and respond to aggressive intervention: ☐ Oxygen requirement $\geq$ 40%, or ☐ Hypotension requiring high dose of a single vasopressor (<i>e.g.</i> , norepinephrine $\geq$ 20 $\mu\text{g/kg/min}$, dopamine $\geq$ 10 $\mu\text{g/kg/min}$, phenylephrine $\geq$ 200 $\mu\text{g/kg/min}$, or epinephrine $\geq$ 10 $\mu\text{g/kg/min}$), or ☐ Hypotension requiring multiple vasopressors (<i>e.g.</i> , vasopressin + one of the above agents, or combination vasopressors equivalent to $\geq$ 20 $\mu\text{g/kg/min}$ norepinephrine), or ☐ Grade 3 organ toxicity or Grade 4 transaminitis (by CTCAE v4.0)
4 Life-threatening	Life-threatening: ☐ Requirement for ventilator support, or ☐ Grade 4 organ toxicity (excluding transaminitis)
5 Fatal	Death

[0359] In some embodiments, a criteria reflective of CRS grade are those detailed in **Table 2** below.

Table 2. Exemplary Grading Criteria for CRS				
Symptoms/Signs	Grade 1 (mild)	Grade 2 (moderate)	Grade 3 (severe)	Grade 4 (life-threatening)
		CRS grade is defined by the most severe symptom (excluding fever)		
Temperature $\geq$ 38.5°C/101.3°F	Any	Any	Any	Any
Systolic blood pressure $\leq$ 90 mm Hg	N/A	Responds to fluid or single low-dose vasopressor	Needs high-dose or multiple vasopressors	Life-threatening

Need for oxygen to reach $\text{SaO}_2 > 90\%$	N/A	$\text{FiO}_2 < 40\%$	$\text{FiO}_2 \geq 40\%$	Needs ventilator support
Organ toxicity	N/A	Grade 2	Grade 3 or transaminitis	Grade 4 (excluding transaminitis)

[0360] In some embodiments, high-dose vasopressor therapy includes those described in **Table 3** below.

Table 3. High dose vasopressors (all doses required for ≥ 3 hours)	
Vasopressor	Dose
Norepinephrine monotherapy	$\geq 20 \mu\text{g}/\text{min}$
Dopamine monotherapy	$\geq 10 \mu\text{g}/\text{kg}/\text{min}$
Phenylephrine monotherapy	$\geq 200 \mu\text{g}/\text{min}$
Epinephrine monotherapy	$\geq 10 \mu\text{g}/\text{min}$
If on vasopressin	Vasopressin + norepinephrine equivalent (NE) of $\geq 10 \mu\text{g}/\text{min}^a$
If on combination vasopressors (not vasopressin)	Norepinephrine equivalent of $\geq 20 \mu\text{g}/\text{min}^a$
^a VASST Trial Vasopressor Equivalent Equation: Norepinephrine equivalent dose = $[\text{norepinephrine } (\mu\text{g}/\text{min})] + [\text{dopamine } (\mu\text{g}/\text{kg}/\text{min}) \div 2] + [\text{epinephrine } (\mu\text{g}/\text{min})] + [\text{phenylephrine } (\mu\text{g}/\text{min}) \div 10]$	

[0361] In some embodiments, the toxic outcome is a severe CRS. In some embodiments, the toxic outcome is the absence of severe CRS (*e.g.*, moderate or mild CRS). In some embodiments, a subject is deemed to develop “severe CRS” (“sCRS”) in response to or secondary to administration of a cell therapy or dose of cells thereof, if, following administration, the subject displays: (1) fever of at least 38 degrees Celsius for at least three days; (2) cytokine elevation that includes either (a) a max fold change of at least 75 for at least two of the following group of seven cytokines compared to the level immediately following the administration: interferon gamma ($\text{IFN}\gamma$), GM-CSF, IL-6, IL-10, Flt-3L, fracktalkine, and IL-5 and/or (b) a max fold change of at least 250 for at least one of the following group of seven cytokines compared to the level immediately following the administration: interferon gamma ($\text{IFN}\gamma$), GM-CSF, IL-6, IL-10, Flt-3L, fracktalkine, and IL-5; and (c) at least one clinical sign of toxicity such as hypotension (requiring at least one intravenous vasoactive pressor) or hypoxia ($\text{PO}_2 < 90\%$) or one or more neurologic disorder(s) (including mental status changes, obtundation, and/or seizures). In some embodiments, severe CRS includes CRS with a grade of 3 or greater, such as set forth in **Table 1** and **Table 2**.

[0362] In some embodiments, the level of the toxic outcome, *e.g.*, the CRS-related outcome, *e.g.*, the serum level of an indicator of CRS, is measured by ELISA. In some embodiments, fever and/or levels of C-reactive protein (CRP) can be measured. In some embodiments, subjects with a fever and a CRP ≥ 15 mg/dL may be considered high-risk for developing severe CRS. In some embodiments, the CRS-associated serum factors or CRS-related outcomes include an increase in the level and/or concentration of inflammatory cytokines and/or chemokines, including Flt-3L, fractalkine, granulocyte macrophage colony stimulating factor (GM-CSF), interleukin-1 beta (IL-1 β), IL-2, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, interferon gamma (IFN- γ), macrophage inflammatory protein (MIP)-1, MIP-1, sIL-2R α , or tumor necrosis factor alpha (TNF α). In some embodiments, the factor or outcome includes C reactive protein (CRP). In addition to being an early and easily measurable risk factor for CRS, CRP also is a marker for cell expansion. In some embodiments, subjects that are measured to have high levels of CRP, such as ≥ 15 mg/dL, have CRS. In some embodiments, subjects that are measured to have high levels of CRP do not have CRS. In some embodiments, a measure of CRS includes a measure of CRP and another factor indicative of CRS.

[0363] In some embodiments, outcomes associated with severe CRS or grade 3 CRS or greater, such as grade 4 or greater, include one or more of: persistent fever, *e.g.*, fever of a specified temperature, *e.g.*, greater than at or about 38 degrees Celsius, for two or more, *e.g.*, three or more, *e.g.*, four or more days or for at least three consecutive days; fever greater than at or about 38 degrees Celsius; elevation of cytokines, such as a max fold change, *e.g.*, of at least at or about 75, compared to pre-treatment levels of at least two cytokines (*e.g.*, at least two of the group consisting of interferon gamma (IFN γ), GM-CSF, IL-6, IL-10, Flt-3L, fractalkine, and IL-5, and/or tumor necrosis factor alpha (TNF α)), or a max fold change, *e.g.*, of at least at or about 250 of at least one of such cytokines; and/or at least one clinical sign of toxicity, such as hypotension (*e.g.*, as measured by at least one intravenous vasoactive pressor); hypoxia (*e.g.*, plasma oxygen (PO₂) levels of less than at or about 90%); and/or one or more neurologic disorders (including mental status changes, obtundation, and seizures). In some embodiments, severe CRS includes CRS that requires management or care in the intensive care unit (ICU).

[0364] In some embodiments, the CRS, such as severe CRS, encompasses a combination of (1) persistent fever (fever of at least 38 degrees Celsius for at least three days) and (2) a serum level of CRP of at least at or about 20 mg/dL. In some embodiments, the CRS encompasses hypotension requiring the use of two or more vasopressors or respiratory failure requiring

mechanical ventilation. In some embodiments, the dosage of vasopressors is increased in a second or subsequent administration.

[0365] In some embodiments, severe CRS or grade 3 CRS encompasses an increase in alanine aminotransferase, an increase in aspartate aminotransferase, chills, febrile neutropenia, headache, left ventricular dysfunction, encephalopathy, hydrocephalus, and/or tremor.

[0366] The method of measuring or detecting the various outcomes may be specified.

[0367] In some aspects, the toxic outcome is or is associated with neurotoxicity. In some embodiments, symptoms associated with a clinical risk of neurotoxicity include confusion, delirium, aphasia, expressive aphasia, obtundation, myoclonus, lethargy, altered mental status, convulsions, seizure-like activity, seizures (optionally as confirmed by electroencephalogram (EEG)), elevated levels of beta amyloid ($A\beta$), elevated levels of glutamate, and elevated levels of oxygen radicals. In some embodiments, neurotoxicity is graded based on severity (*e.g.*, using a Grade 1-5 scale (see, *e.g.*, Guido Cavaletti & Paola Marmiroli *Nature Reviews Neurology* 6, 657-666 (December 2010); National Cancer Institute—Common Toxicity Criteria version 4.03 (NCI-CTCAE v4.03)).

[0368] In some instances, neurologic symptoms may be the earliest symptoms of sCRS. In some embodiments, neurologic symptoms are seen to begin 5 to 7 days after cell therapy infusion. In some embodiments, duration of neurologic changes may range from 3 to 19 days. In some cases, recovery of neurologic changes occurs after other symptoms of sCRS have resolved. In some embodiments, time or degree of resolution of neurologic changes is not hastened by treatment with anti-IL-6 and/or steroid(s).

[0369] In some embodiments, a subject is deemed to develop “severe neurotoxicity” in response to or secondary to administration of a cell therapy or dose of cells thereof, if, following administration, the subject displays symptoms that limit self-care (*e.g.*, bathing, dressing and undressing, feeding, using the toilet, taking medications) from among: 1) symptoms of peripheral motor neuropathy, including inflammation or degeneration of the peripheral motor nerves; 2) symptoms of peripheral sensory neuropathy, including inflammation or degeneration of the peripheral sensory nerves, dysesthesia, such as distortion of sensory perception, resulting in an abnormal and unpleasant sensation, neuralgia, such as intense painful sensation along a nerve or a group of nerves, and/or paresthesia, such as functional disturbances of sensory neurons resulting in abnormal cutaneous sensations of tingling, numbness, pressure, cold and

warmth in the absence of stimulus. In some embodiments, severe neurotoxicity includes neurotoxicity with a grade of 3 or greater, such as set forth in **Table 4**.

Table 4: Exemplary Grading Criteria for neurotoxicity	
Grade	Description of Symptoms
1 Asymptomatic or Mild	Mild or asymptomatic symptoms
2 Moderate	Presence of symptoms that limit instrumental activities of daily living (ADL), such as preparing meals, shopping for groceries or clothes, using the telephone, managing money
3 Severe	Presence of symptoms that limit self-care ADL, such as bathing, dressing and undressing, feeding self, using the toilet, taking medications
4 Life-threatening	Symptoms that are life-threatening, requiring urgent intervention
5 Fatal	Death

[0370] In some embodiments, the methods reduce symptoms associated with CRS or neurotoxicity compared to other methods. In some aspects, the provided methods reduce symptoms, outcomes or factors associated with CRS, including symptoms, outcomes or factors associated with severe CRS or grade 3 or higher CRS, compared to other methods. For example, subjects treated according to the present methods may lack detectable and/or have reduced symptoms, outcomes or factors of CRS, *e.g.*, severe CRS or grade 3 or higher CRS, such as any described, *e.g.*, set forth in **Table 1** and **Table 2**. In some embodiments, subjects treated according to the present methods may have reduced symptoms of neurotoxicity, such as limb weakness or numbness, loss of memory, vision, and/or intellect, uncontrollable obsessive and/or compulsive behaviors, delusions, headache, cognitive and behavioral problems including loss of motor control, cognitive deterioration, and autonomic nervous system dysfunction, and sexual dysfunction, compared to subjects treated by other methods. In some embodiments, subjects treated according to the present methods may have reduced symptoms associated with peripheral motor neuropathy, peripheral sensory neuropathy, dysethesia, neuralgia or paresthesia.

[0371] In some embodiments, the methods reduce outcomes associated with neurotoxicity including damages to the nervous system and/or brain, such as the death of neurons. In some

aspects, the methods reduce the level of factors associated with neurotoxicity such as beta amyloid (A β), glutamate, and oxygen radicals.

[0372] In some embodiments, the toxicity outcome is a dose-limiting toxicity (DLT). In some embodiments, the toxic outcome is a dose-limiting toxicity. In some embodiments, the toxic outcome is the absence of a dose-limiting toxicity. In some embodiments, a dose-limiting toxicity (DLT) is defined as any grade 3 or higher toxicity as assessed by any known or published guidelines for assessing the particular toxicity, such as any described above and including the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

[0373] In some embodiments, the low rate, risk or likelihood of developing a toxicity, *e.g.*, CRS or neurotoxicity or severe CRS or neurotoxicity, *e.g.*, grade 3 or higher CRS or neurotoxicity, observed with administering a dose of T cells in accord with the provided methods, and/or with the provided articles of manufacture or compositions, permits administration of the cell therapy on an outpatient basis. In some embodiments, the administration of the cell therapy, *e.g.*, dose of T cells (*e.g.*, CAR⁺ T cells) in accord with the provided methods, and/or with the provided articles of manufacture or compositions, is performed on an outpatient basis or does not require admission to the hospital, such as admission to the hospital requiring an overnight stay.

[0374] In some aspects, subjects administered the cell therapy, *e.g.*, dose of T cells (*e.g.*, CAR⁺ T cells) in accord with the provided methods, and/or with the provided articles of manufacture or compositions, including subjects treated on an outpatient basis, are not administered an intervention for treating any toxicity prior to or with administration of the cell dose, unless or until the subject exhibits a sign or symptom of a toxicity, such as of a neurotoxicity or CRS. Exemplary agents for treating, delaying, attenuating or ameliorating a toxicity are described in Section I-C.

[0375] In some embodiments, if a subject administered the cell therapy, *e.g.*, dose of T cells (*e.g.*, CAR⁺ T cells), including subjects treated on an outpatient basis, exhibits a fever the subject is given or is instructed to receive or administer a treatment to reduce the fever. In some embodiments, the fever in the subject is characterized as a body temperature of the subject that is (or is measured at) at or above a certain threshold temperature or level. In some aspects, the threshold temperature is that associated with at least a low-grade fever, with at least a moderate fever, and/or with at least a high-grade fever. In some embodiments, the threshold temperature is

a particular temperature or range. For example, the threshold temperature may be at or about or at least at or about 38, 39, 40, 41, or 42 degrees Celsius, and/or may be a range of at or about 38 degrees Celsius to at or about 39 degrees Celsius, a range of at or about 39 degrees Celsius to at or about 40 degrees Celsius, a range of at or about 40 degrees Celsius to at or about 41 degrees, or a range of at or about 41 degrees Celsius to at or about 42 degrees Celsius.

[0376] In some embodiments, the treatment designed to reduce fever includes treatment with an antipyretic. An antipyretic may include any agent, *e.g.*, compound, composition, or ingredient, that reduces fever, such as one of any number of agents known to have antipyretic effects, such as NSAIDs (such as ibuprofen, naproxen, ketoprofen, and nimesulide), salicylates, such as aspirin, choline salicylate, magnesium salicylate, and sodium salicylate, paracetamol, acetaminophen, Metamizole, Nabumetone, Phenaxone, antipyrine, febrifuges. In some embodiments, the antipyretic is acetaminophen. In some embodiments, acetaminophen can be administered at a dose of 12.5 mg/kg orally or intravenously up to every four hours. In some embodiments, it is or comprises ibuprofen or aspirin.

[0377] In some embodiments, if the fever is a sustained fever, the subject is administered an alternative treatment for treating the toxicity. For subjects treated on an outpatient basis, the subject is instructed to return to the hospital if the subject has and/or is determined to or to have a sustained fever. In some embodiments, the subject has, and/or is determined to or considered to have, a sustained fever if he or she exhibits a fever at or above the relevant threshold temperature, and where the fever or body temperature of the subject is not reduced, or is not reduced by or by more than a specified amount (*e.g.*, by more than 1 °C, and generally does not fluctuate by about, or by more than about, 0.5 °C, 0.4 °C, 0.3 °C, or 0.2 °C), following a specified treatment, such as a treatment designed to reduce fever such as treatment with an antipyretic, *e.g.*, NSAID or salicylates, *e.g.*, ibuprofen, acetaminophen or aspirin. For example, a subject is considered to have a sustained fever if he or she exhibits or is determined to exhibit a fever of at least at or about 38 or 39 degrees Celsius, which is not reduced by or is not reduced by more than at or about 0.5 °C, 0.4 °C, 0.3 °C, or 0.2 °C, or by at or about 1%, 2%, 3%, 4%, or 5%, over a period of 6 hours, over a period of 8 hours, or over a period of 12 hours, or over a period of 24 hours, even following treatment with the antipyretic such as acetaminophen. In some embodiments, the dosage of the antipyretic is a dosage ordinarily effective in such as subject to reduce fever or fever of a particular type such as fever associated with a bacterial or viral infection, *e.g.*, a localized or systemic infection.

[0378] In some embodiments, the subject has, and/or is determined to or considered to have, a sustained fever if he or she exhibits a fever at or above the relevant threshold temperature, and where the fever or body temperature of the subject does not fluctuate by about, or by more than about, 1 °C, and generally does not fluctuate by about, or by more than about, 0.5 °C, 0.4 °C, 0.3 °C, or 0.2 °C. Such absence of fluctuation above or at a certain amount generally is measured over a given period of time (such as over a 24-hour, 12-hour, 8-hour, 6-hour, 3-hour, or 1-hour period of time, which may be measured from the first sign of fever or the first temperature above the indicated threshold). For example, in some embodiments, a subject is considered to or is determined to exhibit sustained fever if he or she exhibits a fever of at least at or about or at least at or about 38 or 39 degrees Celsius, which does not fluctuate in temperature by more than at or about 0.5°C, 0.4 °C, 0.3 °C, or 0.2 °C, over a period of 6 hours, over a period of 8 hours, or over a period of 12 hours, or over a period of 24 hours.

[0379] In some embodiments, the fever is a sustained fever; in some aspects, the subject is treated at a time at which a subject has been determined to have a sustained fever, such as within one, two, three, four, five six, or fewer hours of such determination or of the first such determination following the initial therapy having the potential to induce the toxicity, such as the cell therapy, such as dose of T cells, *e.g.*, CAR⁺ T cells.

[0380] In some embodiments, one or more interventions or agents for treating the toxicity, such as a toxicity-targeting therapies, is administered at a time at which or immediately after which the subject is determined to or confirmed to (such as is first determined or confirmed to) exhibit sustained fever, for example, as measured according to any of the aforementioned embodiments. In some embodiments, the one or more toxicity-targeting therapies is administered within a certain period of time of such confirmation or determination, such as within 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, or 8 hours thereof.

[0381] In some embodiments, the provided methods do not result in a high rate or likelihood of toxicity or toxic outcomes or reduces the rate or likelihood of toxicity or toxic outcomes, such as such as immune effector cell-associated neurotoxicity syndrome (ICANS), compared to certain other cell therapies. In some embodiments, the methods do not result in, or do not increase the risk of ICANS.

[0382] Exemplary ICANS-related outcomes include a grading scheme for ICANS developed by the CAR T-Cell Therapy–Associated TOXicity (CARTOX) consensus group consisting of a 10-point grading (CARTOX-10) combining important components of the Mini Mental State

Assessment to assess the grade of encephalopathy by variations in concentration, speech, handwriting and orientation (see *e.g.*, Neelapu et al. Nat Rev Clin Oncol., 2018, 15:47-62).

[0383] In some embodiments, outcomes associated with a method to classify the severity of ICANS by using immune effector cell encephalopathy (ICE) scores. In some embodiments, ICE scoring includes evaluating receptive aphasia.

II. CELL THERAPY AND ENGINEERING CELLS

[0384] In some embodiments, the cell therapy (*e.g.*, T cell therapy) methods disclosed herein includes administering engineered cells expressing recombinant receptors (*e.g.*, CAR) designed to recognize and/or specifically bind to antigens associated with the disease or condition, such as severe and refractory SLE. In particular embodiments, the antigen that is bound or recognized by the recombinant receptor (*e.g.*, CAR) is CD19. In some embodiments, binding to the antigen results in a response, such as an immune response against such antigens. In some embodiments, the cells contain or are engineered to contain the recombinant receptor, such as a chimeric antigen receptor (CAR). The recombinant receptor, such as a CAR, generally includes an extracellular antigen (or ligand) binding domain specific to the antigen that is linked to one or more intracellular signaling components, in some aspects via linkers and/or transmembrane domain(s). In some aspects, the engineered cells are provided as pharmaceutical compositions and formulations suitable for administration to a subjects, such as for adoptive cell therapy. Also provided are therapeutic methods for administering the cells and compositions to subjects, *e.g.*, patients.

[0385] In some embodiments, the cells include one or more nucleic acids introduced via genetic engineering, and thereby express recombinant or genetically engineered products of such nucleic acids. In some embodiments, gene transfer is accomplished by first stimulating the cells, such as by combining it with a stimulus that induces a response such as proliferation, survival, and/or activation, *e.g.*, as measured by expression of a cytokine or activation marker, followed by transduction of the activated cells, and expansion in culture to numbers sufficient for clinical applications.

A. Chimeric Antigen Receptors (*e.g.*, CD19-targeted CARs)

[0386] In some embodiments of the provided methods and uses, chimeric receptors, such as a chimeric antigen receptors, contain one or more domains that combine a ligand-binding domain (*e.g.*, antibody or antibody fragment) that provides specificity for a desired antigen (*e.g.*,

CD19) with intracellular signaling domains. In some embodiments, the intracellular signaling domain is a stimulating or an activating intracellular domain portion, such as a T cell stimulating or activating domain, providing a primary activation signal or a primary signal. In some embodiments, the intracellular signaling domain contains or additionally contains a costimulatory signaling domain to facilitate effector functions. In some embodiments, chimeric receptors when genetically engineered into immune cells can modulate T cell activity, and, in some cases, can modulate T cell differentiation or homeostasis, thereby resulting in genetically engineered cells with improved longevity, survival and/or persistence *in vivo*, such as for use in adoptive cell therapy methods.

[0387] Exemplary antigen receptors, including CARs, and methods for engineering and introducing such receptors into cells, include those described, for example, in international patent application publication numbers WO200014257, WO2013126726, WO2012/129514, WO2014031687, WO2013/166321, WO2013/071154, WO2013/123061 U.S. patent application publication numbers US2002131960, US2013287748, US20130149337, U.S. Patent Nos.: 6,451,995, 7,446,190, 8,252,592, 8,339,645, 8,398,282, 7,446,179, 6,410,319, 7,070,995, 7,265,209, 7,354,762, 7,446,191, 8,324,353, and 8,479,118, and European patent application number EP2537416, and/or those described by Sadelain et al., *Cancer Discov.* 2013 April; 3(4): 388–398; Davila et al. (2013) *pLoS ONE* 8(4): e61338; Turtle et al., *Curr. Opin. Immunol.*, 2012 October; 24(5): 633–39; Wu et al., *Cancer*, 2012 March 18(2): 160–75. In some aspects, the antigen receptors include a CAR as described in U.S. Patent No.: 7,446,190, and those described in International Patent Application Publication No.: WO/2014055668 A1. Examples of the CARs include CARs as disclosed in any of the aforementioned publications, such as WO2014031687, US 8,339,645, US 7,446,179, US 2013/0149337, U.S. Patent No.: 7,446,190, US Patent No.: 8,389,282, Kochenderfer et al., 2013, *Nature Reviews Clinical Oncology*, 10, 267–276 (2013); Wang et al. (2012) *J. Immunother.* 35(9): 689–701; and Brentjens et al., *Sci Transl Med.* 2013 5(177). See also WO2014031687, US 8,339,645, US 7,446,179, US 2013/0149337, U.S. Patent No.: 7,446,190, and US Patent No.: 8,389,282.

[0388] The chimeric receptors, such as CARs, generally include an extracellular antigen binding domain, such as a portion of an antibody molecule, generally a variable heavy (V_H) chain region and/or variable light (V_L) chain region of the antibody, *e.g.*, an scFv antibody fragment. In some embodiments, the antibody or antigen-binding portion thereof is expressed on

cells as part of a recombinant receptor, such as a chimeric receptor (*e.g.*, CAR), that binds, such as specifically binds, to the antigen (*e.g.*, CD19).

[0389] In some embodiments, the antigen targeted by the receptor is a polypeptide. In particular embodiments, the antigen target is CD19. In some embodiments, the antigen is selectively expressed on B cells targeted for treating the autoimmune or inflammatory condition, such as lupus. In some embodiments, the CAR typically includes in its extracellular portion one or more antibody or antigen-binding fragment or portion that targets CD19.

[0390] The chimeric receptors, such as CARs, generally include an extracellular antigen binding domain that is an antigen-binding portion or portions of an antibody molecule. In some embodiments, the antigen-binding domain is a portion of an antibody molecule, generally a variable heavy (V_H) chain region and/or variable light (V_L) chain region of the antibody, *e.g.*, an scFv antibody fragment. In some embodiments, the CAR includes an antigen-binding portion or portions of an antibody molecule, such as a single-chain antibody fragment (scFv) derived from the variable heavy (V_H) and variable light (V_L) chains of a monoclonal antibody (mAb). In some embodiments, the antigen-binding domain is a single domain antibody (sdAb), such as sdFv, nanobody, V_{HH} and V_{NAR} . In some embodiments, an antigen-binding fragment comprises antibody variable regions joined by a flexible linker.

[0391] In some embodiments, the antibody or an antigen-binding fragment (*e.g.*, scFv or V_H domain) specifically recognizes an antigen, such as CD19. In some embodiments, the antibody or antigen-binding fragment is derived from, or is a variant of, antibodies or antigen-binding fragment that specifically binds to CD19. In some embodiments, the antigen is CD19. In some embodiments, the antibody or an antigen-binding fragment (*e.g.*, scFv) contains a variable heavy chain and a variable light chain with six CDRs, CDRH1-3 and CDRL1-3, that confer binding to CD19.

[0392] The terms “complementarity determining region,” and “CDR,” synonymous with “hypervariable region” or “HVR,” are known, in some cases, to refer to non-contiguous sequences of amino acids within antibody variable regions, which confer antigen specificity and/or binding affinity. In general, there are three CDRs in each heavy chain variable region (CDR-H1, CDR-H2, CDR-H3) and three CDRs in each light chain variable region (CDR-L1, CDR-L2, CDR-L3). “Framework regions” and “FR” are known, in some cases, to refer to the non-CDR portions of the variable regions of the heavy and light chains. In general, there are

four FRs in each full-length heavy chain variable region (FR-H1, FR-H2, FR-H3, and FR-H4), and four FRs in each full-length light chain variable region (FR-L1, FR-L2, FR-L3, and FR-L4).

[0393] The precise amino acid sequence boundaries of a given CDR or FR can be readily determined using any of a number of well-known schemes, including those described by Kabat *et al.* (1991), “Sequences of Proteins of Immunological Interest,” 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (“Kabat” numbering scheme); Al-Lazikani *et al.*, (1997) JMB 273,927-948 (“Chothia” numbering scheme); MacCallum *et al.*, J. Mol. Biol. 262:732-745 (1996), “Antibody-antigen interactions: Contact analysis and binding site topography,” J. Mol. Biol. 262, 732-745.” (“Contact” numbering scheme); Lefranc MP *et al.*, “IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains,” Dev Comp Immunol, 2003 Jan;27(1):55-77 (“IMGT” numbering scheme); Honegger A and Plückthun A, “Yet another numbering scheme for immunoglobulin variable domains: an automatic modeling and analysis tool,” J Mol Biol, 2001 Jun 8;309(3):657-70, (“Aho” numbering scheme); and Martin *et al.*, “Modeling antibody hypervariable loops: a combined algorithm,” PNAS, 1989, 86(23):9268-9272, (“AbM” numbering scheme).

[0394] The boundaries of a given CDR or FR may vary depending on the scheme used for identification. For example, the Kabat scheme is based on structural alignments, while the Chothia scheme is based on structural information. Numbering for both the Kabat and Chothia schemes is based upon the most common antibody region sequence lengths, with insertions accommodated by insertion letters, for example, “30a,” and deletions appearing in some antibodies. The two schemes place certain insertions and deletions (“indels”) at different positions, resulting in differential numbering. The Contact scheme is based on analysis of complex crystal structures and is similar in many respects to the Chothia numbering scheme. The AbM scheme is a compromise between Kabat and Chothia definitions based on that used by Oxford Molecular’s AbM antibody modeling software.

[0395] **Table 5**, below, lists exemplary position boundaries of CDR-L1, CDR-L2, CDR-L3 and CDR-H1, CDR-H2, CDR-H3 as identified by Kabat, Chothia, AbM, and Contact schemes, respectively. For CDR-H1, residue numbering is listed using both the Kabat and Chothia numbering schemes. FRs are located between CDRs, for example, with FR-L1 located before CDR-L1, FR-L2 located between CDR-L1 and CDR-L2, FR-L3 located between CDR-L2 and CDR-L3 and so forth. It is noted that because the shown Kabat numbering scheme places insertions at H35A and H35B, the end of the Chothia CDR-H1 loop when numbered using the

shown Kabat numbering convention varies between H32 and H34, depending on the length of the loop.

Table 5. Boundaries of CDRs according to various numbering schemes.

CDR	Kabat	Chothia	AbM	Contact
CDR-L1	L2-L34	L2-L34	L2-L34	L3-L36
CDR-L2	L5-L56	L5-L56	L5-L56	L4-L55
CDR-L3	L8-L97	L8-L97	L8-L97	L8-L96
CDR-H1 (Kabat Numbering ¹)	H3-H35B	H2-H32..34	H2-H35B	H3-H35B
CDR-H1 (Chothia Numbering ²)	H3-H35	H2-H32	H2-H35	H3-H35
CDR-H2	H5-H65	H5-H56	H5-H58	H4-H58
CDR-H3	H9-H102	H9-H102	H9-H102	H9-H101

1— Kabat *et al.* (1991), “Sequences of Proteins of Immunological Interest,” 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD

2— Al-Lazikani *et al.*, (1997) JMB 273,927-948

[0396] Thus, unless otherwise specified, a “CDR” or “complementary determining region,” or individual specified CDRs (*e.g.*, CDR-H1, CDR-H2, CDR-H3), of a given antibody or region thereof, such as a variable region thereof, should be understood to encompass a (or the specific) complementary determining region as defined by any of the aforementioned schemes, or other known schemes. For example, where it is stated that a particular CDR (*e.g.*, a CDR-H3) contains the amino acid sequence of a corresponding CDR in a given V_H or V_L region amino acid sequence, it is understood that such a CDR has a sequence of the corresponding CDR (*e.g.*, CDR-H3) within the variable region, as defined by any of the aforementioned schemes, or other known schemes. In some embodiments, specific CDR sequences are specified. Exemplary CDR sequences of provided antibodies are described using various numbering schemes, although it is understood that a provided antibody can include CDRs as described according to any of the other aforementioned numbering schemes or other numbering schemes known to a skilled artisan.

[0397] Likewise, unless otherwise specified, a FR or individual specified FR(s) (*e.g.*, FR-H1, FR-H2, FR-H3, FR-H4), of a given antibody or region thereof, such as a variable region thereof, should be understood to encompass a (or the specific) framework region as defined by any of the known schemes. In some instances, the scheme for identification of a particular CDR, FR, or FRs or CDRs is specified, such as the CDR as defined by the Kabat, Chothia, AbM

or Contact method, or other known schemes. In other cases, the particular amino acid sequence of a CDR or FR is given.

[0398] The term “variable region” or “variable domain” refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable regions of the heavy chain and light chain (V_H and V_L , respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three CDRs. (See, *e.g.*, Kindt *et al.* Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007). A single V_H or V_L domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a V_H or V_L domain from an antibody that binds the antigen to screen a library of complementary V_L or V_H domains, respectively. See, *e.g.*, Portolano *et al.*, J. Immunol. 150:880-887 (1993); Clarkson *et al.*, Nature 352:624-628 (1991).

[0399] Among the provided antibodies are antibody fragments. An “antibody fragment” refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies; linear antibodies; variable heavy chain (V_H) regions, single-chain antibody molecules such as scFvs and single-domain V_H single antibodies; and multispecific antibodies formed from antibody fragments. In particular embodiments, the antibodies are single-chain antibody fragments comprising a variable heavy chain region and/or a variable light chain region, such as scFvs.

[0400] Single-domain antibodies (sdAb) are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody. In some embodiments, the CAR comprises an antibody heavy chain domain that specifically binds the antigen, such as an antigen on a B cell, such as CD19. Exemplary single-domain antibodies include sdFv, nanobody, V_{HH} or V_{NAR} .

[0401] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells. In some embodiments, the antibodies are recombinantly produced fragments, such as fragments comprising arrangements that do not occur naturally, such as those with two or more antibody regions or chains joined by synthetic linkers, *e.g.*, peptide linkers. In some embodiments, the

antibody fragments are fragments that are not produced by enzyme digestion of a naturally-occurring intact antibody. In some embodiments, the antibody fragments are scFvs.

[0402] A “humanized” antibody is an antibody in which all or substantially all CDR amino acid residues are derived from non-human CDRs and all or substantially all FR amino acid residues are derived from human FRs. A humanized antibody optionally may include at least a portion of an antibody constant region derived from a human antibody. A “humanized form” of a non-human antibody, refers to a variant of the non-human antibody that has undergone humanization, typically to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (*e.g.*, the antibody from which the CDR residues are derived), *e.g.*, to restore or improve antibody specificity or affinity.

[0403] In some embodiments, the scFv contains a V_H and a V_L derived from an antibody or an antibody fragment specific to CD19. In some embodiments, the extracellular binding domain of the CD19 CAR is derived from an antibody specific to CD19, including, for example, SJ25C1 (Bejcek et al., Cancer Res. 55:2346-2351 (1995)), HD37 (Pezutto et al., J. Immunol. 138(9):2793-2799 (1987)), 4G7 (Meeker et al., Hybridoma 3:305-320 (1984)), B43 (Bejcek (1995)), BLY3 (Bejcek (1995)), B4 (Freedman et al., 70:418-427 (1987)), B4 HB12b (Kansas & Tedder, J. Immunol. 147:4094-4102 (1991); Yazawa et al., Proc. Natl. Acad. Sci. USA 102:15178-15183 (2005); Herbst et al., J. Pharmacol. Exp. Ther. 335:213-222 (2010)), BU12 (Callard et al., J. Immunology, 148(10): 2983-2987 (1992)), and CLB-CD19 (De Rie Cell. Immunol. 118:368-381(1989)). In any of these embodiments, the extracellular binding domain of the CD19 CAR can comprise or consist of the V_H, the V_L, and/or one or more CDRs of any of the antibodies. In some embodiments, the antibody or antibody fragment that binds CD19 is a mouse derived antibody such as FMC63 and SJ25C1. In some embodiments, the antibody or antibody fragment is a human antibody, *e.g.*, as described in U.S. Patent Publication No. US 2016/0152723.

[0404] In some embodiments the antigen-binding domain includes a V_H and/or V_L derived from FMC63, which, in some aspects, can be an scFv. FMC63 generally refers to a mouse monoclonal IgG1 antibody raised against Nalm-1 and -16 cells expressing CD19 of human origin (Ling, N. R., *et al.* (1987). *Leucocyte typing III*. 302). In some embodiments, the FMC63 antibody comprises CDR-H1 and CDR-H2 set forth in SEQ ID NO: 38 and 39, respectively, and

CDR-H3 set forth in SEQ ID NO: 40 or 54 and CDR-L1 set forth in SEQ ID NO: 35 and CDR-L2 set forth in SEQ ID NO: 36 or 55 and CDR-L3 sequences set forth in SEQ ID NO: 37 or 56. In some embodiments, the FMC63 antibody comprises the heavy chain variable region (V_H) comprising the amino acid sequence of SEQ ID NO: 41 and the light chain variable region (V_L) comprising the amino acid sequence of SEQ ID NO: 42.

[0405] In some embodiments, the scFv comprises a variable light chain containing the CDR-L1 sequence of SEQ ID NO:35, a CDR-L2 sequence of SEQ ID NO:36, and a CDR-L3 sequence of SEQ ID NO:37 and/or a variable heavy chain containing a CDR-H1 sequence of SEQ ID NO:38, a CDR-H2 sequence of SEQ ID NO:39, and a CDR-H3 sequence of SEQ ID NO:40, or a variant of any of the foregoing having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto. In some embodiments, the scFv comprises a variable heavy chain region of FMC63 set forth in SEQ ID NO:41 and a variable light chain region of FMC63 set forth in SEQ ID NO:42, or a variant of any of the foregoing having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto.

[0406] In some embodiments, the FMC63 antibody comprises CDR-H1 and CDR-H2 set forth in SEQ ID NO: 38 and 39, respectively, and CDR-H3 set forth in SEQ ID NO: 40 or 54 and CDR-L1 set forth in SEQ ID NO: 35 and CDR-L2 set forth in SEQ ID NO: 36 or 55 and CDR-L3 sequences set forth in SEQ ID NO: 37 or 56. In some embodiments, the FMC63 antibody comprises the heavy chain variable region (V_H) comprising the amino acid sequence of SEQ ID NO: 41 and the light chain variable region (V_L) comprising the amino acid sequence of SEQ ID NO: 42. In some embodiments, the scFv comprises a variable light chain containing the CDR-L1 sequence of SEQ ID NO:35, a CDR-L2 sequence of SEQ ID NO:36, and a CDR-L3 sequence of SEQ ID NO:37 and/or a variable heavy chain containing a CDR-H1 sequence of SEQ ID NO:38, a CDR-H2 sequence of SEQ ID NO:39, and a CDR-H3 sequence of SEQ ID NO:40, or a variant of any of the foregoing having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto. In some embodiments, the scFv comprises a variable heavy chain region of FMC63 set forth in SEQ ID NO:41 and a variable light chain region of FMC63 set forth in SEQ ID NO:42, or a variant of any of the foregoing having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto.

[0407] In some embodiments, the variable heavy and variable light chains are connected by a linker. In some embodiments, the linker is set forth in SEQ ID NO:24. In some embodiments, the scFv comprises, in order, a V_H, a linker, and a V_L. In some embodiments, the scFv comprises, in order, a V_L, a linker, and a V_H. In some embodiments, the scFv is encoded by a sequence of nucleotides set forth in SEQ ID NO:25 or a sequence that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:25. In some embodiments, the scFv comprises the sequence of amino acids set forth in SEQ ID NO:43 or a sequence that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:43.

[0408] In some embodiments the antigen-binding domain includes a V_H and/or V_L derived from SJ25C1, which, in some aspects, can be an scFv. SJ25C1 is a mouse monoclonal IgG1 antibody raised against Nalm-1 and -16 cells expressing CD19 of human origin (Ling, N. R., *et al.* (1987). *Leucocyte typing III*. 302). In some embodiments, the SJ25C1 antibody comprises CDR-H1, CDR-H2 and CDR-H3 set forth in SEQ ID NOS: 47-49, respectively, and CDR-L1, CDR-L2 and CDR-L3 sequences set forth in SEQ ID NOS: 44-46, respectively. In some embodiments, the SJ25C1 antibody comprises the heavy chain variable region (V_H) comprising the amino acid sequence of SEQ ID NO: 50 and the light chain variable region (V_L) comprising the amino acid sequence of SEQ ID NO: 51. In some embodiments, the svFv comprises a variable light chain containing a CDR-L1 sequence of SEQ ID NO:44, a CDR-L2 sequence of SEQ ID NO: 45, and a CDR-L3 sequence of SEQ ID NO:46 and/or a variable heavy chain containing a CDR-H1 sequence of SEQ ID NO:47, a CDR-H2 sequence of SEQ ID NO:48, and a CDR-H3 sequence of SEQ ID NO:49, or a variant of any of the foregoing having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto. In some embodiments, the scFv comprises a variable heavy chain region of SJ25C1 set forth in SEQ ID NO:50 and a variable light chain region of SJ25C1 set forth in SEQ ID NO:51, or a variant of any of the foregoing having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto. In some embodiments, the variable heavy and variable light chains are connected by a linker. In some embodiments, the linker is set forth in SEQ ID NO:52. In some embodiments, the scFv comprises, in order, a V_H, a linker, and a V_L. In some embodiments, the scFv comprises, in order, a V_L, a linker, and a V_H. In some embodiments, the scFv comprises the

sequence of amino acids set forth in SEQ ID NO:53 or a sequence that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:53.

[0409] In some embodiments, the linker is set forth in SEQ ID NO:23. In some embodiments, the linker has the formula -PGGG-(SGGGG)₅-P- wherein P is proline, G is glycine and S is serine the linker is set forth in SEQ ID NO:22.

[0410] In some embodiments, the anti-CD19 CAR includes an antigen-binding domain described in PCT Pub. No. WO2015187528. In some embodiments, the anti-CD19 CAR is a CAR described in PCT Pub. No. WO2015187528.

[0411] In some embodiments, the anti-CD19 CAR includes an antigen-binding domain that is a single chain antibody derived from a fully human antibody. In some embodiments, the single chain antibody is an scFv. Exemplary fully human anti-CD19 antibodies are described in PCT Pub. No. WO2016033570, PCT Pub. No. WO2020233589, U.S. Pub. No. US2010/0104509 and U.S. Pub. No. US20220220200.

[0412] Exemplary antigen receptors, *e.g.*, CARs, also include the CARs of FDA-approved products BREYANZI® (lisocabtagene maraleucel), TECARTUS™ (brexucabtagene autoleucel), KYMRIAH™ (tisagenlecleucel), and YESCARTA™ (axicabtagene ciloleucel). In some of any of the provided embodiments, the CAR is the CAR of BREYANZI® (lisocabtagene maraleucel), TECARTUS™ (brexucabtagene autoleucel), KYMRIAH™ (tisagenlecleucel), YESCARTA™ (axicabtagene ciloleucel). In some of any of the provided embodiments, the CAR is the CAR of BREYANZI® (lisocabtagene maraleucel, see Sehgal et al., 2020, Journal of Clinical Oncology 38:15_suppl, 8040; Teoh et al., 2019, Blood 134(Supplement_1):593; and Abramson et al., 2020, The Lancet 396(10254): 839-852). In some of any of the provided embodiments, the CAR is the CAR of TECARTUS™ (brexucabtagene autoleucel, see Mian and Hill, 2021, Expert Opin Biol Ther; 21(4):435-441; and Wang et al., 2021, Blood 138(Supplement 1):744). In some of any of the provided embodiments, the CAR is the CAR of KYMRIAH™ (tisagenlecleucel, see Bishop et al., 2022, N Engl J Med 386:629:639; Schuster et al., 2019, N Engl J Med 380:45-56; Halford et al., 2021, Ann Pharmacother 55(4):466-479; Mueller et al., 2021, Blood Adv. 5(23):4980-4991; and Fowler et al., 2022, Nature Medicine 28:325-332). In some of any of the provided embodiments, the CAR is the CAR of YESCARTA™ (axicabtagene ciloleucel, see Neelapu et al., 2017, N Engl J Med 377(26):2531-

2544; Jacobson et al., 2021, The Lancet 23(1):P91-103; and Locke et al., 2022, N Engl J Med 386:640-654).

[0413] In some aspects, the recombinant receptor, *e.g.*, a chimeric antigen receptor, includes the extracellular portion containing one or more antigen binding domains, such as an antibody or fragment thereof, and one or more intracellular signaling region or domain (also interchangeably called a cytoplasmic signaling domain or region). In some aspects, the recombinant receptor, *e.g.*, CAR, further includes a spacer and/or a transmembrane domain or portion. In some aspects, the spacer and/or transmembrane domain can link the extracellular portion containing the antigen-binding domain and the intracellular signaling region(s) or domain(s).

[0414] In some embodiments, the recombinant receptor such as the CAR, further includes a spacer, which may include a hinge domain. In some embodiments, the spacer in a CD8 α hinge domain, for example, a human CD8 α hinge domain. In some embodiments, the CD8 α hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:93. In some embodiments, the hinge domain comprises a CD28 hinge domain, for example, a human CD28 hinge domain. In some embodiments, the CD28 hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:94. In some embodiments, the CD28 hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:95. In some embodiments, the hinge domain has a sequence of amino acids that has at least 80% sequence identity, such as at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to any of the foregoing.

[0415] In some embodiments, the spacer may be or include at least a portion of an immunoglobulin constant region or variant or modified version thereof, such as a hinge region, *e.g.*, an IgG4 hinge region, and/or a C_H1/C_L and/or Fc region. In some embodiments, the recombinant receptor further comprises a spacer and/or a hinge region. In some embodiments, the constant region or portion is of a human IgG, such as IgG4 or IgG1. In some aspects, the portion of the constant region serves as a spacer region between the antigen-recognition component, *e.g.*, scFv, and transmembrane domain. The spacer can be of a length that provides for increased responsiveness of the cell following antigen binding, as compared to in the absence of the spacer. In some examples, the spacer is at or about 12 amino acids in length or is no more than 12 amino acids in length. Exemplary spacers include those having at least about 10 to 229 amino acids, about 10 to 200 amino acids, about 10 to 175 amino acids, about 10 to 150 amino acids, about 10 to 125 amino acids, about 10 to 100 amino acids, about 10 to 75 amino acids,

about 10 to 50 amino acids, about 10 to 40 amino acids, about 10 to 30 amino acids, about 10 to 20 amino acids, or about 10 to 15 amino acids, and including any integer between the endpoints of any of the listed ranges. In some embodiments, a spacer region has about 12 amino acids or less, about 119 amino acids or less, or about 229 amino acids or less. Exemplary spacers include IgG4 hinge alone, IgG4 hinge linked to CH₂ and CH₃ domains, or IgG4 hinge linked to the CH₃ domain. Exemplary spacers include, but are not limited to, those described in Hudecek et al. (2013) Clin. Cancer Res., 19:3153, Hudecek et al. (2015) Cancer Immunol Res. 3(2): 125–135 or international patent application publication number WO2014031687.

[0416] In some embodiments, the spacer contains only a hinge region of an IgG, such as only a hinge of IgG4 or IgG1, such as the hinge only spacer set forth in SEQ ID NO: 1, and encoded by the sequence set forth in SEQ ID NO: 2. In some embodiments, the spacer is an Ig hinge, *e.g.*, and IgG4 hinge, linked to a C_{H2} and/or C_{H3} domains. In some embodiments, the spacer is an Ig hinge, *e.g.*, an IgG4 hinge, linked to C_{H2} and C_{H3} domains, such as set forth in SEQ ID NO: 4. In some embodiments, the spacer the spacer is an Ig hinge, *e.g.*, an IgG4 hinge, linked to a C_{H3} domain only, such as set forth in SEQ ID NO: 3. In some embodiments, the spacer is or comprises a glycine-serine rich sequence or other flexible linker such as known flexible linkers. In some embodiments, the constant region or portion is of IgD. In some embodiments, the spacer has the sequence set forth in SEQ ID NO: 5. In some embodiments, the spacer has a sequence of amino acids that exhibits at least or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 1, 3, 4 and 5.

[0417] In some aspects, the spacer is a polypeptide spacer that (a) comprises or consists of all or a portion of an immunoglobulin hinge or a modified version thereof or comprises about 15 amino acids or less, and does not comprise a CD28 extracellular region or a CD8 extracellular region, (b) comprises or consists of all or a portion of an immunoglobulin hinge, optionally an IgG4 hinge, or a modified version thereof and/or comprises about 15 amino acids or less, and does not comprise a CD28 extracellular region or a CD8 extracellular region, or (c) is at or about 12 amino acids in length and/or comprises or consists of all or a portion of an immunoglobulin hinge, optionally an IgG4, or a modified version thereof; or (d) consists or comprises the sequence of amino acids set forth in SEQ ID NOS: 1, 3-5, 27-34 or 24, or a variant of any of the foregoing having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto, or (e) comprises or consists of the formula

X₁PPX₂P, where X₁ is glycine, cysteine or arginine and X₂ is cysteine or threonine, as set forth in SEQ ID NO: 26.

[0418] In some embodiments, the antigen receptor comprises an intracellular domain linked directly or indirectly to the extracellular domain. In some embodiments, the chimeric antigen receptor includes a transmembrane domain linking the extracellular domain and the intracellular signaling domain. In some embodiments, the intracellular signaling domain comprises an ITAM. For example, in some aspects, the antigen recognition domain (*e.g.*, extracellular domain) generally is linked to one or more intracellular signaling components, such as signaling components that mimic activation through an antigen receptor complex, such as a TCR complex, in the case of a CAR, and/or signal via another cell surface receptor. In some embodiments, the chimeric receptor comprises a transmembrane domain linked or fused between the extracellular domain (*e.g.*, scFv) and intracellular signaling domain. Thus, in some embodiments, the antigen-binding component (*e.g.*, antibody) is linked to one or more transmembrane and intracellular signaling domains.

[0419] In one embodiment, a transmembrane domain that naturally is associated with one of the domains in the receptor, *e.g.*, CAR, is used. In some instances, the transmembrane domain is selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex.

[0420] The transmembrane domain in some embodiments is derived either from a natural or from a synthetic source. Where the source is natural, the domain in some aspects is derived from any membrane-bound or transmembrane protein. Transmembrane regions include those derived from (*i.e.* comprise at least the transmembrane region(s) of) the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 (4-1BB), or CD154. Alternatively, the transmembrane domain in some embodiments is synthetic. In some aspects, the synthetic transmembrane domain comprises predominantly hydrophobic residues such as leucine and valine. In some aspects, a triplet of phenylalanine, tryptophan and valine will be found at each end of a synthetic transmembrane domain. In some embodiments, the linkage is by linkers, spacers, and/or transmembrane domain(s). In some aspects, the transmembrane domain contains a transmembrane portion of CD28 or a variant thereof. The extracellular domain and

transmembrane can be linked directly or indirectly. In some embodiments, the extracellular domain and transmembrane are linked by a spacer, such as any described herein.

[0421] In some embodiments, the transmembrane domain is a transmembrane domain of human CD28 or variant thereof, *e.g.*, a 27-amino acid transmembrane domain of a human CD28 (Accession No.: P10747.1). In some embodiments, the transmembrane domain is a transmembrane domain that comprises the sequence of amino acids set forth in SEQ ID NO: 8 or a sequence of amino acids that exhibits at least or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:8. In some embodiments, the transmembrane-domain containing portion of the recombinant receptor comprises the sequence of amino acids set forth in SEQ ID NO: 9 or a sequence of amino acids having at least or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto.

[0422] In some embodiments, the transmembrane domain of the is a transmembrane domain of a human CD8 α . In some embodiments, the transmembrane domain is a transmembrane domain that comprises the sequence of amino acids set forth in SEQ ID NO: 96 or a sequence of amino acids that exhibits at least or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:96.

[0423] In some embodiments, the recombinant receptor, *e.g.*, CAR, includes at least one intracellular signaling component or components, such as an intracellular signaling region or domain. T cell activation is in some aspects described as being mediated by two classes of cytoplasmic signaling sequences: those that initiate antigen-dependent primary activation through the TCR (primary cytoplasmic signaling sequences), and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal (secondary cytoplasmic signaling sequences). In some aspects, the CAR includes one or both of such signaling components. Among the intracellular signaling region are those that mimic or approximate a signal through a natural antigen receptor, a signal through such a receptor in combination with a costimulatory receptor, and/or a signal through a costimulatory receptor alone. In some embodiments, a short oligo- or polypeptide linker, for example, a linker of between 2 and 10 amino acids in length, such as one containing glycines and serines, *e.g.*, glycine-serine doublet, is present and forms a linkage between the transmembrane domain and the cytoplasmic signaling domain of the CAR.

[0424] In some embodiments, upon ligation of the CAR, the cytoplasmic domain or intracellular signaling region of the CAR activates at least one of the normal effector functions or responses of the immune cell, *e.g.*, T cell engineered to express the CAR. For example, in some contexts, the CAR induces a function of a T cell such as cytolytic activity or T-helper activity, such as secretion of cytokines or other factors. In some embodiments, a truncated portion of an intracellular signaling region of an antigen receptor component or costimulatory molecule is used in place of an intact immunostimulatory chain, for example, if it transduces the effector function signal. In some embodiments, the intracellular signaling regions, *e.g.*, comprising intracellular domain or domains, include the cytoplasmic sequences of the T cell receptor (TCR), and in some aspects also those of co-receptors that in the natural context act in concert with such receptor to initiate signal transduction following antigen receptor engagement, and/or any derivative or variant of such molecules, and/or any synthetic sequence that has the same functional capability. In some embodiments, the intracellular signaling regions, *e.g.*, comprising intracellular domain or domains, include the cytoplasmic sequences of a region or domain that is involved in providing costimulatory signal.

[0425] In some aspects, the CAR includes a primary cytoplasmic signaling sequence that regulates primary activation of the TCR complex. Primary cytoplasmic signaling sequences that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs. Examples of ITAM containing primary cytoplasmic signaling sequences include those derived from CD3 zeta chain, FcR gamma, CD3 gamma, CD3 delta and CD3 epsilon. In some embodiments, cytoplasmic signaling molecule(s) in the CAR contain(s) a cytoplasmic signaling domain, portion thereof, or sequence derived from CD3 zeta.

[0426] In some embodiments, the receptor includes an intracellular component of a TCR complex, such as a TCR CD3 chain that mediates T-cell activation and cytotoxicity, *e.g.*, CD3 zeta chain. Thus, in some aspects, the antigen-binding portion is linked to one or more cell signaling modules. In some embodiments, cell signaling modules include CD3 transmembrane domain, CD3 intracellular signaling domains, and/or other CD transmembrane domains. In some embodiments, the receptor, *e.g.*, further includes a portion of one or more additional molecules such as Fc receptor γ , CD8alpha, CD8beta, CD4, CD25, or CD16. For example, in some aspects, the CAR or other chimeric receptor includes a chimeric molecule between CD3-zeta (CD3- ζ) or Fc receptor γ and CD8alpha, CD8beta, CD4, CD25 or CD16.

[0427] In some embodiments, the intracellular (or cytoplasmic) signaling region comprises a human CD3 chain, optionally a CD3 zeta stimulatory signaling domain or functional variant thereof, such as an 112 AA cytoplasmic domain of isoform 3 of human CD3 ζ (Accession No.: P20963.2) or a CD3 zeta signaling domain as described in U.S. Patent No.: 7,446,190 or U.S. Patent No. 8,911,993. In some embodiments, the intracellular signaling region comprises the sequence of amino acids set forth in SEQ ID NO: 13, 14 or 15 or a sequence of amino acids that exhibits at least or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 13, 14 or 15. In some embodiments, the CD3 ζ signaling domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:13. In some embodiments, the CD3 ζ signaling domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:14. In some embodiments, the CD3 ζ signaling domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:15.

[0428] In the context of a natural TCR, full activation generally requires not only signaling through the TCR, but also a costimulatory signal. Thus, in some embodiments, to promote full activation, a component for generating secondary or co-stimulatory signal is also included in the CAR. In other embodiments, the CAR does not include a component for generating a costimulatory signal. In some aspects, an additional CAR is expressed in the same cell and provides the component for generating the secondary or costimulatory signal.

[0429] In some embodiments, the chimeric antigen receptor contains an intracellular domain of a T cell costimulatory molecule. In some embodiments, the CAR includes a signaling domain and/or transmembrane portion of a costimulatory receptor, such as CD28, 4-1BB, OX40 (CD134), CD27, DAP10, DAP12, ICOS and/or other costimulatory receptors. In some embodiments, the CAR includes a costimulatory region or domain of CD28 or 4-1BB, such as of human CD28 or human 4-1BB.

[0430] In some embodiments, the intracellular signaling region or domain comprises an intracellular costimulatory signaling domain of human CD28 or functional variant or portion thereof, such as a 41 amino acid domain thereof and/or such a domain with an LL to GG substitution at positions 186-187 of a native CD28 protein. In some embodiments, the intracellular signaling domain can comprise the sequence of amino acids set forth in SEQ ID NO: 10 or 11 or a sequence of amino acids that exhibits at least or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 10 or 11. In some embodiments, the intracellular region comprises an

intracellular costimulatory signaling domain of 4-1BB or functional variant or portion thereof, such as a 42-amino acid cytoplasmic domain of a human 4-1BB (Accession No. Q07011.1) or functional variant or portion thereof, such as the sequence of amino acids set forth in SEQ ID NO: 12 or a sequence of amino acids that exhibits at least or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 12.

[0431] In some aspects, the same CAR includes both the primary (or activating) cytoplasmic signaling regions and costimulatory signaling components.

[0432] In some embodiments, the activating domain is included within one CAR, whereas the costimulatory component is provided by another CAR recognizing another antigen. In some embodiments, the CARs include activating or stimulatory CARs, costimulatory CARs, both expressed on the same cell (see WO2014/055668). In some aspects, the cells include one or more stimulatory or activating CAR and/or a costimulatory CAR. In some embodiments, the cells further include inhibitory CARs (iCARs, see Fedorov et al., *Sci. Transl. Medicine*, 5(215) (December, 2013)), such as a CAR recognizing an antigen other than the one associated with and/or specific for the disease or condition whereby an activating signal delivered through the disease-targeting CAR is diminished or inhibited by binding of the inhibitory CAR to its ligand, *e.g.*, to reduce off-target effects.

[0433] In some embodiments, the two receptors induce, respectively, an activating and an inhibitory signal to the cell, such that ligation of one of the receptors to its antigen activates the cell or induces a response, but ligation of the second inhibitory receptor to its antigen induces a signal that suppresses or dampens that response. Examples are combinations of activating CARs and inhibitory CARs (iCARs). Such a strategy may be used, for example, to reduce the likelihood of off-target effects in the context in which the activating CAR binds an antigen expressed in a disease or condition but which is also expressed on normal cells, and the inhibitory receptor binds to a separate antigen which is expressed on the normal cells but not cells of the disease or condition.

[0434] In some aspects, the chimeric receptor is or includes an inhibitory CAR (*e.g.*, iCAR) and includes intracellular components that dampen or suppress an immune response, such as an ITAM- and/or co stimulatory-promoted response in the cell. Exemplary of such intracellular signaling components are those found on immune checkpoint molecules, including PD-1, CTLA4, LAG3, BTLA, OX2R, TIM-3, TIGIT, LAIR-1, PGE2 receptors, EP2/4 Adenosine

receptors including A2AR. In some aspects, the engineered cell includes an inhibitory CAR including a signaling domain of or derived from such an inhibitory molecule, such that it serves to dampen the response of the cell, for example, that induced by an activating and/or costimulatory CAR.

[0435] In some cases, CARs are referred to as first, second, and/or third generation CARs. In some aspects, a first generation CAR is one that solely provides a CD3-chain induced signal upon antigen binding; in some aspects, a second-generation CARs is one that provides such a signal and costimulatory signal, such as one including an intracellular signaling domain from a costimulatory receptor such as CD28 or CD137; in some aspects, a third generation CAR in some aspects is one that includes multiple costimulatory domains of different costimulatory receptors.

[0436] In some embodiments, the CAR encompasses one or more, *e.g.*, two or more, costimulatory domains and an activation domain, *e.g.*, primary activation domain, in the cytoplasmic portion. Exemplary CARs include intracellular components of CD3-zeta, CD28, and 4-1BB.

[0437] In some embodiments, the antigen receptor further includes a marker and/or cells expressing the CAR or other antigen receptor further includes a surrogate marker, such as a cell surface marker, which may be used to confirm transduction or engineering of the cell to express the receptor. In some aspects, the marker includes all or part (*e.g.*, truncated form) of CD34, a NGFR, or epidermal growth factor receptor, such as truncated version of such a cell surface receptor (*e.g.*, tEGFR). In some embodiments, the nucleic acid encoding the marker is operably linked to a polynucleotide encoding for a linker sequence, such as a cleavable linker sequence, *e.g.*, T2A. For example, a marker, and optionally a linker sequence, can be any as disclosed in published patent application No. WO2014031687. For example, the marker can be a truncated EGFR (tEGFR) that is, optionally, linked to a linker sequence, such as a T2A cleavable linker sequence.

[0438] An exemplary polypeptide for a truncated EGFR (*e.g.*, tEGFR) comprises the sequence of amino acids set forth in SEQ ID NO: 7 or 16 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 7 or 16. An exemplary T2A linker sequence comprises the sequence of amino acids set forth in SEQ ID NO: 6 or 17 or a sequence

of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 6 or 17.

[0439] In some embodiments, the marker is a molecule, *e.g.*, cell surface protein, not naturally found on T cells or not naturally found on the surface of T cells, or a portion thereof. In some embodiments, the molecule is a non-self molecule, *e.g.*, non-self protein, *i.e.*, one that is not recognized as “self” by the immune system of the host into which the cells will be adoptively transferred.

[0440] In some embodiments, the marker serves no therapeutic function and/or produces no effect other than to be used as a marker for genetic engineering, *e.g.*, for selecting cells successfully engineered. In other embodiments, the marker may be a therapeutic molecule or molecule otherwise exerting some desired effect, such as a ligand for a cell to be encountered *in vivo*, such as a costimulatory or immune checkpoint molecule to enhance and/or dampen responses of the cells upon adoptive transfer and encounter with ligand.

[0441] In some embodiments, the chimeric antigen receptor includes an extracellular portion containing the antibody or fragment described herein. In some aspects, the chimeric antigen receptor includes an extracellular portion containing the antibody or fragment described herein and an intracellular signaling domain. In some embodiments, the antibody or fragment includes an scFv or a single-domain V_H antibody and the intracellular domain contains an ITAM. In some aspects, the intracellular signaling domain includes a signaling domain of a zeta chain of a CD3-zeta (CD3ζ) chain. In some embodiments, the CD3-zeta chain is a human CD3-zeta chain. In some embodiments, the intracellular signaling region further comprises a CD28 and CD137 (4-1BB, TNFRSF9) co-stimulatory domains, linked to a CD3 zeta intracellular domain. In some embodiments, the CD28 is a human CD28. In some embodiments, the 4-1BB is a human 4-1BB. In some embodiments, the chimeric antigen receptor includes a transmembrane domain disposed between the extracellular domain and the intracellular signaling region. In some aspects, the transmembrane domain contains a transmembrane portion of CD28. The extracellular domain and transmembrane can be linked directly or indirectly. In some embodiments, the extracellular domain and transmembrane are linked by a spacer, such as any described herein.

[0442] In some embodiments, the CAR contains an antibody, *e.g.*, an antibody fragment, a transmembrane domain that is or contains a transmembrane portion of CD28 or a functional variant thereof, and an intracellular signaling domain containing a signaling portion of CD28 or functional variant thereof and a signaling portion of CD3 zeta or functional variant thereof. For

example, in some embodiments, the CAR includes an antibody such as an antibody fragment, including scFvs, *e.g.*, specific for CD19 such as any described above, a spacer, such as a spacer containing a portion of an immunoglobulin molecule, such as a hinge region and/or one or more constant regions of a heavy chain molecule, such as an Ig-hinge containing spacer, a transmembrane domain containing all or a portion of a CD28-derived transmembrane domain, a CD28-derived intracellular signaling domain, and a CD3 zeta signaling domain.

[0443] In some embodiments, the CAR contains an antibody, *e.g.*, antibody fragment, a transmembrane domain that is or contains a transmembrane portion of CD28 or a functional variant thereof, and an intracellular signaling domain containing a signaling portion of a 4-1BB or functional variant thereof and a signaling portion of CD3 zeta or functional variant thereof. In some such embodiments, the receptor further includes a spacer containing a portion of an Ig molecule, such as a human Ig molecule, such as an Ig hinge, *e.g.*, an IgG4 hinge, such as a hinge-only spacer. In some embodiments, the CAR includes an antibody or fragment, such as scFv, *e.g.*, specific for CD19 such as any described above, a spacer such as any of the Ig-hinge containing spacers, a CD28-derived transmembrane domain, a 4-1BB-derived intracellular signaling domain, and a CD3 zeta-derived signaling domain.

[0444] In particular embodiments, the CAR is a CD19-directed CAR containing an scFv antigen-binding domain from FMC63; an immunoglobulin hinge spacer, a transmembrane domain, and an intracellular signaling domain containing a costimulatory signaling region that is a signaling domain of 4-1BB and a signaling domain of a CD3-zeta (CD3ζ) chain. In some embodiments, the scFv contains the sequence set forth in SEQ ID NO:43. In some embodiments, the scFv has a VL having CDRs having an amino acid sequences RASQDISKYLN (SEQ ID NO: 35), an amino acid sequence of SRLHSGV (SEQ ID NO: 36), and an amino acid sequence of GNTLPYTFG (SEQ ID NO: 37); and a VH with CDRs having an amino acid sequence of DYGVVS (SEQ ID NO: 38), an amino acid sequence of VIWGSETTYNSALKS (SEQ ID NO: 39) and YAMDYWG (SEQ ID NO: 40). In some embodiments, the transmembrane domain has the sequence set forth in SEQ ID NO:8. In some embodiments, the transmembrane domain has a sequence that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:8. In some embodiments, the 4-1BB costimulatory signaling domain has the sequence set forth in SEQ ID NO:12. In some embodiments, the 4-1BB costimulatory signaling domain has a sequence at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%,

96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:12. In some embodiments, the CD3-zeta domain has the sequence set forth in SEQ ID NO: 13. In some embodiments, the CD3zeta signaling domain has a sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto. In some embodiments, the CAR contains a hinge-containing immunoglobulin spacer between the scFv and the transmembrane domain. In some embodiments, the spacer is set forth in SEQ ID NO:1.

[0445] In particular embodiments of any of the provided methods, the CAR contains in order from N-terminus to C-terminus: an extracellular antigen-binding domain that is the scFv set forth in SEQ ID NO: 43, the spacer set forth in SEQ ID NO:1, the transmembrane domain set forth in SEQ ID NO:8, the 4-1BB costimulatory signaling domain set forth in SEQ ID NO:12, and the signaling domain of a CD3-zeta (CD3 ζ) chain set forth in SEQ ID NO:13.

[0446] In some embodiments, the CAR has a sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:91. In some embodiments, the CAR comprises the sequence set forth in SEQ ID NO:91. In some embodiments, the CAR is set forth in SEQ ID NO:91. In some embodiments, the CAR is the CD19 CAR as present in Lisocabtagene maraleucel.

[0447] In some embodiments, the CAR is encoded by a sequence of nucleotides having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:92. In some embodiments, the CAR is encoded by a sequence of nucleotides set forth in SEQ ID NO: 92.

[0448] In some embodiments, the CAR contains in order from N-terminus to C-terminus: an extracellular antigen-binding domain that is an scFv comprising a variable heavy chain region of FMC63 set forth in SEQ ID NO:41 and a variable light chain region of FMC63 set forth in SEQ ID NO:42, such as the scFv set forth in SEQ ID NO: 43, the CD8 α hinge domain of SEQ ID NO:93, the CD8 α transmembrane domain of SEQ ID NO:96, the 4-1BB costimulatory domain of SEQ ID NO:12, the CD3 ζ signaling domain of SEQ ID NO:13. In some embodiments, the CAR has a sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of the foregoing sequences. In some embodiments, the CAR has a sequence of amino acids having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 97. In some embodiments, the CAR has the sequence set forth in SEQ ID NO: 97. In some embodiments, the CAR is the CD19 CAR as present in Tisagenlecleucel.

[0449] In some embodiments, the CAR contains in order from N-terminus to C-terminus: an extracellular antigen-binding domain that is an scFv comprising a variable heavy chain region of FMC63 set forth in SEQ ID NO:41 and a variable light chain region of FMC63 set forth in SEQ ID NO:42, such as the scFv set forth in SEQ ID NO: 43, the CD28 hinge domain of SEQ ID NO:94, the CD28 transmembrane domain of SEQ ID NO:8 or 9, the CD28 costimulatory domain of SEQ ID NO:10, the CD3 ζ signaling domain of SEQ ID NO:13. In some embodiments, the CAR has a sequence of amino acids having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 98. In some embodiments, the CAR has the sequence set forth in SEQ ID NO: 98. In some embodiments, the CAR is the CD19 CAR as present in *Axicahtagene ciloleucel*.

[0450] In some embodiments, the CAR contains an extracellular binding domain composed of an scFv derived from the anti-CD19 antibody known as Hu19. In some embodiments, the CAR contains the scFv derived from Hu19, a CD8 α hinge and transmembrane domain (*e.g.*, SEQ ID NO:111), a CD28 costimulatory domain (*e.g.*, SEQ ID NO: 10) and a CD3 ζ signaling domain (*e.g.*, SEQ ID NO: 13). In some embodiments, the scFv designated Hu19 contains a light chain variable region (SEQ ID NO: 112), a linker peptide (GSTSGSGKPGSGEGSTKG [SEQ ID NO: 113]), and a heavy chain variable region (SEQ ID NO: 114). The scFv also can include a human CD8 α leader sequence (SEQ ID NO: 115). In some embodiments, the CAR has the sequence set forth in SEQ ID NO:116. In some embodiments, the CAR has the sequence set forth in SEQ ID NO:117. In some embodiments, the CAR contains the scFv derived from Hu19, a CD8 α hinge and transmembrane domain, a 4-1BB costimulatory domain and a CD3 ζ signaling domain. In some embodiments, the CAR has the sequence set forth in SEQ ID NO:118. In some embodiments, the CAR does not include a signal sequence.

[0451] In some embodiments, the CAR contains an extracellular binding domain composed of an scFv derived from a fully human antibody, and an intracellular signaling domain comprising a 4-1BB costimulatory domain and a CD3 ζ signaling domain. In some embodiments, the the light chain variable region of the scFv comprises an amino acid sequence set forth in SEQ ID NO: 106, and the heavy chain variable region of the scFv comprises an amino acid sequence set forth in SEQ ID NO: 107. In some embodiments, the light chain variable region of the scFv comprises an amino acid sequence set forth in SEQ ID NO: 109, and the heavy chain variable region of the scFv comprises an amino acid sequence set forth in SEQ ID NO: 110. In

some embodiments, the scFv has the sequence set forth in SEQ ID NO:105. In some embodiments, the scFv has the sequence set forth in SEQ ID NO:108.

[0452] In some embodiments, the CAR contains a fully human anti-CD19 antibody, a CD8 α hinge and transmembrane domains, a CD28 costimulatory domain and a CD3 ζ activation domain. In some embodiments, the CAR has the sequence set forth in SEQ ID NO:119 or a sequence that has at least 85%, at least 90%, at least 95% or at least 98% sequence identity to SEQ ID NO:119. In some embodiments, the CAR has the sequence set forth in SEQ ID NO:120 or a sequence that has at least 85%, at least 90%, at least 95% or at least 98% sequence identity to SEQ ID NO:120. In some embodiments, the CAR has the sequence set forth in SEQ ID NO:121 or a sequence that has at least 85%, at least 90%, at least 95% or at least 98% sequence identity to SEQ ID NO:121. In some embodiments, the CAR has the sequence set forth in SEQ ID NO:122 or a sequence that has at least 85%, at least 90%, at least 95% or at least 98% sequence identity to SEQ ID NO:122. In some embodiments, the CAR has the sequence set forth in SEQ ID NO:123 or a sequence that has at least 85%, at least 90%, at least 95% or at least 98% sequence identity to SEQ ID NO:123. In some embodiments, the CAR has the sequence set forth in SEQ ID NO:124 or a sequence that has at least 85%, at least 90%, at least 95% or at least 98% sequence identity to SEQ ID NO:124. In some embodiments, the CAR has the sequence set forth in SEQ ID NO:125 or a sequence that has at least 85%, at least 90%, at least 95% or at least 98% sequence identity to SEQ ID NO:125. In some embodiments, the CAR has the sequence set forth in SEQ ID NO:126 or a sequence that has at least 85%, at least 90%, at least 95% or at least 98% sequence identity to SEQ ID NO:126. In some embodiments, the CAR has the sequence set forth in SEQ ID NO:127 or a sequence that has at least 85%, at least 90%, at least 95% or at least 98% sequence identity to SEQ ID NO:127. In some embodiments, the CAR has the sequence set forth in SEQ ID NO:128 or a sequence that has at least 85%, at least 90%, at least 95% or at least 98% sequence identity to SEQ ID NO:128. In some embodiments, the CAR has the sequence set forth in SEQ ID NO:129 or a sequence that has at least 85%, at least 90%, at least 95% or at least 98% sequence identity to SEQ ID NO:129. In some embodiments, the CAR has the sequence set forth in SEQ ID NO:130 or a sequence that has at least 85%, at least 90%, at least 95% or at least 98% sequence identity to SEQ ID NO:130. In some embodiments, the CAR has the sequence set forth in SEQ ID NO:131 or a sequence that has at least 85%, at least 90%, at least 95% or at least 98% sequence identity to SEQ ID NO:131. In some embodiments, the CAR is a CAR described in U.S. Patent No.

10287350, *e.g.*, Table 1 therein. In some embodiments, the CAR is a Hu19-CD828Z (KYV-101) which has a scFv from a fully-human anti-CD19 monoclonal antibody, CD8 α hinge and transmembrane domains, a CD28 costimulatory domain and a CD3 ζ activation domain.

[0453] In some embodiments, the CAR targets CD19 and at least one other antigen expressed on B cells. In some embodiments, the antigen associated with the disease or disorder is selected from CD20, CD19, CD22, ROR1, BCMA, CD45, CD21, CD5, CD33, Igkappa, Iglambda, CD79a, CD79b or CD30. In some embodiments, the other antigen is CD20 and the CAR is a CD20/CD19 directed CAR product. In some embodiments, the CAR is a bispecific CAR in which the extracellular antigen-binding domain binds CD19 and the one other antigen (*e.g.* CD20). In some embodiments, the bispecific CAR is a tandem CAR containing a first antigen binding domain that binds CD19 and a second antigen binding domain that binds the other antigen (*e.g.* CD20). In some embodiments, the CD19 directed scFv comprises a variable heavy chain region and a variable light chain region of FMC63 (*e.g.* variable heavy chain region set forth in SEQ ID NO:41 and a variable light chain region set forth in SEQ ID NO:42). In some embodiments, the CD19 scFv is Hu19 and comprises the variable heavy chain region set forth in SEQ ID NO:114 and the variable light chain region set forth in SEQ ID NO:112. In some embodiments, CD20 directed scFv comprises a variable heavy chain region and a variable light chain region of Leu16 (*e.g.* variable heavy chain region set forth in SEQ ID NO:103 and a variable light chain region set forth in SEQ ID NO:104). In some embodiments, the CD20 directed scFv comprises a variable heavy chain region and a variable light chain region of Ofatumumab (*e.g.*, variable heavy chain region set forth in SEQ ID NO:132 and a variable light chain region set forth in SEQ ID NO:133). In some embodiments, the antigen binding domain is an scFv derived from a CD20 antibody described in U.S. patent publ. No. US2021/0363245. In some embodiments, the antigen binding domain is an scFv derived from the CD20 antibody C2B8 (*e.g.*, described in U.S. Patent No. 5,736,137), 11B8 (*e.g.*, described in U.S. patent application 2004/0167319), 8G6-5 (*e.g.*, described in U.S. patent application 2009/0035322), 2.1.2 (*e.g.*, described in WO 2006/130458), or GA101 (*e.g.*, described in U.S. Pat. No. 9,539,251). In some embodiments, the antigen binding domain is an scFv derived from a BCMA directed antibody, such as any described herein. In some embodiments, the antigen binding domain is an scFv targeting BCMA that comprises the heavy chain variable region shown in SEQ ID NO: 136, and an antibody light chain variable region shown in SEQ ID NO: 137. In some embodiments, the first and second antigen binding domain can be positioned in

any order, in which one antigen binding domain is distal and the other is proximal to the spacer and transmembrane domain. In some embodiments, each antigen binding domain comprises a variable heavy (VH) chain and a variable light (VL) chain for targeting the antigen. In some embodiments, the VH chain is N-terminal to the VL chain of the scFv. In some embodiments, the VL chain is C-terminal to the VL chain of the scFv. In some embodiments, each antigen binding domain is an scFv. In some embodiments, each antigen binding domain is a single domain antibody, such as a VHH. Exemplary dual binding CARs are known, such as described in PCT publ. No. WO2019/028051 and Zhu et al. (2018) *Cytotherapy*, 20:394-406.

[0454] In some embodiments, the CAR is a CD19/CD20 tandem CAR. In some embodiments, the CAR contains an extracellular antigen binding domain composed of a CD19 scFv in tandem with a scFv from the Leu16 antibody specific for CD20; a CD8-derived spacer hinge and transmembrane region, a 4-1BB costimulatory domain and a CD3zeta signaling domain. In some embodiments, the CD20 scFv is distal and the CD19 scFv is proximal to the hinge and transmembrane region. In some embodiments, the CAR is set forth in SEQ ID NO:100. In some embodiments, the nucleotide sequence encoding the CAR is the set forth in SEQ ID NO:99. In some embodiments, the CAR is set forth in SEQ ID NO:102. In some embodiments, the nucleotide sequence encoding the CAR is the set forth in SEQ ID NO:101. In some embodiments, the CAR does not include the leader sequence. In some embodiments, the CD20/CD19 CAR is the CD20/CD19 CAR present in zamtocabtagene autoleucel.

[0455] In some embodiments, the CAR is a CD19/CD20 tandem bispecific CAR. In some embodiments, the CAR contains an extracellular antigen binding domain composed of an anti-CD19 scFv from the FMC63 antibody specific to CD19, an anti-CD20 scFv from the Leu16 antibody specific for CD20; a CD8-derived spacer hinge and transmembrane region, a 4-1BB costimulatory domain and a and a CD3zeta signaling domain.

[0456] In some embodiments, the CAR is a CD19/CD20 tandem CAR containing an extracellular antigen binding domain composed of a CD19 scFv in tandem with a scFv from the Leu16 antibody specific for CD20; a CD8-derived spacer hinge and transmembrane region, a 4-1BB costimulatory domain and a CD3zeta signaling domain. In some embodiments, the CD19 scFv contains the VH sequence set forth in SEQ ID NO:41 and the VL chain region set forth in SEQ ID NO:42. In some embodiments, the VH is N-terminal to the VL in the CD19 scFv. In some embodiments the VH is C-terminal to the VL in the CD19 scFv. In some embodiments, the CD20 scFv contains the VH sequence set forth in SEQ ID NO:103 and the VL chain region

set forth in SEQ ID NO:104. In some embodiments, the VH is N-terminal to the VL in the CD20 scFv. In some embodiments the VH is C-terminal to the VL in the CD20 scFv. In some embodiments, the CD20 scFv is distal and the CD19 scFv is proximal to the hinge and transmembrane region, such that the CAR as a tandem link of the anti-CD20 scFv followed by the FMC63 scFv. In some embodiments, the CAR has the sequence set forth in SEQ ID NO: 135 or a sequence that has at least 85%, 90%, 95% or 98% sequence identity to SEQ ID NO: 135. In some embodiments, the CD20/CD19 CAR is TN-LEU-19, for example as described in WO2021/188681.

[0457] In some embodiments, the CAR is a CD19/CD20 tandem CAR containing an extracellular antigen binding domain composed of a CD19 scFv in tandem with a scFv from the Ofatumumab antibody specific for CD20; a CD8-derived spacer hinge and transmembrane region, a 4-1BB costimulatory domain and a CD3zeta signaling domain. In some embodiments, the CD19 scFv contains the VH sequence set forth in SEQ ID NO:41 and the VL chain region set forth in SEQ ID NO:42. In some embodiments, the VH is N-terminal to the VL in the CD19 scFv. In some embodiments the VH is C-terminal to the VL in the CD19 scFv. In some embodiments, the CD20 scFv contains the VH sequence set forth in SEQ ID NO:132 and the VL chain region set forth in SEQ ID NO:133. In some embodiments, the VH is N-terminal to the VL in the CD20 scFv. In some embodiments the VH is C-terminal to the VL in the CD20 scFv. In some embodiments, the CD20 scFv is distal and the CD19 scFv is proximal to the hinge and transmembrane region, such that the CAR as a tandem link of the anti-CD20 scFv followed by the FMC63 scFv. In some embodiments, the CAR has the sequence set forth in SEQ ID NO: 134 or a sequence that has at least 85%, 90%, 95% or 98% sequence identity to SEQ ID NO: 134. In some embodiments, the CD20/CD19 CAR is TN-OF-19 (C-CAR039), for example as described in WO2021/188681.

[0458] In some embodiments, the CAR is a CD19/BCMA tandem CAR. In some embodiments, the CAR contains an extracellular antigen binding domain targeting CD19 and BCMA, in which the antigen binding domain (scFv) targeting CD19 in the bispecific CAR comprises an antibody heavy chain variable region shown in SEQ ID NO: 41 and an antibody light chain variable region shown in SEQ ID NO: 42, and the antigen binding domain (scFv) targeting BCMA in the bispecific CAR comprises an antibody heavy chain variable region shown in SEQ ID NO: 136 and an antibody light chain variable region shown in SEQ ID NO: 137. In some embodiments, the bispecific CAR for targeting CD19 and BCMA antigens

includes in order the anti-CD19 scFv, the anti-BCMA scFv, a hinge region (*e.g.* CD8 hinge), a transmembrane region, and an intracellular T cell signal region including a costimulatory signaling domain (*e.g.* CD28 domain or 4-1BB domain) and a CD3zeta signaling domain. In some embodiments, the CD19scFv and BCMAscFv are connected by a short peptide segment (G4S)_xN. In some embodiments, the CAR is a CAR as described in US2022/0202864.

[0459] In some embodiments, the CD19-directed CAR binds to CD19 and mediates cytokine production and/or cytotoxic activity against CD19+ target cells when expressed in a T cell and stimulated via the CAR, such as by binding to CD19.

[0460] In some embodiments, nucleic acid molecules encoding such CAR constructs further includes a sequence encoding a T2A ribosomal skip element and/or a tEGFR sequence, *e.g.*, downstream of the sequence encoding the CAR. In some embodiments, the sequence encodes a T2A ribosomal skip element set forth in SEQ ID NO: 6 or 17, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 6 or 17. In some embodiments, T cells expressing an antigen receptor (*e.g.*, CAR) can also be generated to express a truncated EGFR (EGFRt) as a non-immunogenic selection epitope (*e.g.*, by introduction of a construct encoding the CAR and EGFRt separated by a T2A ribosome switch to express two proteins from the same construct), which then can be used as a marker to detect such cells (see *e.g.*, U.S. Patent No. 8,802,374). In some embodiments, the sequence encodes an tEGFR sequence set forth in SEQ ID NO: 7 or 16, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 7 or 16. In some cases, the peptide, such as T2A, can cause the ribosome to skip (ribosome skipping) synthesis of a peptide bond at the C-terminus of a 2A element, leading to separation between the end of the 2A sequence and the next peptide downstream (*see*, for example, de Felipe. *Genetic Vaccines and Ther.* 2:13 (2004) and deFelipe *et al. Traffic* 5:616-626 (2004)). Many 2A elements are known. Examples of 2A sequences that can be used in the methods and nucleic acids disclosed herein, without limitation, 2A sequences from the foot-and-mouth disease virus (F2A, *e.g.*, SEQ ID NO: 21), equine rhinitis A virus (E2A, *e.g.*, SEQ ID NO: 20), Thosea asigna virus (T2A, *e.g.*, SEQ ID NO: 6 or 17), and porcine teschovirus-1 (P2A, *e.g.*, SEQ ID NO: 18 or 19) as described in U.S. Patent Publication No. 20070116690.

[0461] The recombinant receptors, such as CARs, expressed by the cells administered to the subject generally recognize or specifically bind to a molecule that is expressed in, associated

with, and/or specific for the disease or condition or cells thereof being treated. Upon specific binding to the molecule, *e.g.*, antigen, the receptor generally delivers an immunostimulatory signal, such as an ITAM-transduced signal, into the cell, thereby promoting an immune response targeted to the disease or condition. For example, in some embodiments, the cells express a CAR that specifically binds to an antigen expressed by a cell or tissue of the disease or condition or associated with the disease or condition.

B. Nucleic Acids, Vectors and Methods for Genetic Engineering

[0462] In some embodiments, the cells, *e.g.*, T cells, are genetically engineered to express a recombinant receptor. In some embodiments, the engineering is carried out by introducing polynucleotides that encode the recombinant receptor. Also provided are polynucleotides encoding a recombinant receptor, and vectors or constructs containing such nucleic acids and/or polynucleotides.

[0463] In some cases, the nucleic acid sequence encoding the recombinant receptor contains a signal sequence that encodes a signal peptide. In some aspects, the signal sequence may encode a signal peptide derived from a native polypeptide. In other aspects, the signal sequence may encode a heterologous or non-native signal peptide, such as the exemplary signal peptide of the GMCSFR alpha chain set forth in SEQ ID NO:65 and encoded by the nucleotide sequence set forth in SEQ ID NO:66. In some cases, the nucleic acid sequence encoding the recombinant receptor, *e.g.*, chimeric antigen receptor (CAR) contains a signal sequence that encodes a signal peptide. Non-limiting exemplary examples of signal peptides include, for example, the GMCSFR alpha chain signal peptide set forth in SEQ ID NO: 65 and encoded by the nucleotide sequence set forth in SEQ ID NO:66, or the CD8 alpha signal peptide set forth in SEQ ID NO:67.

[0464] In some embodiments, the polynucleotide encoding the recombinant receptor contains at least one promoter that is operatively linked to control expression of the recombinant receptor. In some examples, the polynucleotide contains two, three, or more promoters operatively linked to control expression of the recombinant receptor.

[0465] In certain cases where nucleic acid molecules encode two or more different polypeptide chains, *e.g.*, a recombinant receptor and a marker, each of the polypeptide chains can be encoded by a separate nucleic acid molecule. For example, two separate nucleic acids are provided, and each can be individually transferred or introduced into the cell for expression in

the cell. In some embodiments, the nucleic acid encoding the recombinant receptor and the nucleic acid encoding the marker are operably linked to the same promoter and are optionally separated by an internal ribosome entry site (IRES), or a nucleic acid encoding a self-cleaving peptide or a peptide that causes ribosome skipping, which optionally is a T2A, a P2A, an E2A or an F2A. In some embodiments, the nucleic acids encoding the marker and the nucleic acid encoding the recombinant receptor are operably linked to two different promoters. In some embodiments, the nucleic acid encoding the marker and the nucleic acid encoding the recombinant receptor are present or inserted at different locations within the genome of the cell. In some embodiments, the polynucleotide encoding the recombinant receptor is introduced into a composition containing cultured cells, such as by retroviral transduction, transfection, or transformation.

[0466] In some embodiments, such as those where the polynucleotide contains a first and second nucleic acid sequence, the coding sequences encoding each of the different polypeptide chains can be operatively linked to a promoter, which can be the same or different. In some embodiments, the nucleic acid molecule can contain a promoter that drives the expression of two or more different polypeptide chains. In some embodiments, such nucleic acid molecules can be multicistronic (bicistronic or tricistronic, *see e.g.*, U.S. Patent No. 6,060,273). In some embodiments, transcription units can be engineered as a bicistronic unit containing an IRES (internal ribosome entry site), which allows coexpression of gene products (*e.g.*, encoding the marker and encoding the recombinant receptor) by a message from a single promoter. Alternatively, in some cases, a single promoter may direct expression of an RNA that contains, in a single open reading frame (ORF), two or three genes (*e.g.*, encoding the marker and encoding the recombinant receptor) separated from one another by sequences encoding a self-cleavage peptide (*e.g.*, 2A sequences) or a protease recognition site (*e.g.*, furin). The ORF thus encodes a single polypeptide, which, either during (in the case of 2A) or after translation, is processed into the individual proteins. In some cases, the peptide, such as a T2A, can cause the ribosome to skip (ribosome skipping) synthesis of a peptide bond at the C-terminus of a 2A element, leading to separation between the end of the 2A sequence and the next peptide downstream (*see*, for example, de Felipe, Genetic Vaccines and Ther. 2:13 (2004) and de Felipe *et al.* Traffic 5:616-626 (2004)). Various 2A elements are known. Examples of 2A sequences that can be used in the methods and system disclosed herein, without limitation, 2A sequences from the foot-and-mouth disease virus (F2A, *e.g.*, SEQ ID NO: 21), equine rhinitis A virus

(E2A, *e.g.*, SEQ ID NO: 20), Thosea asigna virus (T2A, *e.g.*, SEQ ID NO: 6 or 17), and porcine teschovirus-1 (P2A, *e.g.*, SEQ ID NO: 18 or 19) as described in U.S. Patent Publication No. 20070116690.

[0467] Any of the recombinant receptors described herein can be encoded by polynucleotides containing one or more nucleic acid sequences encoding recombinant receptors, in any combinations or arrangements. For example, one, two, three or more polynucleotides can encode one, two, three or more different polypeptides, *e.g.*, recombinant receptors. In some embodiments, one vector or construct contains a nucleic acid sequence encoding marker, and a separate vector or construct contains a nucleic acid sequence encoding a recombinant receptor, *e.g.*, CAR. In some embodiments, the nucleic acid encoding the marker and the nucleic acid encoding the recombinant receptor are operably linked to two different promoters. In some embodiments, the nucleic acid encoding the recombinant receptor is present downstream of the nucleic acid encoding the marker.

[0468] In some embodiments, the vector backbone contains a nucleic acid sequence encoding one or more marker(s). In some embodiments, the one or more marker(s) is a transduction marker, surrogate marker and/or a selection marker.

[0469] In some embodiments, the marker is a transduction marker or a surrogate marker. A transduction marker or a surrogate marker can be used to detect cells that have been introduced with the polynucleotide, *e.g.*, a polynucleotide encoding a recombinant receptor. In some embodiments, the transduction marker can indicate or confirm modification of a cell. In some embodiments, the surrogate marker is a protein that is made to be co-expressed on the cell surface with the recombinant receptor, *e.g.*, CAR. In particular embodiments, such a surrogate marker is a surface protein that has been modified to have little or no activity. In certain embodiments, the surrogate marker is encoded on the same polynucleotide that encodes the recombinant receptor. In some embodiments, the nucleic acid sequence encoding the recombinant receptor is operably linked to a nucleic acid sequence encoding a marker, optionally separated by an internal ribosome entry site (IRES), or a nucleic acid encoding a self-cleaving peptide or a peptide that causes ribosome skipping, such as a 2A sequence, such as a T2A, a P2A, an E2A or an F2A. Extrinsic marker genes may in some cases be utilized in connection with engineered cell to permit detection or selection of cells and, in some cases, also to promote cell suicide.

[0470] Exemplary surrogate markers can include truncated forms of cell surface polypeptides, such as truncated forms that are non-functional and do not transduce or are not capable of transducing a signal or a signal ordinarily transduced by the full-length form of the cell surface polypeptide, and/or do not or are not capable of internalizing. Exemplary truncated cell surface polypeptides including truncated forms of growth factors or other receptors such as a truncated human epidermal growth factor receptor 2 (tHER2), a truncated epidermal growth factor receptor (tEGFR, exemplary tEGFR sequence set forth in SEQ ID NO:7 or 16) or a prostate-specific membrane antigen (PSMA) or modified form thereof. tEGFR may contain an epitope recognized by the antibody cetuximab (Erbix®) or other therapeutic anti-EGFR antibody or binding molecule, which can be used to identify or select cells that have been engineered with the tEGFR construct and an encoded exogenous protein, and/or to eliminate or separate cells expressing the encoded exogenous protein. *See* U.S. Patent No. 8,802,374 and Liu *et al.*, *Nature Biotech.* 2016 April; 34(4): 430–434). In some aspects, the marker, *e.g.*, surrogate marker, includes all or part (*e.g.*, truncated form) of CD34, a NGFR, a CD19 or a truncated CD19, *e.g.*, a truncated non-human CD19, or epidermal growth factor receptor (*e.g.*, tEGFR).

[0471] In some embodiments, the marker is or comprises a fluorescent protein, such as green fluorescent protein (GFP), enhanced green fluorescent protein (EGFP), such as super-fold GFP (sfGFP), red fluorescent protein (RFP), such as tdTomato, mCherry, mStrawberry, AsRed2, DsRed or DsRed2, cyan fluorescent protein (CFP), blue green fluorescent protein (BFP), enhanced blue fluorescent protein (EBFP), and yellow fluorescent protein (YFP), and variants thereof, including species variants, monomeric variants, and codon-optimized and/or enhanced variants of the fluorescent proteins. In some embodiments, the marker is or comprises an enzyme, such as a luciferase, the lacZ gene from *E. coli*, alkaline phosphatase, secreted embryonic alkaline phosphatase (SEAP), chloramphenicol acetyl transferase (CAT). Exemplary light-emitting reporter genes include luciferase (luc), β -galactosidase, chloramphenicol acetyltransferase (CAT), β -glucuronidase (GUS) or variants thereof.

[0472] In some embodiments, the marker is a selection marker. In some embodiments, the selection marker is or comprises a polypeptide that confers resistance to exogenous agents or drugs. In some embodiments, the selection marker is an antibiotic resistance gene. In some embodiments, the selection marker is an antibiotic resistance gene confers antibiotic resistance to a mammalian cell. In some embodiments, the selection marker is or comprises a Puromycin resistance gene, a Hygromycin resistance gene, a Blasticidin resistance gene, a Neomycin

resistance gene, a Geneticin resistance gene or a Zeocin resistance gene or a modified form thereof.

[0473] In some embodiments, the molecule is a non-self molecule, *e.g.*, non-self protein, *i.e.*, one that is not recognized as “self” by the immune system of the host into which the cells will be adoptively transferred.

[0474] In some embodiments, the marker serves no therapeutic function and/or produces no effect other than to be used as a marker for genetic engineering, *e.g.*, for selecting cells successfully engineered. In other embodiments, the marker may be a therapeutic molecule or molecule otherwise exerting some desired effect, such as a ligand for a cell to be encountered *in vivo*, such as a costimulatory or immune checkpoint molecule to enhance and/or dampen responses of the cells upon adoptive transfer and encounter with ligand.

[0475] In some embodiments, the nucleic acid encoding the marker is operably linked to a polynucleotide encoding for a linker sequence, such as a cleavable linker sequence, *e.g.*, a T2A. For example, a marker, and optionally a linker sequence, can be any as disclosed in PCT Pub. No. WO2014031687. For example, the marker can be a truncated EGFR (tEGFR) that is, optionally, linked to a linker sequence, such as a T2A cleavable linker sequence. An exemplary polypeptide for a truncated EGFR (*e.g.*, tEGFR) comprises the sequence of amino acids set forth in SEQ ID NO: 7 or 16 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 7 or 16.

[0476] In some embodiments, the marker is or comprises a fluorescent protein, such as green fluorescent protein (GFP), enhanced green fluorescent protein (EGFP), such as super-fold GFP (sfGFP), red fluorescent protein (RFP), such as tdTomato, mCherry, mStrawberry, AsRed2, DsRed or DsRed2, cyan fluorescent protein (CFP), blue green fluorescent protein (BFP), enhanced blue fluorescent protein (EBFP), and yellow fluorescent protein (YFP), and variants thereof, including species variants, monomeric variants, and codon-optimized and/or enhanced variants of the fluorescent proteins. In some embodiments, the marker is or comprises an enzyme, such as a luciferase, the lacZ gene from *E. coli*, alkaline phosphatase, secreted embryonic alkaline phosphatase (SEAP), chloramphenicol acetyl transferase (CAT). Exemplary light-emitting reporter genes include luciferase (luc), β -galactosidase, chloramphenicol acetyltransferase (CAT), β -glucuronidase (GUS) or variants thereof.

[0477] In some embodiments, the marker is a selection marker. In some embodiments, the selection marker is or comprises a polypeptide that confers resistance to exogenous agents or drugs. In some embodiments, the selection marker is an antibiotic resistance gene. In some embodiments, the selection marker is an antibiotic resistance gene confers antibiotic resistance to a mammalian cell. In some embodiments, the selection marker is or comprises a Puromycin resistance gene, a Hygromycin resistance gene, a Blasticidin resistance gene, a Neomycin resistance gene, a Geneticin resistance gene or a Zeocin resistance gene or a modified form thereof.

[0478] In some embodiments, recombinant nucleic acids are transferred into cells using recombinant infectious virus particles, such as, *e.g.*, vectors derived from simian virus 40 (SV40), adenoviruses, adeno-associated virus (AAV). In some embodiments, recombinant nucleic acids are transferred into T cells using recombinant lentiviral vectors or retroviral vectors, such as gamma-retroviral vectors (*see, e.g.*, Koste *et al.* (2014) *Gene Therapy*, 2014 Apr 3. doi: 10.1038/gt.2014.25; Carlens *et al.* (2000) *Exp. Hematol.*, 28(10): 1137-46; Alonso-Camino *et al.* (2013) *Mol. Ther. Nucl. Acids.*, 2, e93; Park *et al.*, *Trends Biotechnol.*, 2011 November 29(11): 550–557).

[0479] In some embodiments, the viral vector is an adeno-associated virus (AAV).

[0480] In some embodiments, the retroviral vector has a long terminal repeat sequence (LTR), *e.g.*, a retroviral vector derived from the Moloney murine leukemia virus (MoMLV), myeloproliferative sarcoma virus (MPSV), murine embryonic stem cell virus (MESV), murine stem cell virus (MSCV) or spleen focus forming virus (SFFV). Most retroviral vectors are derived from murine retroviruses. In some embodiments, the retroviruses include those derived from any avian or mammalian cell source. The retroviruses typically are amphotropic, meaning that they are capable of infecting host cells of several species, including humans. In one embodiment, the gene to be expressed replaces the retroviral gag, pol and/or env sequences. A number of illustrative retroviral systems have been described (*e.g.*, U.S. Pat. Nos. 5,219,740; 6,207,453; 5,219,740; Miller and Rosman (1989) *BioTechniques* 7:980-990; Miller, A. D. (1990) *Human Gene Therapy* 1:5-14; Scarpa *et al.* (1991) *Virology* 180:849-852; Burns *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:8033-8037; and Boris-Lawrie and Temin (1993) *Cur. Opin. Genet. Develop.* 3:102-109).

[0481] Methods of lentiviral transduction are known. Exemplary methods are described in, *e.g.*, Wang *et al.* (2012) *J. Immunother.* 35(9): 689-701; Cooper *et al.* (2003) *Blood*. 101:1637–

1644; Verhoeven *et al.* (2009) *Methods Mol Biol.* 506: 97-114; and Cavalieri *et al.* (2003) *Blood.* 102(2): 497-505.

[0482] In some embodiments, recombinant nucleic acids are transferred into T cells via electroporation (*see, e.g.,* Chicaybam *et al.*, (2013) *PLoS ONE* 8(3): e60298 and Van Tedeloo *et al.* (2000) *Gene Therapy* 7(16): 1431-1437). In some embodiments, recombinant nucleic acids are transferred into T cells via transposition (*see, e.g.,* Manuri *et al.* (2010) *Hum Gene Ther* 21(4): 427-437; Sharma *et al.* (2013) *Molec Ther Nucl Acids* 2, e74; and Huang *et al.* (2009) *Methods Mol Biol* 506: 115-126). Other methods of introducing and expressing genetic material in immune cells include calcium phosphate transfection (*e.g.,* as described in *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, N.Y.), protoplast fusion, cationic liposome-mediated transfection; tungsten particle-facilitated microparticle bombardment (Johnston, *Nature*, 346: 776-777 (1990)); and strontium phosphate DNA co-precipitation (Brash *et al.*, *Mol. Cell Biol.*, 7: 2031-2034 (1987)).

[0483] Other approaches and vectors for transfer of the nucleic acids encoding the recombinant products are those described, *e.g.,* in international patent application, Publication No.: WO2014055668, and U.S. Patent No. 7,446,190.

[0484] In some embodiments, the cells, *e.g.,* T cells, may be transfected either during or after expansion *e.g.,* with a chimeric antigen receptor (CAR). This transfection for the introduction of the gene of the desired receptor can be carried out with any suitable retroviral vector, for example. The genetically modified cell population can then be liberated from the initial stimulus (the anti-CD3/anti-CD28 stimulus, for example) and subsequently be stimulated with a second type of stimulus *e.g.,* via a de novo introduced receptor). This second type of stimulus may include an antigenic stimulus in form of a peptide/MHC molecule, the cognate (cross-linking) ligand of the genetically introduced receptor (*e.g.,* natural ligand of a CAR) or any ligand (such as an antibody) that directly binds within the framework of the new receptor (*e.g.,* by recognizing constant regions within the receptor). *See, for example,* Cheadle *et al.*, “Chimeric antigen receptors for T-cell based therapy” *Methods Mol Biol.* 2012; 907:645-66 or Barrett *et al.*, *Chimeric Antigen Receptor Therapy for Cancer Annual Review of Medicine* Vol. 65: 333-347 (2014).

[0485] In some cases, a vector may be used that does not require that the cells, *e.g.,* T cells, are activated. In some such instances, the cells may be selected and/or transduced prior to

activation. Thus, the cells may be engineered prior to, or subsequent to culturing of the cells, and in some cases at the same time as or during at least a portion of the culturing.

[0486] Among additional nucleic acids, *e.g.*, genes for introduction are those to improve the efficacy of therapy, such as by promoting viability and/or function of transferred cells; genes to provide a genetic marker for selection and/or evaluation of the cells, such as to assess *in vivo* survival or localization; genes to improve safety, for example, by making the cell susceptible to negative selection *in vivo* as described by Lupton S. D. *et al.*, *Mol. and Cell Biol.*, 11:6 (1991); and Riddell *et al.*, *Human Gene Therapy* 3:319-338 (1992); *see also* the publications of PCT/US91/08442 and PCT/US94/05601 by Lupton *et al.* describing the use of bifunctional selectable fusion genes derived from fusing a dominant positive selectable marker with a negative selectable marker. *See, e.g.*, Riddell *et al.*, US Patent No. 6,040,177, at columns 14-17.

C. Methods of Manufacturing Engineered Cells

[0487] In particular embodiments, the engineered cells are produced by a process that generates an output composition of enriched T cells from one or more input compositions and/or from a single biological sample. In certain embodiments, the output composition contains cells that express a recombinant receptor, *e.g.*, a CAR, such as an anti-CD19 CAR. In particular embodiments, the cells of the output compositions are suitable for administration to a subject as a therapy, *e.g.*, an autologous cell therapy. In some embodiments, the output composition is a composition of enriched CD3+ T cells, or enriched CD4+ and CD8+ T cells. The T cells are engineered by methods that involve introduction of a nucleic acid encoding the CAR, *e.g.*, anti-CD19 CAR into cells under conditions in which the nucleic acid is integrated into the genome of the cells. In some embodiments, the engineering methods include transduction with viral vectors, such as lentiviral vectors.

[0488] In some embodiments, the process for generating or producing engineered cells is by a process that includes some or all of the steps of: collecting or obtaining a biological sample; isolating, selecting, or enriching input cells from the biological sample; cryopreserving and storing the input cells; thawing and/or incubating the input cells under stimulating conditions; engineering the stimulated cells to express or contain a recombinant polynucleotide, *e.g.*, a polynucleotide encoding a recombinant receptor such as a CAR; cultivating the engineered cells, *e.g.*, to a threshold amount, density, or expansion; formulating the cultivated cells in an output

composition; and/or cryopreserving and storing the formulated output cells until the cells are released for infusion and/or are suitable to be administered to a subject.

[0489] In certain embodiments, the process for producing engineered cells further can include one or more of: activating and/or stimulating a cells, *e.g.*, cells of an input composition; genetically engineering the activated and/or stimulated cells, *e.g.*, to introduce a polynucleotide encoding a recombinant protein by transduction or transfection; and/or cultivating the engineered cells, *e.g.*, under conditions that promote proliferation and/or expansion. In particular embodiments, the provided methods may be used in connection with harvesting, collecting, and/or formulating output compositions produced after the cells have been incubated, activated, stimulated, engineered, transduced, transfected, and/or cultivated.

[0490] In some embodiments, engineered cells, such as those that express an anti-CD19 CAR as described, used in accord with the provided methods and uses are produced or generated by a process for selecting, isolating, activating, stimulating, expanding, cultivating, and/or formulating cells. In some embodiments, such methods include any as described.

[0491] In some embodiments, engineered cells, such as those that express an anti-CD19 CAR as described, used in accord with the provided methods and uses are produced or generated by exemplary processes as described in, for example, PCT/US2019/046062, WO 2019/089855 and WO 2015/164675.

[0492] In particular embodiments, the T cells are activated or stimulated by contacting the cells with an oligomeric reagent, *e.g.*, a streptavidin mutein oligomer. In some embodiments, the cells are engineered by a process that is completed within 96 hours or less, of stimulating the cells with an oligomeric reagent, *e.g.*, a streptavidin mutein oligomer. In some embodiments, the provided methods do not include a step to expand or increase the number of cells during the process. Exemplary methods of manufacturing and engineered cells produced by such methods are disclosed in PCT/US2019/046062, which is incorporated by reference in its entirety.

[0493] In particular embodiments, the provided methods are used in connection with an entire process for generating or producing output cells and/or an output populations of engineered T cells, such as a process including some or all of the steps of: stimulating cells from an input population; engineering, transforming, transducing, or transfecting the stimulated cells to express or contain a heterologous or recombinant polynucleotide, *e.g.*, a polynucleotide encoding a recombinant receptor such as a CAR; incubating the cells, removing or separating a

stimulatory reagent from the cells, and harvesting and collecting the cells, in some aspects thereby generating an output population of engineered T cells.

[0494] In some embodiments, the provided methods are used in connection with an entire process for generating or producing output cells and/or output compositions of enriched T cells, such as a process including some or all of the steps of: collecting or obtaining a biological sample; isolating, selecting, or enriching input cells from the biological sample; cryofreezing and storing and then thawing the input cells; stimulating the cells; genetically engineering the stimulated cells to express or contain a recombinant polynucleotide, *e.g.*, a polynucleotide encoding a recombinant receptor such as a CAR; formulating the engineered cells in an output composition; and cryofreezing and storing the formulated output cells until the cells are released for infusion and or administration to a subject. In some embodiments, the provided methods do not include a step to expand or increase the number of cells during the process, such as by cultivating the cells in a bioreactor under conditions where the cells expand, such as to a threshold amount that is at least 3, 4, 5, or more times the amount, level, or concentration of the cells as compared to the input population. In some embodiments, genetically engineering the cells is or includes steps for transducing the cells with a viral vector, such as by spinoculating the cells in the presence of viral particles and then incubating the cells under static conditions in the presence of the viral particles.

[0495] In certain embodiments, the total duration of the provided process for generating engineered cells, from the initiation of the stimulation to collecting, harvesting, or formulating the cells is, is about, or is less than 36 hours, 42 hours, 48 hours, 54 hours, 60 hours, 72 hours, 84 hours, 96 hours, 108 hours, or 120 hours. In certain embodiments, the total duration of the provided process for generating engineered cells, from the initiation of the stimulation to collecting, harvesting, or formulating the cells is, is about, or is less than 1.5 days, 2 days, 3 days, 4 days, or 5 days. In some embodiments, the total duration of the provided process for generating engineered cells, from the initiation of the stimulation to collecting, harvesting, or formulating the cells is between or between about 36 hours and 120 hours, 48 hours and 96 hours, or 48 hours and 72 hours, inclusive, or between or between about 1.5 days and 5 days, 2 days and 4 days, or 2 days and 3 days, inclusive. In particular embodiments, the amount of time to complete the provided process as measured from the initiation of incubation to harvesting, collecting, or formulating the cells is, is about, or is less than 48 hours, 72 hours, or 96 hours, or is, is about, or is less than 2 days, 3 days, or 4 days. In particular embodiments, the amount of

time to complete the provided process as measured from the initiation of incubation to harvesting, collecting, or formulating the cells is 48 hours $\pm$ 6 hours, 72 hours $\pm$ 6 hours, or 96 hours $\pm$ 6 hours.

[0496] In some embodiments, the incubation, *e.g.*, as disclosed in Section II-C-4, is completed between or between about 24 hour and 120 hours, 36 hour and 108 hours, 48 hours and 96 hours, or 48 hours and 72 hours, inclusive, after the initiation of the stimulation. In some embodiments, the incubation is completed at, about, or within 120 hours, 108 hours, 96 hours, 72 hours, 48 hours, or 36 hours from the initiation of the stimulation. In particular embodiments, the incubation is completed after 24 hours $\pm$ 6 hours, 48 hours $\pm$ 6 hours, or 72 hours $\pm$ 6 hours. In some embodiments, the incubation is completed between or between about one day and 5 days, 1.5 days and 4.5 days, 2 days and 4 days, or 2 day and 3 days, inclusive, after the initiation of the stimulation. In some embodiments, the incubation is completed at, about, or within 5 days, 4 days, 3 days, 2 days, or 1.5 days from the initiation of the stimulation.

[0497] In some embodiments, the entire process is performed with a single population of enriched T cells, *e.g.*, CD4+ and CD8+ T cells. In certain embodiments, the process is performed with two or more input populations of enriched T cells (*e.g.*, CD4+ and CD8+ cells) that are combined prior to and/or during the process to generate or produce a single output population of enriched T cells. In some embodiments, the enriched T cells are or include engineered T cells, *e.g.*, T cells transduced to express a recombinant receptor.

[0498] In some embodiments, an output population, *e.g.*, a population of engineered T cells, is generated by (i) incubating an input population of or containing T cells under stimulating conditions for between or between about 18 and 30 hours, inclusive, (ii) introducing a heterologous or recombinant polynucleotide encoding a recombinant receptor into T cells of the stimulated population, (iii) incubating the cells, and then (iv) collecting or harvesting the incubated cells.

[0499] In some embodiments, the cells are collected or harvested within between 36 and 108 hours or between 1.5 days and 4.5 days after the incubation under stimulatory conditions is initiated. In particular embodiments, the cells are collected or harvested within 48 hours or two days after the transformed (*e.g.*, genetically engineered, transduced, or transfected) T cells achieve a stable integrated vector copy number (iVCN) per genome that does not increase or decrease by more than 20% within a span of 24-48 hours or one to two days. In some embodiments, the integration is considered stable when the measured iVCN of a cell population

is within or within about 20%, 15%, 10%, or 5% of the total vector copy number (VCN) measured in the population. Particular embodiments contemplate that to achieve a stable integration, the cells must be incubated for, for about, or for at least 48 hours, 60 hours, or 72 hours, or one day, 2 days, or 3 days, after the viral vector is contacted or introduced to the cells. In some embodiments, the stable integration occurs within or with about 72 hours of the incubation. In some embodiments, the cells are collected or harvested at a time when the total number of transformed T cells is at or less than the total number of cells of the input population. In various embodiments, the cells are collected or harvested at a time before the cells of the input population have doubled more than three, two, or one time(s). Exemplary methods and compositions for the VCN and iVCN assays are disclosed in PCT/US2019/046048, which is incorporated herein by reference in its entirety.

[0500] In certain embodiments, an output population, *e.g.*, a population of engineered T cells, is generated by (i) incubating an input population comprising T cells under stimulating conditions for between 18 and 30 hours, inclusive, in the presence of a stimulatory reagent, *e.g.*, a stimulatory reagent described herein, such as in Section II-C-2, (ii) transducing the stimulated T cells with a viral vector encoding a recombinant receptor, such as by spinoculating the stimulated T cells in the presence of the viral vector, (iii) incubating the transduced T cells under static conditions for between or between 18 hours and 96 hours, inclusive, and (iv) harvesting T cells of the transformed population within between or between about 36 and 108 hours after the incubation under stimulatory conditions is initiated.

[0501] In some embodiments, the duration or amount of time required to complete the provided process, as measured from the isolation, enrichment, and/or selection input cells (*e.g.*, CD4+ or CD8+ T cells) from a biological sample to the time at which a the output cells are collected, formulated, and/or cryoprotected is, is about, or is less than 48 hours, 72 hours, 96 hours, 120 hours, 2 days, 3 days, 4 days, 5 days, 7 days, or 10 days. In some embodiments, isolated, selected, or enriched cells are not cryoprotected prior to the stimulation, and the duration or amount of time required to complete the provided process, as measured from the isolation, enrichment, and/or selection input cells (to the time at which a the output cells are collected, formulated, and/or cryoprotected is, is about, or is less than 48 hours, 72 hours, 96 hours, or 120 hours, or 2 days, 3 days, 4 days, or 5 days.

[0502] In certain embodiments, the provided processes are performed on a population of cells, *e.g.*, CD4+ and CD8+ T cells, that were isolated, enriched, or selected from a biological

sample. In some aspects, the provided methods can produce or generate a composition of engineered T cells from when a biological sample is collected from a subject within a shortened amount of time as compared to other methods or processes. In some embodiments, the provided methods can produce or generate engineered T cells, including any or all times where biological samples, or enriched, isolated, or selected cells are cryopreserved and stored prior to steps for stimulation or transduction, within or within about 10 days, 9 days, 8 days, 7 days, 6 days, 5 days, 4 days, 3 days, 2 days, or within or within about 120 hours, 96 hours, 72 hours, or 48 hours, from when a biological sample is collected from a subject to when the engineered T cells are collected, harvested, or formulated (*e.g.*, for cryopreservation or administration). In particular embodiments, the provided methods can produce or generate engineered T cells, including any or all times where biological samples, or enriched, isolated, or selected cells are cryopreserved and stored prior to steps for stimulation or transduction, within between or between about 6 days and 8 days, inclusive, from when the biological sample is collected from a subject to when the engineered T cells are collected, harvested, or formulated.

[0503] In certain embodiments, the provided methods are used in connection with a process for generating or producing output cells and/or output populations of enriched T cells. In particular embodiments, the output cells and/or output populations of enriched T cells are or include cells that were collected, obtained, isolated, selected, and/or enriched from the biological sample, such as a blood sample or leukapheresis sample; incubated under stimulating conditions; engineered, *e.g.*, transduced, to express or contain a recombinant polynucleotide, *e.g.*, a polynucleotide encoding a recombinant receptor such as a CAR; incubated to a threshold cell amount or density; and/or formulated. In some embodiments, the output population have been previously cryoprotected and thawed, *e.g.*, during, prior to, and/or after one or more steps of the process. In some embodiments, the output population contains T cells, *e.g.*, CD4⁺ T cells and CD8⁺ T cells, that express a recombinant receptor, *e.g.*, a CAR.

[0504] In some embodiments, at least 30%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90%, at least 95%, of the cells of the output population express the recombinant receptor. In certain embodiments, at least 50% of the cells of the output composition express the recombinant receptor. In certain embodiments, at least 30%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%, of the CD3⁺ T cells of the output composition express the

recombinant receptor. In some embodiments, at least 50% of the CD3+ T cells of the output composition express the recombinant receptor. In particular embodiments, at least at least 30%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or more than 99% of the CD4+ T cells of the output composition express the recombinant receptor. In particular embodiments, at least 50% of the CD4+ T cells of the output composition express the recombinant receptor. In some embodiments, at least at least 30%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or more than 99% of the CD8+ T cells of the output composition express the recombinant receptor. In certain embodiments, at least 50% of the CD8+ T cells of the output composition express the recombinant receptor.

[0505] In particular embodiments, the cells of the output composition have cytolytic activity towards cells expressing an antigen bound by and/or recognized by the recombinant receptor (e.g., CAR). In some embodiments, when the cells of the output composition are exposed to the cells that express the antigen, e.g., the target cells, the cells of the output composition kill, kill about, or kill at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of cells that express the antigen.

[0506] In particular embodiments, a majority of the cells of the output population are naïve-like, central memory, and/or effector memory cells. In particular embodiments, a majority of the cells of the output population are naïve-like or central memory cells. In some embodiments, a majority of the cells of the output population are positive for one or more of CCR7 or CD27 expression. In certain embodiments, the cells of the output population have a greater portion of naïve-like or central memory cells that output populations generated from alternative processes, such as processes that involve expansion.

[0507] In certain embodiments, the cells of the output population have a low portion and/or frequency of cells that are exhausted and/or senescent. In particular embodiments, the cells of the output population have a low portion and/or frequency of cells that are exhausted and/or senescent. In some embodiments, less than 40%, less than 35%, less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, less than 5%, or less than 1% of the cells of the output population are exhausted and/or senescent. In certain embodiments, less than 25% of the

cells of the output population are exhausted and/or senescent. In certain embodiments, less than 10% of the cells of the output population are exhausted and/or senescent.

[0508] In some embodiments, the cells of the output population have a low portion and/or frequency of cells that are negative for CD27 and CCR7 expression, *e.g.*, surface expression. In particular embodiments, the cells of the output population have a low portion and/or frequency of CD27- CCR7- cells. In some embodiments, less than 40%, less than 35%, less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, less than 5%, or less than 1% of the cells of the output population are CD27- CCR7- cells. In certain embodiments, less than 25% of the cells of the output population are CD27- CCR7- cells. In certain embodiments, less than 10% of the cells of the output population are CD27- CCR7- cells. In embodiments, less than 5% of the cells of the output population are CD27- CCR7- cells.

[0509] In some embodiments, the cells of the output population have a high portion and/or frequency of cells that are positive for one or both of CD27 and CCR7 expression, *e.g.*, surface expression. In some embodiments, the cells of the output population have a high portion and/or frequency of cells that are positive for one or both of CD27 and CCR7. In some embodiments, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells of the output population are positive for one or both of CD27 and CCR7. In various embodiments, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or greater than 95% of the CD4+ CAR+ cells of the output population are positive for one or both of CD27 and CCR7. In some embodiments, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or greater than 95% of the CD8+ CAR+ cells of the output population are positive for one or both of CD27 and CCR7.

[0510] In certain embodiments, the cells of the output population have a high portion and/or frequency of cells that are positive for CD27 and CCR7 expression, *e.g.*, surface expression. In some embodiments, the cells of the output population have a high portion and/or frequency of CD27+ CCR7+ cells. In some embodiments, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells of the output population are CD27+ CCR7+ cells. In various embodiments, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or greater than 95% of the CD4+ CAR+ cells of the output population are CD27+ CCR7+ cells. In some embodiments, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%,

at least 90%, at least 95% or greater than 95% of the CD8+ CAR+ cells of the output population are CD27+ CCR7+ cells.

[0511] In certain embodiments, the cells of the output population have a low portion and/or frequency of cells that are negative for CCR7 and positive for CD45RA expression, *e.g.*, surface expression. In some embodiments, the cells of the output population have a low portion and/or frequency of CCR7-CD45RA+ cells. In particular embodiments, less than 40%, less than 35%, less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, less than 5%, or less than 1% of the cells of the output population are CCR7-CD45RA+ cells. In some embodiments, less than 25% of the cells of the output population are CCR7-CD45RA+ cells. In particular embodiments, less than less than 10% of the cells of the output population are CCR7-CD45RA+ cells. In certain embodiments, less than 5% of the cells of the output population are CCR7-CD45RA+ cells.

[0512] In particular embodiments, the cells are harvested prior to, prior to about, or prior to at least one, two, three, four, five, six, eight, ten, twenty, or more cell doublings of the cell population, *e.g.*, doublings that occur during the incubating. In certain embodiments, the cells are harvested prior to any doubling of the population, *e.g.*, doubling that occurs during the incubation. In some aspects, reducing the doubling that may occur during an engineering process will, in some embodiments, increase the portion of engineered T cells that are naïve-like. In some embodiments, increasing the doubling during an engineering process increases T cell differentiation that may occur during the engineering process.

[0513] In some aspects, it is contemplated that, for a process for generating or producing engineered cell compositions, reducing the expansion or cell doublings that occur during the process, *e.g.*, during the incubation, increases the amount or portion of naïve-like T cells of the resulting engineered cell composition. In particular aspects, increasing the expansion or cell doublings that occur during the process increases the amount or portion of differentiated T cells of the resulting engineered cell composition. In some aspects, it is contemplated that process, such as the processes provided herein, that increase or enlarge the portion of naïve-like cells in the resulting engineered cell composition may increase the potency, efficacy, and persistence, *e.g.*, *in vivo* after administration, of the engineered cell composition.

1. Cells and Preparation of Cells for Genetic Engineering

[0514] In some embodiments, cells, such as T cells, used in connection with the provided methods, uses, articles of manufacture or compositions are cells have been genetically engineered to express a recombinant receptor, *e.g.*, a CAR described herein. In some embodiments, the engineered cells are used in the context of cell therapy, *e.g.*, adoptive cell therapy. In some embodiments, the engineered cells are immune cells. In some embodiments, the engineered cells are T cells, such as CD4+ or CD8+ T cells.

[0515] In some embodiments, preparation of the engineered cells includes one or more culture and/or preparation steps. The cells for introduction of the nucleic acid encoding the transgenic receptor such as the CAR, may be isolated from a sample, such as a biological sample, *e.g.*, one obtained from or derived from a subject. In some embodiments, the subject from which the cell is isolated is one having the disease or condition or in need of a cell therapy or to which cell therapy will be administered. The subject in some embodiments is a human in need of a particular therapeutic intervention, such as the adoptive cell therapy for which cells are being isolated, processed, and/or engineered.

[0516] Accordingly, the cells in some embodiments are primary cells, *e.g.*, primary human cells. The samples include tissue, fluid, and other samples taken directly from the subject, as well as samples resulting from one or more processing steps, such as separation, centrifugation, genetic engineering (*e.g.*, transduction with viral vector), washing, and/or incubation. The biological sample can be a sample obtained directly from a biological source or a sample that is processed. Biological samples include, but are not limited to, body fluids, such as blood, plasma, serum, cerebrospinal fluid, synovial fluid, urine and sweat, tissue and organ samples, including processed samples derived therefrom.

[0517] In some aspects, the sample is blood or a blood-derived sample, or is derived from an apheresis or leukapheresis product. Exemplary samples include whole blood, peripheral blood mononuclear cells (PBMCs), leukocytes, bone marrow, thymus, tissue biopsy, tumor, leukemia, lymphoma, lymph node, gut associated lymphoid tissue, mucosa associated lymphoid tissue, spleen, other lymphoid tissues, liver, lung, stomach, intestine, colon, kidney, pancreas, breast, bone, prostate, cervix, testes, ovaries, tonsil, or other organ, and/or cells derived therefrom. Samples include, in the context of cell therapy, *e.g.*, adoptive cell therapy, samples from autologous and allogeneic sources.

[0518] In some examples, cells from the circulating blood of a subject are obtained, *e.g.*, by apheresis or leukapheresis. The samples, in some aspects, contain lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and/or platelets, and in some aspects contains cells other than red blood cells and platelets.

[0519] In some embodiments, the blood cells collected from the subject are washed, *e.g.*, to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In some embodiments, the cells are washed with phosphate buffered saline (PBS). In some embodiments, the wash solution lacks calcium and/or magnesium and/or many or all divalent cations. In some aspects, a washing step is accomplished a semi-automated “flow-through” centrifuge (for example, the Cobe 2991 cell processor, Baxter) according to the manufacturer's instructions. In some aspects, a washing step is accomplished by tangential flow filtration (TFF) according to the manufacturer's instructions. In some embodiments, the cells are resuspended in a variety of biocompatible buffers after washing, such as, for example, $\text{Ca}^{++}/\text{Mg}^{++}$ free PBS. In certain embodiments, components of a blood cell sample are removed and the cells directly resuspended in culture media.

[0520] In some embodiments, the sample containing cells (*e.g.*, an apheresis product or a leukapheresis product) is washed in order to remove one or more anti-coagulants, such as heparin, added during apheresis or leukapheresis.

[0521] In some embodiments, the sample containing cells (*e.g.*, 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) is cryopreserved and/or cryoprotected (*e.g.*, frozen) and then thawed and optionally washed prior to any steps for isolating, selecting, activating, stimulating, engineering, transducing, transfecting, incubating, culturing, harvesting, formulating a population of the cells, and/or administering the formulated cell population to a subject.

[0522] In some embodiments, a sample containing autologous Peripheral Blood Mononuclear Cells (PBMCs) from a subject is collected in a method suitable to ensure appropriate quality for manufacturing. In one aspect, the sample containing PBMCs is derived from fractionated whole blood. In some embodiments, whole blood from a subject is fractionated by leukapheresis using a centrifugal force and making use of the density differences between cellular phenotypes, when autologous mononuclear cells (MNCs) are preferentially enriched while other cellular phenotypes, such as red blood cells, are reduced in the collected

cell composition. In some embodiments, autologous plasma is concurrently collected during the MNC collection, which in some aspects can allow for extended leukapheresis product stability. In one aspect, the autologous plasma is added to the leukapheresis product to improve the buffering capacity of the leukapheresis product matrix. In some aspects, a total volume of whole blood processed in order to generate the leukapheresis product is or is about 2L, 4L, 6L, 8L, 10L, 12L, 14L, 16L, 18L, or 20L, or is any value between any of the foregoing. In some embodiments, the volume of autologous plasma collected is or is about 10mL, 50mL, 100mL, 150mL, 200mL, 250mL, or 300mL, or more, or is a volume between any of the foregoing. In some embodiments, the leukapheresis product is subjected to a procedure, *e.g.*, washing and formulation for in-process cryopreservation, within about 48 hours of the leukapheresis collection completion. In some embodiments, the leukapheresis product is subjected to one or more wash steps, *e.g.*, within about 2 hours, 6 hours, 12 hours, 18 hours, 24 hours, 36 hours, or 48 hours of the leukapheresis collection completion. In some aspects, the one or more wash step removes the anticoagulant during leukapheresis collection, cellular waste that may have accumulated in the leukapheresis product, residual platelets and/or cellular debris. In some embodiments, one or more buffer exchange is performed during the one or more wash step.

[0523] In particular embodiments, an apheresis product or a leukapheresis product is cryopreserved and/or cryoprotected (*e.g.*, frozen) and then thawed before being subject to a cell selection or isolation step (*e.g.*, a T cell selection or isolation step) as described *infra*. In some embodiments, after a cryopreserved and/or cryoprotected apheresis product or leukapheresis product is subject to a T cell selection or isolation step, no additional cryopreservation and/or cryoprotection step is performed during or between any of the subsequent steps, such as the steps of activating, stimulating, engineering, transducing, transfecting, incubating, culturing, harvesting, formulating a population of the cells, and/or administering the formulated cell population to a subject. For example, T cells selected from a thawed cryopreserved and/or cryoprotected apheresis product or leukapheresis product are not again cryopreserved and/or cryoprotected before being thawed and optionally washed for a downstream process, such as T cell activation/stimulation or transduction.

[0524] In particular embodiments, an apheresis product or a leukapheresis product is cryopreserved and/or cryoprotected (*e.g.*, frozen) at a density of, of about, or at least 5×10^6 cells/mL, 10×10^6 cells/mL, 20×10^6 cells/mL, 30×10^6 cells/mL, 40×10^6 cells/mL, 50×10^6 cells/mL, 60×10^6 cells/mL, 70×10^6 cells/mL, 80×10^6 cells/mL, 90×10^6 cells/mL, 100×10^6

cells/mL, 110×10^6 cells/mL, 120×10^6 cells/mL, 130×10^6 cells/mL, 140×10^6 cells/mL, or 150×10^6 cells/mL, or any value between any of the foregoing, in a cryopreservation solution or buffer. In some embodiments, the cryopreservation solution or buffer is or contains, for example, a DMSO solution optionally comprising human serum albumin (HSA), or other suitable cell freezing media.

[0525] Exemplary methods and systems for cryogenic storage and processing of cells from a sample, such as an apheresis sample, can include those described in WO2018170188. In some embodiments, the method and systems involve collecting apheresis before the patient needs cell therapy, and then subjecting the apheresis sample to cryopreservation for later use in a process for engineering the cells, *e.g.*, T cells, with a recombinant receptor (*e.g.*, CAR). In some cases, such processes can include those described herein. In some embodiments, an apheresis sample is collected from a subject and cryopreserved prior to subsequent T cell selection, activation, stimulation, engineering, transduction, transfection, incubation, culturing, harvest, formulation of a population of the cells, and/or administration of the formulated cell population to a subject. In such examples, the cryopreserved apheresis sample is thawed prior to subjecting the sample to one or more selection steps, such as any as described herein.

[0526] In some embodiments, the cryopreserved and/or cryoprotected sample of cells (*e.g.*, apheresis or leukapheresis sample) is thawed prior to its use for downstream processes for manufacture of a cell population for cell therapy, for example, a T cell population containing CAR+ T cells. In some embodiments, such a cryopreserved and/or cryoprotected sample of cells (*e.g.*, apheresis or leukapheresis sample) is used in connection with the process provided herein for engineered a T cell therapy, such as a CAR+ T cell therapy. In particular examples, no further step of cryopreservation is carried out prior to or during the harvest/formulation steps.

[0527] In some embodiments, selection, isolation, or enrichment of the cells or populations includes one or more preparation and/or non-affinity based cell separation steps. In some examples, cells are washed, centrifuged, and/or incubated in the presence of one or more reagents, for example, to remove unwanted components, enrich for desired components, lyse or remove cells sensitive to particular reagents. In some examples, cells are separated based on one or more property, such as density, adherent properties, size, sensitivity and/or resistance to particular components.

[0528] In some examples, cells from the circulating blood of a subject are obtained, *e.g.*, by apheresis or leukapheresis. The samples, in some aspects, contain lymphocytes, including T

cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and/or platelets, and in some aspects contains cells other than red blood cells and platelets.

[0529] In some embodiments, the blood cells collected from the subject are washed, *e.g.*, to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In some embodiments, the cells are washed with phosphate buffered saline (PBS). In some embodiments, the wash solution lacks calcium and/or magnesium and/or many or all divalent cations. In some aspects, a washing step is accomplished a semi-automated “flow-through” centrifuge (for example, the Cobe 2991 cell processor, Baxter) according to the manufacturer’s instructions. In some aspects, a washing step is accomplished by tangential flow filtration (TFF) according to the manufacturer’s instructions. In some embodiments, the cells are resuspended in a variety of biocompatible buffers after washing, such as, for example, $\text{Ca}^{++}/\text{Mg}^{++}$ free PBS. In certain embodiments, components of a blood cell sample are removed and the cells directly resuspended in culture media.

[0530] In some embodiments, the methods include density-based cell separation methods, such as the preparation of white blood cells from peripheral blood by lysing the red blood cells and centrifugation through a Percoll or Ficoll gradient.

[0531] In some embodiments, at least a portion of the selection step includes incubation of cells with a selection reagent. The incubation with a selection reagent or reagents, *e.g.*, as part of selection methods which may be performed using one or more selection reagents for selection of one or more different cell types based on the expression or presence in or on the cell of one or more specific molecules, such as surface markers, *e.g.*, surface proteins, intracellular markers, or nucleic acid. In some embodiments, any known method using a selection reagent or reagents for separation based on such markers may be used. In some embodiments, the selection reagent or reagents result in a separation that is affinity- or immunoaffinity-based separation. For example, the selection in some aspects includes incubation with a reagent or reagents for separation of cells and cell populations based on the cells’ expression or expression level of one or more markers, typically cell surface markers, for example, by incubation with an antibody or binding partner that specifically binds to such markers, followed generally by washing steps and separation of cells having bound the antibody or binding partner, from those cells having not bound to the antibody or binding partner.

[0532] In some embodiments, the isolation methods include the separation of different cell types based on the expression or presence in the cell of one or more specific molecules, such as

surface markers, *e.g.*, surface proteins, intracellular markers, or nucleic acid. In some embodiments, any known method for separation based on such markers may be used. In some embodiments, the separation is affinity- or immunoaffinity-based separation. For example, the isolation in some aspects includes separation of cells and cell populations based on the cells' expression or expression level of one or more markers, typically cell surface markers, for example, by incubation with an antibody or binding partner that specifically binds to such markers, followed generally by washing steps and separation of cells having bound the antibody or binding partner, from those cells having not bound to the antibody or binding partner.

[0533] Such separation steps can be based on positive selection, in which the cells having bound the reagents are retained for further use, and/or negative selection, in which the cells having not bound to the antibody or binding partner are retained. In some examples, both fractions are retained for further use. In some aspects, negative selection can be particularly useful where no antibody is available that specifically identifies a cell type in a heterogeneous population, such that separation is best carried out based on markers expressed by cells other than the desired population.

[0534] The separation need not result in 100% enrichment or removal of a particular cell population or cells expressing a particular marker. For example, positive selection of or enrichment for cells of a particular type, such as those expressing a marker, refers to increasing the number or percentage of such cells, but need not result in a complete absence of cells not expressing the marker. Likewise, negative selection, removal, or depletion of cells of a particular type, such as those expressing a marker, refers to decreasing the number or percentage of such cells, but need not result in a complete removal of all such cells.

[0535] In some examples, multiple rounds of separation steps are carried out, where the positively or negatively selected fraction from one step is subjected to another separation step, such as a subsequent positive or negative selection. In some examples, a single separation step can deplete cells expressing multiple markers simultaneously, such as by incubating cells with a plurality of antibodies or binding partners, each specific for a marker targeted for negative selection. Likewise, multiple cell types can simultaneously be positively selected by incubating cells with a plurality of antibodies or binding partners expressed on the various cell types.

[0536] For example, in some aspects, specific subpopulations of T cells, such as cells positive or expressing high levels of one or more surface markers, *e.g.*, CD28⁺, CD62L⁺,

CCR7⁺, CD27⁺, CD127⁺, CD4⁺, CD8⁺, CD45RA⁺, and/or CD45RO⁺ T cells, are isolated by positive or negative selection techniques.

[0537] For example, CD3⁺, CD28⁺ T cells can be positively selected using anti-CD3/anti-CD28 conjugated magnetic beads (*e.g.*, DYNABEADS® M-450 CD3/CD28 T Cell Expander).

[0538] In some embodiments, isolation is carried out by enrichment for a particular cell population by positive selection, or depletion of a particular cell population, by negative selection. In some embodiments, positive or negative selection is accomplished by incubating cells with one or more antibodies or other binding agent that specifically bind to one or more surface markers expressed or expressed (marker⁺) at a relatively higher level (marker^{high}) on the positively or negatively selected cells, respectively.

[0539] In some embodiments, a biological sample, *e.g.*, a sample of PBMCs or other white blood cells, are subjected to selection of T cells. In some embodiments, the selection results in an enriched composition of input cells in which at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the cells in the composition are T cells. In some embodiments, a biological sample, *e.g.*, a sample of PBMCs or other white blood cells, are subjected to selection of CD3⁺ T cells. In some embodiments, the selection results in an enriched composition of input cells in which at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the cells in the composition are CD3⁺ T cells. In some embodiments, a biological sample, *e.g.*, a sample of PBMCs or other white blood cells, are subjected to selection of CD4⁺ T cells and CD8⁺ T cells. In some embodiments, the selection results in an enriched composition of input cells in which at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the cells in the composition are CD4⁺ and CD8⁺ T cells.

[0540] In particular embodiments, a biological sample, *e.g.*, a sample of PBMCs or other white blood cells, are subjected to selection of CD4⁺ T cells, where both the negative and positive fractions are retained. In certain embodiments, CD8⁺ T cells are selected from the negative fraction. In some embodiments, a biological sample is subjected to selection of CD8⁺ T cells, where both the negative and positive fractions are retained. In certain embodiments, CD4⁺ T cells are selected from the negative fraction.

[0541] In some embodiments, T cells are separated from a PBMC sample by negative selection of markers expressed on non-T cells, such as B cells, monocytes, or other white blood cells, such as CD14. In some aspects, a CD4⁺ or CD8⁺ selection step is used to separate CD4⁺ helper and CD8⁺ cytotoxic T cells. Such CD4⁺ and CD8⁺ populations can be further sorted into

sub-populations by positive or negative selection for markers expressed or expressed to a relatively higher degree on one or more naive, memory, and/or effector T cell subpopulations.

[0542] In some embodiments, CD8⁺ cells are further enriched for or depleted of naive, central memory, effector memory, and/or central memory stem cells, such as by positive or negative selection based on surface antigens associated with the respective subpopulation. In some embodiments, enrichment for central memory T (T_{CM}) cells is carried out to increase efficacy, such as to improve long-term survival, expansion, and/or engraftment following administration, which in some aspects is particularly robust in such sub-populations. *See* Terakura et al. (2012) *Blood*.1:72–82; Wang et al. (2012) *J Immunother.* 35(9):689-701. In some embodiments, combining T_{CM}-enriched CD8⁺ T cells and CD4⁺ T cells further enhances efficacy.

[0543] In embodiments, memory T cells are present in both CD62L⁺ and CD62L⁻ subsets of CD8⁺ peripheral blood lymphocytes. PBMC can be enriched for or depleted of CD62L⁻CD8⁺ and/or CD62L⁺CD8⁺ fractions, such as using anti-CD8 and anti-CD62L antibodies.

[0544] In some embodiments, the enrichment for central memory T (T_{CM}) cells is based on positive or high surface expression of CD45RO, CD62L, CCR7, CD28, CD3, and/or CD127; in some aspects, it is based on negative selection for cells expressing or highly expressing CD45RA and/or granzyme B. In some aspects, isolation of a CD8⁺ population enriched for T_{CM} cells is carried out by depletion of cells expressing CD4, CD14, CD45RA, and positive selection or enrichment for cells expressing CD62L. In one aspect, enrichment for central memory T (T_{CM}) cells is carried out starting with a negative fraction of cells selected based on CD4 expression, which is subjected to a negative selection based on expression of CD14 and CD45RA, and a positive selection based on CD62L. Such selections in some aspects are carried out simultaneously and in other aspects are carried out sequentially, in either order. In some aspects, the same CD4 expression-based selection step used in preparing the CD8⁺ cell population or subpopulation, also is used to generate the CD4⁺ cell population or subpopulation, such that both the positive and negative fractions from the CD4-based separation are retained and used in subsequent steps of the methods, optionally following one or more further positive or negative selection steps.

[0545] In a particular example, a sample of PBMCs or other white blood cell sample is subjected to selection of CD4⁺ cells, where both the negative and positive fractions are retained. The negative fraction then is subjected to negative selection based on expression of CD14 and

CD45RA or CD19, and positive selection based on a marker characteristic of central memory T cells, such as CD62L or CCR7, where the positive and negative selections are carried out in either order.

[0546] CD4⁺ T helper cells are sorted into naïve, central memory, and effector cells by identifying cell populations that have cell surface antigens. CD4⁺ lymphocytes can be obtained by standard methods. In some embodiments, naïve CD4⁺ T lymphocytes are CD45RO⁻, CD45RA⁺, CD62L⁺, CD4⁺ T cells. In some embodiments, central memory CD4⁺ cells are CD62L⁺ and CD45RO⁺. In some embodiments, effector CD4⁺ cells are CD62L⁻ and CD45RO⁻. In one example, to enrich for CD4⁺ cells by negative selection, a monoclonal antibody cocktail typically includes antibodies to CD14, CD20, CD11b, CD16, HLA-DR, and CD8.

[0547] In some aspects of such processes, a volume of cells is mixed with an amount of a desired affinity-based selection reagent. The immunoaffinity-based selection can be carried out using any system or method that results in a favorable energetic interaction between the cells being separated and the molecule specifically binding to the marker on the cell, *e.g.*, the antibody or other binding partner on the solid surface, *e.g.*, particle. In some embodiments, methods are carried out using particles such as beads, *e.g.*, magnetic beads, that are coated with a selection agent (*e.g.*, antibody) specific to the marker of the cells. The particles (*e.g.*, beads) can be incubated or mixed with cells in a container, such as a tube or bag, while shaking or mixing, with a constant cell density-to-particle (*e.g.*, bead) ratio to aid in promoting energetically favored interactions.

[0548] In some embodiments, the antibody or binding partner is bound to a solid support or matrix, such as a magnetic bead or paramagnetic bead, to allow for separation of cells for positive and/or negative selection. For example, in some embodiments, the cells and cell populations are separated or isolated using immunomagnetic (or affinitymagnetic) separation techniques (reviewed in *Methods in Molecular Medicine*, vol. 58: *Metastasis Research Protocols*, Vol. 2: *Cell Behavior In Vitro and In Vivo*, p 17-25 Edited by: S. A. Brooks and U. Schumacher © Humana Press Inc., Totowa, NJ).

[0549] In some aspects, the sample or composition of cells to be separated is incubated with small, magnetizable or magnetically responsive material, such as magnetically responsive particles or microparticles, such as paramagnetic beads (*e.g.*, such as Dynalbeads or MACS beads). The magnetically responsive material, *e.g.*, particle, generally is directly or indirectly attached to a binding partner, *e.g.*, an antibody, that specifically binds to a molecule, *e.g.*,

surface marker, present on the cell, cells, or population of cells that it is desired to separate, *e.g.*, that it is desired to negatively or positively select.

[0550] In some embodiments, the magnetic particle or bead comprises a magnetically responsive material bound to a specific binding member, such as an antibody or other binding partner. There are many well-known magnetically responsive materials used in magnetic separation methods. Suitable magnetic particles include those described in Molday, U.S. Pat. No. 4,452,773, and in European Patent Specification EP 452342 B, which are hereby incorporated by reference. Colloidal sized particles, such as those described in Owen U.S. Pat. No. 4,795,698, and Liberti et al., U.S. Pat. No. 5,200,084 are other examples.

[0551] The incubation generally is carried out under conditions whereby the antibodies or binding partners, or molecules, such as secondary antibodies or other reagents, which specifically bind to such antibodies or binding partners, which are attached to the magnetic particle or bead, specifically bind to cell surface molecules if present on cells within the sample.

[0552] In some aspects, the sample is placed in a magnetic field, and those cells having magnetically responsive or magnetizable particles attached thereto will be attracted to the magnet and separated from the unlabeled cells. For positive selection, cells that are attracted to the magnet are retained; for negative selection, cells that are not attracted (unlabeled cells) are retained. In some aspects, a combination of positive and negative selection is performed during the same selection step, where the positive and negative fractions are retained and further processed or subject to further separation steps.

[0553] In certain embodiments, the magnetically responsive particles are coated in primary antibodies or other binding partners, secondary antibodies, lectins, enzymes, or streptavidin. In certain embodiments, the magnetic particles are attached to cells via a coating of primary antibodies specific for one or more markers. In certain embodiments, the cells, rather than the beads, are labeled with a primary antibody or binding partner, and then cell-type specific secondary antibody- or other binding partner (*e.g.*, streptavidin)-coated magnetic particles, are added. In certain embodiments, streptavidin-coated magnetic particles are used in conjunction with biotinylated primary or secondary antibodies.

[0554] In some embodiments, the magnetically responsive particles are left attached to the cells that are to be subsequently incubated, cultured and/or engineered; in some aspects, the particles are left attached to the cells for administration to a patient. In some embodiments, the magnetizable or magnetically responsive particles are removed from the cells. Methods for

removing magnetizable particles from cells are known and include, *e.g.*, the use of competing non-labeled antibodies, and magnetizable particles or antibodies conjugated to cleavable linkers. In some embodiments, the magnetizable particles are biodegradable.

[0555] In some embodiments, the affinity-based selection is via magnetic-activated cell sorting (MACS) (Miltenyi Biotec, Auburn, CA). Magnetic Activated Cell Sorting (MACS) systems are capable of high-purity selection of cells having magnetized particles attached thereto. In certain embodiments, MACS operates in a mode wherein the non-target and target species are sequentially eluted after the application of the external magnetic field. That is, the cells attached to magnetized particles are held in place while the unattached species are eluted. Then, after this first elution step is completed, the species that were trapped in the magnetic field and were prevented from being eluted are freed in some manner such that they can be eluted and recovered. In certain embodiments, the non-target cells are labelled and depleted from the heterogeneous population of cells.

[0556] In certain embodiments, the isolation or separation is carried out using a system, device, or apparatus that carries out one or more of the isolation, cell preparation, separation, processing, incubation, culture, and/or formulation steps of the methods. In some aspects, the system is used to carry out each of these steps in a closed or sterile environment, for example, to minimize error, user handling and/or contamination. In one example, the system is a system as described in International Patent Application, Publication Number WO2009/072003, or US 20110003380 A1.

[0557] In other cases, the methods include selection of cells in which all or a portion of the selection is carried out in the internal cavity of a centrifugal chamber, for example, under centrifugal rotation. In some embodiments, incubation of cells with selection reagents, such as immunoaffinity-based selection reagents, is performed in a centrifugal chamber. In certain embodiments, the isolation or separation is carried out using a system, device, or apparatus described in International Patent Application, Publication Number WO2009/072003, or US 20110003380 A1. In one example, the system is a system as described in International Publication Number WO2016/073602.

[0558] In some embodiments, the system or apparatus carries out one or more, *e.g.*, all, of the isolation, processing, engineering, and formulation steps in an integrated or self-contained system, and/or in an automated or programmable fashion. In some aspects, the system or apparatus includes a computer and/or computer program in communication with the system or

apparatus, which allows a user to program, control, assess the outcome of, and/or adjust various aspects of the processing, isolation, engineering, and formulation steps.

[0559] In some aspects, the separation and/or other steps is carried out using CliniMACS system (Miltenyi Biotec), for example, for automated separation of cells on a clinical-scale level in a closed and sterile system. Components can include an integrated microcomputer, magnetic separation unit, peristaltic pump, and various pinch valves. The integrated computer in some aspects controls all components of the instrument and directs the system to perform repeated procedures in a standardized sequence. The magnetic separation unit in some aspects includes a movable permanent magnet and a holder for the selection column. The peristaltic pump controls the flow rate throughout the tubing set and, together with the pinch valves, ensures the controlled flow of buffer through the system and continual suspension of cells.

[0560] The CliniMACS® system in some aspects uses antibody-coupled magnetizable particles that are supplied in a sterile, non-pyrogenic solution. In some embodiments, after labelling of cells with magnetic particles the cells are washed to remove excess particles. A cell preparation bag is then connected to the tubing set, which in turn is connected to a bag containing buffer and a cell collection bag. The tubing set consists of pre-assembled sterile tubing, including a pre-column and a separation column, and are for single use only. After initiation of the separation program, the system automatically applies the cell sample onto the separation column. Labelled cells are retained within the column, while unlabeled cells are removed by a series of washing steps. In some embodiments, the cell populations for use with the methods described herein are unlabeled and are not retained in the column. In some embodiments, the cell populations for use with the methods described herein are labeled and are retained in the column. In some embodiments, the cell populations for use with the methods described herein are eluted from the column after removal of the magnetic field, and are collected within the cell collection bag.

[0561] In certain embodiments, separation and/or other steps are carried out using the CliniMACS Prodigy system (Miltenyi Biotec). The CliniMACS Prodigy® system in some aspects is equipped with a cell processing unit that permits automated washing and fractionation of cells by centrifugation. The CliniMACS Prodigy® system can also include an onboard camera and image recognition software that determines the optimal cell fractionation endpoint by discerning the macroscopic layers of the source cell product. For example, peripheral blood is automatically separated into erythrocytes, white blood cells and plasma

layers. The CliniMACS Prodigy® system can also include an integrated cell cultivation chamber which accomplishes cell culture protocols such as, *e.g.*, cell differentiation and expansion, antigen loading, and long-term cell culture. Input ports can allow for the sterile removal and replenishment of media and cells can be monitored using an integrated microscope. See, *e.g.*, Klebanoff et al. (2012) *J Immunother.* 35(9): 651–660, Terakura et al. (2012) *Blood*.1:72–82, and Wang et al. (2012) *J Immunother.* 35(9):689-701.

[0562] In some embodiments, cells, *e.g.*, T cells, are isolated, selected, or enriched by chromatographic isolation, such as by column chromatography including affinity chromatography or gel permeations chromatography. In some embodiments, the method employs a receptor binding reagent that binds to a receptor molecule that is located on the surface of a target cell, *e.g.*, the cell to be isolated, selected, or enriched. Such methods may be described as (traceless) cell affinity chromatography technology (CATCH). In certain embodiments, methods, techniques, and reagents for selection, isolation, and enrichment are described, for example, in WO2013124474 and WO2015164675, which are hereby incorporated by reference in their entirety.

[0563] In some embodiments, a cell population described herein is collected and enriched (or depleted) via flow cytometry, in which cells stained for multiple cell surface markers are carried in a fluidic stream. In some embodiments, a cell population described herein is collected and enriched (or depleted) via preparative scale (FACS)-sorting. In certain embodiments, a cell population described herein is collected and enriched (or depleted) by use of microelectromechanical systems (MEMS) chips in combination with a FACS-based detection system (see, *e.g.*, WO 2010/033140, Cho et al. (2010) *Lab Chip* 10, 1567-1573; and Godin et al. (2008) *J Biophoton.* 1(5):355–376. In both cases, cells can be labeled with multiple markers, allowing for the isolation of well-defined T cell subsets at high purity.

[0564] In some embodiments, the antibodies or binding partners are labeled with one or more detectable marker, to facilitate separation for positive and/or negative selection. For example, separation may be based on binding to fluorescently labeled antibodies. In some examples, separation of cells based on binding of antibodies or other binding partners specific for one or more cell surface markers are carried in a fluidic stream, such as by fluorescence-activated cell sorting (FACS), including preparative scale (FACS) and/or microelectromechanical systems (MEMS) chips, *e.g.*, in combination with a flow-cytometric

detection system. Such methods allow for positive and negative selection based on multiple markers simultaneously.

2. Activation/Stimulation

[0565] In some embodiments, the cells are incubated and/or cultured prior to or in connection with genetic engineering. The incubation steps can include culture, stimulation, activation, and/or propagation. The incubation and/or engineering may be carried out in a culture vessel, such as a unit, chamber, well, column, tube, tubing set, valve, vial, culture dish, bag, or other container for culture. In some embodiments, the compositions or cells are incubated in the presence of stimulating conditions or a stimulatory agent. Such conditions include those designed to induce proliferation, expansion, activation, and/or survival of cells in the population, to mimic antigen exposure, and/or to prime the cells for genetic engineering, such as for the introduction of a recombinant antigen receptor.

[0566] In some embodiments, between at or about 1×10^5 and at or about $500,000 \times 10^6$ cells, In some embodiments, between at or about 1×10^5 and at or about $500,000 \times 10^6$ cells, between at or about 1×10^6 and at or about $50,000 \times 10^6$ cells, between at or about 10×10^6 and at or about $5,000 \times 10^6$ cells, between at or about 1×10^6 and at or about $1,000 \times 10^6$ cells, between at or about 50×10^6 and at or about $5,000 \times 10^6$ cells, between at or about 10×10^6 and at or about $1,000 \times 10^6$ cells, between at or about 100×10^6 and at or about $2,500 \times 10^6$ cells, *e.g.*, cells of the input composition, are incubated *e.g.*, under stimulating conditions such as in the presence of a stimulatory reagent. In particular embodiments, at least, at, or at about 50×10^6 cells, 100×10^6 cells, 150×10^6 cells, 200×10^6 cells, 250×10^6 cells, 300×10^6 cells, 350×10^6 cells, 400×10^6 cells, 450×10^6 cells, 500×10^6 cells, 600×10^6 cells, 700×10^6 cells, 800×10^6 cells, 900×10^6 cells, 1000×10^6 cells, 2000×10^6 cells, 3000×10^6 cells, 4000×10^6 cells, 5000×10^6 cells, or any value between any of the foregoing, are incubated, *e.g.*, under stimulating conditions. In particular embodiments, the cells are or include CD4+ T cells and CD8+ T cells.

[0567] The conditions can include one or more of particular media, temperature, oxygen content, carbon dioxide content, time, agents, *e.g.*, nutrients, amino acids, antibiotics, ions, and/or stimulatory factors, such as cytokines, chemokines, antigens, binding partners, fusion proteins, recombinant soluble receptors, and any other agents designed to activate the cells. In some embodiments, the stimulating conditions include temperature suitable for the growth of

human T lymphocytes, for example, at least about 25 degrees Celsius, generally at least about 30 degrees Celsius, and generally at or about 37 degrees Celsius.

[0568] In some embodiments, the T cells are expanded by adding to a culture-initiating composition feeder cells, such as non-dividing peripheral blood mononuclear cells (PBMC), (*e.g.*, such that the resulting population of cells contains at least about 5, 10, 20, or 40 or more PBMC feeder cells for each T lymphocyte in the initial population to be expanded); and incubating the culture (*e.g.*, for a time sufficient to expand the numbers of T cells). In some aspects, the non-dividing feeder cells can comprise gamma-irradiated PBMC feeder cells. In some embodiments, the PBMC are irradiated with gamma rays in the range of about 3000 to 3600 rads to prevent cell division. In some aspects, the feeder cells are added to culture medium prior to the addition of the populations of T cells. In some cases, the incubation may further comprise adding non-dividing EBV-transformed lymphoblastoid cells (LCL) as feeder cells. LCL can be irradiated with gamma rays in the range of about 6000 to 10,000 rads. The LCL feeder cells in some aspects is provided in any suitable amount, such as a ratio of LCL feeder cells to initial T lymphocytes of at least about 10:1.

[0569] In embodiments, antigen-specific T cells, such as antigen-specific CD4⁺ and/or CD8⁺ T cells, are obtained by stimulating naive or antigen specific T lymphocytes with antigen. For example, antigen-specific T cell lines or clones can be generated to cytomegalovirus antigens by isolating T cells from infected subjects and stimulating the cells in vitro with the same antigen.

[0570] In some embodiments, the stimulating conditions or agents include one or more agent, *e.g.*, ligand, which is capable of stimulating or activating an intracellular signaling domain of a TCR complex. In some aspects, the agent turns on or initiates TCR/CD3 intracellular signaling cascade in a T cell. Such agents can include antibodies, such as those specific for a TCR, *e.g.*, anti-CD3. In some embodiments, the stimulating conditions include one or more agent, *e.g.*, ligand, which is capable of stimulating a costimulatory receptor, *e.g.*, anti-CD28. In some embodiments, such agents and/or ligands may be, bound to solid support such as a bead, and/or one or more cytokines. Optionally, the expansion method may further comprise the step of adding anti-CD3 and/or anti-CD28 antibody to the culture medium (*e.g.*, at a concentration of at least about 0.5 ng/ml). In some embodiments, the stimulating agents include IL-2, IL-15 and/or IL-7. In some aspects, the IL-2 concentration is at least about 10 units/mL.

[0571] In some aspects, incubation is carried out in accordance with techniques such as those described in US Patent No. 6,040,177 to Riddell et al., Klebanoff et al.(2012) J

Immunother. 35(9): 651–660, Terakura et al. (2012) Blood.1:72–82, and/or Wang et al. (2012) J Immunother. 35(9):689-701.

[0572] In some embodiments, the stimulating conditions or stimulatory reagents include one or more reagent, *e.g.*, ligand, which is capable of stimulating or activating an intracellular signaling domain of a TCR complex. In some aspects, the agent turns on or initiates TCR/CD3 intracellular signaling cascade in a T cell, such as agents suitable to deliver a primary signal, *e.g.*, to initiate activation of an ITAM-induced signal, such as those specific for a TCR component, *e.g.*, anti-CD3, and/or an agent that promotes a costimulatory signal, such as one specific for a T cell costimulatory receptor, *e.g.*, anti-CD28, or anti-4-1BB, for example, bound to solid support such as a bead, and/or one or more cytokines. Among the stimulatory reagents are anti-CD3/anti-CD28 beads (*e.g.*, DYNABEADS® M-450 CD3/CD28 T Cell Expander, and/or ExpACT® beads). Optionally, the expansion method may further comprise the step of adding anti-CD3 and/or anti CD28 antibody to the culture medium. In some embodiments, the stimulating agents include cytokines.

[0573] In some embodiments, the composition of enriched T cells is incubated at a ratio of stimulatory reagent and/or beads to cells at or at about 3:1, 2.5:1, 2:1, 1.5:1, 1.25:1, 1.2:1, 1.1:1, 1:1, 0.9:1, 0.8:1, 0.75:1, 0.67:1, 0.5:1, 0.3:1, or 0.2:1. In particular embodiments, the ratio of stimulatory reagent and/or beads to cells is between 2.5:1 and 0.2:1, between 2:1 and 0.5:1, between 1.5:1 and 0.75:1, between 1.25:1 and 0.8:1, between 1.1:1 and 0.9:1. In particular embodiments, the ratio of stimulatory reagent to cells is about 1:1 or is 1:1.

[0574] In particular embodiments, the stimulatory reagent contains an oligomeric reagent, *e.g.*, a streptavidin mutein reagent, that is conjugated, linked, or attached to one or more agent, *e.g.*, ligand, which is capable of activating an intracellular signaling domain of a TCR complex. In some embodiments, the one or more agents have an attached binding domain (*e.g.*, a binding partner C) that is capable of binding to oligomeric reagent at a particular binding sites (*e.g.*, binding site Z). In some embodiments, a plurality of the agent is reversibly bound to the oligomeric reagent. In various embodiments, the oligomeric reagent has a plurality of the particular binding sites which, in certain embodiments, are reversibly bound to a plurality of agents at the binding domain (*e.g.*, binding partner C). In some embodiments, the binding interaction between the binding partner C and the at least one binding site Z is a non-covalent interaction. In some embodiments, the binding interaction, such as non-covalent interaction, between the binding partner C and the at least one binding site Z is reversible. In some

embodiments, the bound agents are dissociated from the oligomeric reagent in the presence of a competition reagent, *e.g.*, a reagent that is also capable of binding to the particular binding sites (*e.g.*, binding site Z).

[0575] In particular embodiments, the one or more agents bind to a cell surface receptor and/or an accessory molecule to stimulate the T cell, and may include an antibody targeting the TCR complex or a component thereof, an antibody targeting a co-stimulatory molecule, anti-CD3 antibodies, anti-CD28 antibodies, or an anti-CD3 and/or an anti CD28 Fab), and the one or more agents contain a binding partner C that is a streptavidin binding peptide, *e.g.*, Strep-tag® II. In some embodiments, the agent is an anti-CD3 and/or an anti-CD28 antibody or antigen binding fragment thereof, such as an antibody or antigen fragment thereof that contains a binding partner C that is a streptavidin binding peptide, *e.g.*, Strep-tag® II. In particular embodiments, the stimulatory reagent is a streptavidin-based oligomer, such as a streptavidin mutein oligomer, conjugated to Strep-tagged anti-CD3 and Strep-tagged anti-CD28 Fabs. In some embodiments, the oligomeric particle reagent is any as described in WO2015/158868 or WO2018/197949.

[0576] Substances that may be used as oligomeric reagents in such reversible systems are known, see *e.g.*, U.S. Patent Nos. 5,168,049; 5,506,121; 6,103,493; 7,776,562; 7,981,632; 8,298,782; 8,735,540; 9,023,604; and International published PCT Appl. Nos. WO2013/124474 and WO2014/076277. Non-limiting examples of reagents and binding partners capable of forming a reversible interaction, as well as substances (*e.g.*, competition reagents) capable of reversing such binding, are described below.

[0577] In some embodiments, the oligomeric reagent is an oligomer of streptavidin, streptavidin mutein or analog, avidin, an avidin mutein or analog (such as neutravidin) or a mixture thereof, in which such oligomeric reagent contains one or more binding sites for reversible association with the binding domain of the agent (*e.g.*, a binding partner C). In some embodiments, the binding domain of the agent can be a biotin, a biotin derivative or analog, or a streptavidin-binding peptide or other molecule that is able to specifically bind to streptavidin, a streptavidin mutein or analog, avidin or an avidin mutein or analog.

[0578] In certain embodiments, one or more agents (*e.g.*, agents that are capable of producing a signal in a cell such as a T cell) associate with, such as are reversibly bound to, the oligomeric reagent, such as via the plurality of the particular binding sites (*e.g.*, binding sites Z) present on the oligomeric reagent. In some cases, this results in the agents being closely

arranged to each other such that an avidity effect can take place if a target cell having (at least two copies of) a cell surface molecule that is bound by or recognized by the agent is brought into contact with the agent.

[0579] In some embodiments, the oligomeric reagent is a streptavidin oligomer, a streptavidin mutein oligomer, a streptavidin analog oligomer, an avidin oligomer, an oligomer composed of avidin mutein or avidin analog (such as neutravidin) or a mixture thereof. In particular embodiments, the oligomeric reagents contain particular binding sites that are capable of binding to a binding domain (*e.g.*, the binding partner C) of an agent. In some embodiments, the binding domain can be a biotin, a biotin derivative or analog, or a streptavidin-binding peptide or other molecule that is able to specifically bind to streptavidin, a streptavidin mutein or analog, avidin or an avidin mutein or analog. In some embodiments, the streptavidin can be wild-type streptavidin, streptavidin muteins or analogs, such as streptavidin-like polypeptides. Likewise, avidin, in some aspects, includes wild-type avidin or muteins or analogs of avidin such as neutravidin, a deglycosylated avidin with modified arginines that typically exhibits a more neutral pI and is available as an alternative to native avidin. Generally, deglycosylated, neutral forms of avidin include those commercially available forms such as "Extrav"™, available through Sigma Aldrich, "or "NeutrAv"™ available from Thermo Scientific or Invitrogen, for example

[0580] In some embodiments, the reagent is a streptavidin or a streptavidin mutein or analog. In some embodiments, wild-type streptavidin (wt-streptavidin) has the amino acid sequence disclosed by Argarana et al, Nucleic Acids Res. 14 (1986) 1871-1882 (SEQ ID NO: 68). In general, streptavidin naturally occurs as a tetramer of four identical subunits, i.e. it is a homo-tetramer, where each subunit contains a single binding site for biotin, a biotin derivative or analog or a biotin mimic. An exemplary sequence of a streptavidin subunit is the sequence of amino acids set forth in SEQ ID NO: 68, but such a sequence also can include a sequence present in homologs thereof from other *Streptomyces* species. In particular, each subunit of streptavidin may exhibit a strong binding affinity for biotin with a dissociation constant (K_d) on the order of about 10^{-14} M. In some cases, streptavidin can exist as a monovalent tetramer in which only one of the four binding sites is functional (Howarth et al. (2006) Nat. Methods, 3:267-73; Zhang et al. (2015) Biochem. Biophys. Res. Commun., 463:1059-63)), a divalent tetramer in which two of the four binding sites are functional (Fairhead et al. (2013) J. Mol. Biol., 426:199-214), or can be present in monomeric or dimeric

form (Wu et al. (2005) J. Biol. Chem., 280:23225-31; Lim et al. (2010) Biochemistry, 50:8682-91).

[0581] In some embodiments, streptavidin may be in any form, such as wild-type or unmodified streptavidin, such as a streptavidin from a *Streptomyces* species or a functionally active fragment thereof that includes at least one functional subunit containing a binding site for biotin, a biotin derivative or analog or a biotin mimic, such as generally contains at least one functional subunit of a wild-type streptavidin from *Streptomyces avidinii* set forth in SEQ ID NO: 68 or a functionally active fragment thereof. For example, in some embodiments, streptavidin can include a fragment of wild-type streptavidin, which is shortened at the N- and/or C-terminus. Such minimal streptavidins include any that begin N-terminally in the region of amino acid positions 10 to 16 of SEQ ID NO: 68 and terminate C-terminally in the region of amino acid positions 133 to 142 of SEQ ID NO: 68. In some embodiments, a functionally active fragment of streptavidin contains the sequence of amino acids set forth in SEQ ID NO: 69. In some embodiments, streptavidin, such as set forth in SEQ ID NO: 69, can further contain an N-terminal methionine at a position corresponding to Ala13 with numbering set forth in SEQ ID NO: 68. Reference to the position of residues in streptavidin or streptavidin muteins is with reference to numbering of residues in SEQ ID NO: 68.

[0582] Examples of streptavidins or streptavidin muteins are mentioned, for example, in WO 86/02077, DE 19641876 A1, US 6,022,951, WO 98/40396 or WO 96/24606. Examples of streptavidin muteins are known in the art, see *e.g.*, U.S. Pat. No. 5,168,049; 5,506,121; 6,022,951; 6,156,493; 6,165,750; 6,103,493; or 6,368,813; or International published PCT App. No. WO2014/076277.

[0583] In some embodiments, a streptavidin mutein can contain amino acids that are not part of an unmodified or wild-type streptavidin or can include only a part of a wild-type or unmodified streptavidin. In some embodiments, a streptavidin mutein contains at least one subunit that can have one more amino acid substitutions (replacements) compared to a subunit of an unmodified or wild-type streptavidin, such as compared to the wild-type streptavidin subunit set forth in SEQ ID NO: 68 or a functionally active fragment thereof, *e.g.*, set forth in SEQ ID NO: 69.

[0584] In some embodiments, the binding affinity, such as dissociation constant (K_d), of streptavidin or a streptavidin mutein for a binding domain is less than 1×10^{-4} M, 5×10^{-4} M, 1×10^{-5} M, 5×10^{-5} M, 1×10^{-6} M, 5×10^{-6} M or 1×10^{-7} M, but generally greater than 1×10^{-13} M, 1

$\times 10^{-12}$ M or 1×10^{-11} M. For example, peptide sequences (*e.g.*, Strep-tags), such as disclosed in U.S. Pat. No. 5,506,121, can act as biotin mimics and demonstrate a binding affinity for streptavidin, *e.g.*, with a K_d of approximately between 10^{-4} and 10^{-5} M. In some cases, the binding affinity can be further improved by making a mutation within the streptavidin molecule, see *e.g.*, U.S. Pat. No. 6,103,493 or WO2014/076277. In some embodiments, binding affinity can be determined by methods known in the art, such as any described herein.

[0585] In some embodiments, the reagent, such as a streptavidin or streptavidin mutein, exhibits binding affinity for a peptide ligand binding partner, which peptide ligand binding partner can be the binding partner C present in the agent. In some embodiments, the peptide sequence contains a sequence with the general formula His-Pro-Xaa, where Xaa is glutamine, asparagine, or methionine, such as contains the sequence set forth in SEQ ID NO: 71. In some embodiments, the peptide sequence contains a sequence set forth in SEQ ID NO: 70. In some embodiments, the peptide sequence has the general formula set forth in SEQ ID NO: 72, such as set forth in SEQ ID NO: 73. In one example, the peptide sequence is Trp-Arg-His-Pro-Gln-Phe-Gly-Gly (also called Strep-tag®, set forth in SEQ ID NO: 74). In one example, the peptide sequence is Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (also called Strep-tag® II, set forth in SEQ ID NO: 75). In some embodiments, the peptide ligand contains a sequential arrangement of at least two streptavidin-binding modules, wherein the distance between the two modules is at least 0 and not greater than 50 amino acids, wherein one binding module has 3 to 8 amino acids and contains at least the sequence His-Pro-Xaa, where Xaa is glutamine, asparagine, or methionine, and wherein the other binding module has the same or different streptavidin peptide ligand, such as set forth in SEQ ID NO: 72 (see *e.g.*, International Published PCT Appl. No. WO02/077018; U.S. Patent No. 7,981,632). In some embodiments, the peptide ligand contains a sequence having the formula set forth in any of SEQ ID NO: 76 or 77. In some embodiments, the peptide ligand has the sequence of amino acids set forth in any of SEQ ID NOS: 64, 78-80, and 81-82. In most cases, all these streptavidin binding peptides bind to the same binding site, namely the biotin binding site of streptavidin. If one or more of such streptavidin binding peptides is used as binding partners C, *e.g.*, C1 and C2, the multimerization reagent and/or oligomeric particle reagents bound to the one or more agents via the binding partner C is typically composed of one or more streptavidin muteins.

[0586] In some embodiments, the streptavidin mutein is a mutant as described in U.S. Pat. No. 6,103,493. In some embodiments, the streptavidin mutein contains at least one mutation

within the region of amino acid positions 44 to 53, based on the amino acid sequence of wild-type streptavidin, such as set forth in SEQ ID NO: 68. In some embodiments, the streptavidin mutein contains a mutation at one or more residues 44, 45, 46, and/or 47. In some embodiments, the streptavidin mutein contains a replacement of Glu at position 44 of wild-type streptavidin with a hydrophobic aliphatic amino acid, *e.g.*, Val, Ala, Ile or Leu, any amino acid at position 45, an aliphatic amino acid, such as a hydrophobic aliphatic amino acid at position 46 and/or a replacement of Val at position 47 with a basic amino acid, *e.g.*, Arg or Lys, such as generally Arg. In some embodiments, Ala is at position 46 and/or Arg is at position 47 and/or Val or Ile is at position 44. In some embodiments, the streptavidin mutant contains residues Val44-Thr45-Ala46-Arg47, such as set forth in exemplary streptavidin muteins containing the sequence of amino acids set forth in SEQ ID NO: 83 or SEQ ID NO: 84 or 85 (also known as streptavidin mutant 1, SAM1). In some embodiments, the streptavidin mutein contains residues Ile44-Gly45-Ala46-Arg47, such as set forth in exemplary streptavidin muteins containing the sequence of amino acids set forth in SEQ ID NO: 86, 87, or 59 (also known as SAM2). In some cases, such streptavidin mutein are described, for example, in US patent 6,103,493, and are commercially available under the trademark Strep-Tactin®. In some embodiments, the mutein streptavidin contains the sequence of amino acids set forth in SEQ ID NO: 88 or SEQ ID NO: 89. In particular embodiments, the molecule is a tetramer of streptavidin or a streptavidin mutein comprising a sequence set forth in any of SEQ ID NOS: 69, 84, 87, 88, 90, 85 or 59, which, as a tetramer, is a molecule that contains 20 primary amines, including 1 N-terminal amine and 4 lysines per monomer.

[0587] In some embodiments, streptavidin mutein exhibits a binding affinity characterized by a dissociation constant (K_d) that is or is less than 3.7×10^{-5} M for the peptide ligand (Trp-Arg-His-Pro-Gln-Phe-Gly-Gly; also called Strep-tag®, set forth in SEQ ID NO: 74) and/or that is or is less than 7.1×10^{-5} M for the peptide ligand (Trp-Ser-His-Pro-Gln-Phe-Glu-Lys; also called Strep-tag® II, set forth in SEQ ID NO: 75) and/or that is or is less than 7.0×10^{-5} M, 5.0×10^{-5} M, 1.0×10^{-5} M, 5.0×10^{-6} M, 1.0×10^{-6} M, 5.0×10^{-7} M, or 1.0×10^{-7} M, but generally greater than 1×10^{-13} M, 1×10^{-12} M or 1×10^{-11} M for any of the peptide ligands set forth in any of SEQ ID NOS: 75, 76-77, 81-82, 78-80, 73, 74, 70, 72.

[0588] In some embodiments, the resulting streptavidin mutein exhibits a binding affinity characterized by an association constant (K_a) that is or is greater than 2.7×10^4 M⁻¹ for the peptide ligand (Trp-Arg-His-Pro-Gln-Phe-Gly-Gly; also called Strep-tag®, set forth in SEQ ID

NO: 74) and/or that is or is greater than $1.4 \times 10^4 \text{ M}^{-1}$ for the peptide ligand (Trp-Ser-His-Pro-Gln-Phe-Glu-Lys; also called Strep-tag® II, set forth in SEQ ID NO: 75) and/or that is or is greater than $1.43 \times 10^4 \text{ M}^{-1}$, $1.67 \times 10^4 \text{ M}^{-1}$, $2 \times 10^4 \text{ M}^{-1}$, $3.33 \times 10^4 \text{ M}^{-1}$, $5 \times 10^4 \text{ M}^{-1}$, $1 \times 10^5 \text{ M}^{-1}$, $1.11 \times 10^5 \text{ M}^{-1}$, $1.25 \times 10^5 \text{ M}^{-1}$, $1.43 \times 10^5 \text{ M}^{-1}$, $1.67 \times 10^5 \text{ M}^{-1}$, $2 \times 10^5 \text{ M}^{-1}$, $3.33 \times 10^5 \text{ M}^{-1}$, $5 \times 10^5 \text{ M}^{-1}$, $1 \times 10^6 \text{ M}^{-1}$, $1.11 \times 10^6 \text{ M}^{-1}$, $1.25 \times 10^6 \text{ M}^{-1}$, $1.43 \times 10^6 \text{ M}^{-1}$, $1.67 \times 10^6 \text{ M}^{-1}$, $2 \times 10^6 \text{ M}^{-1}$, $3.33 \times 10^6 \text{ M}^{-1}$, $5 \times 10^6 \text{ M}^{-1}$, $1 \times 10^7 \text{ M}^{-1}$, but generally less than $1 \times 10^{13} \text{ M}^{-1}$, $1 \times 10^{12} \text{ M}^{-1}$ or $1 \times 10^{11} \text{ M}^{-1}$ for any of the peptide ligands set forth in any of SEQ ID NOS: 75, 76-77, 81-82, 78-80, 73, 74, 70, 72.

[0589] In particular embodiments, provided herein is an oligomeric particle reagent that is composed of and/or contains a plurality of streptavidin or streptavidin mutein tetramers. In certain embodiments, the oligomeric particle reagent provided herein contains a plurality of binding sites that reversibly bind or are capable of reversibly binding to one or more agents, *e.g.*, a stimulatory agent and/or a selection agent. In some embodiments, the oligomeric particle has a radius, *e.g.*, an average radius, of between 70 nm and 125 nm, inclusive; a molecular weight of between $1 \times 10^7 \text{ g/mol}$ and $1 \times 10^9 \text{ g/mol}$, inclusive; and/or between 1,000 and 5,000 streptavidin or streptavidin mutein tetramers, inclusive. In some embodiments, the oligomeric particle reagent is bound, *e.g.*, reversibly bound, to one or more agents such as an agent that binds to a molecule, *e.g.*, receptor, on the surface of a T cell. In some embodiments, the agent is an anti-CD3 and/or an anti-CD28 antibody or antigen binding fragment thereof, such as an antibody or antigen fragment thereof that contains a binding partner, *e.g.*, a streptavidin binding peptide, *e.g.*, Strep-tag® II. In particular embodiments, the one or more agents bind to a cell surface receptor and/or an accessory molecule to stimulate the cell, and may include an antibody targeting the TCR complex or a component thereof, an antibody targeting a co-stimulatory molecule, anti-CD3 antibodies, anti-CD28 antibodies, or an anti-CD3 and/or an anti CD28 Fab), and the one or more agents contain a binding partner, *e.g.*, a streptavidin binding peptide, *e.g.*, Strep-tag® II. In particular embodiments, the one or more agents comprise a streptavidin-based oligomer, such as a streptavidin mutein oligomer, conjugated to Strep-tagged anti-CD3 and Strep-tagged anti-CD28 Fabs. In some embodiments, the oligomeric particle reagent is any as described in WO2015/158868 or WO2018/197949.

[0590] In particular embodiments, an oligomeric reagent is prepared by polymerizing an exemplary streptavidin mutein designated STREP-TACTIN® M2 (see *e.g.*, U.S. Patent No. 6,103,493 and Voss and Skerra (1997) Protein Eng., 1:975-982, and Argarana et al. (1986)

Nucleic Acids Research, 1871-1882). In particular embodiments, to prepare streptavidin muteins for oligomerization, streptavidin muteins containing one or more reactive thiol groups are incubated with maleimide activated streptavidin muteins. In particular embodiments, to prepare the thiolated streptavidin mutein, about 100 mg of streptavidin mutein is thiolated by incubation with 2-iminothiolane hydrochloride at a molar ratio of 1:100 at a pH of about 8.5 at 24°C for 1 hour in 100 mM Borate buffer in a total volume of 2.6 mL. For the activation reaction, about 400 mg of streptavidin mutein is incubated with Succinimidyl-6-[(β-maleimidopropionamido) hexanoate (SMPH) at a molar ratio of 1:2 at a pH of about 7.2 at 24°C for 1 hour in a total volume of about 10.4 mL in a sodium phosphate buffer. The thiolation and activation reactions are coordinated to start at about the same time, and the duration of the reactions is controlled. After the reactions, the 2-Iminothiolane hydrochloride and SMPH are removed from the samples by individually carrying out gel filtration of the samples with PD-10 desalting columns (GE Healthcare). For each 2.5 mL volume of sample, a 1 mL PD-10 column is equilibrated and loaded with either thiolated mutein streptavidin or maleimide mutein streptavidin and elution is carried out by adding 3.5 mL of coupling buffer (100 mM NaH₂PO₄, 150 mM NaCl, 5 mM EDTA, pH 7.2). Gel filtration of the maleimide mutein streptavidin is carried out on 4 columns to account for the > 10 mL volume and eluates are pooled. The timing of the activation and thiolation reactions and the timing between the end of the activation and thiolation reactions and the start of the oligomerization reactions are controlled. Generally, no more than ten minutes is allowed to pass from the start of gel filtrations, i.e. the end of the activation and thiolation reactions, to when oligomerization reaction is initiated.

[0591] In particular embodiments, the maleimide streptavidin mutein and thiolated streptavidin mutein samples are then combined into an overall volume of about 17.5 mL and incubated for 1 hour at a pH of 7.2 at 24°C under stirring conditions at about 600 rpm. Because four times more streptavidin mutein was incubated with SMPH than with 2-iminothiolane hydrochloride, the molar ratio of thiolated streptavidin mutein and maleimide streptavidin mutein is 1:4 during the oligomerization reaction. After the reaction, remaining SH groups of the oligomerized streptavidin mutein reagent are saturated by incubation with N-Ethylmaleimide (NEM) for 15 min at 24°C with stirring (about 600 rpm) followed by incubation for a further 16-20 hours at 4°C. After incubation with NEM, the sample containing oligomerized streptavidin mutein is centrifuged and the supernatant is filtered through a 0.45 μm membrane (Millex-HP 0.45 μm from Merck Millopore). The filtered solution is then loaded into a column (Sephacryl

S-300 HR HiPrep 26/60, GE Healthcare) for size exclusion chromatography (SEC) with an AKTA Explorer chromatography system (GE Healthcare). Fractions with a milli absorbance unit (mAU) greater than or equal to 1500 mAU are pooled. The pooled sample containing oligomeric streptavidin mutein is treated with 100 mM hydroxylamine at a pH of 6.35 for 15 minutes at room temperature. To remove the hydroxylamine after treatment, sample is loaded onto a PD10 column (2.5 mL per column) and eluted with 3.5 mL of buffer containing 100 mM NaH₂PO₄, 140 mM NaCl, 1 mM EDTA, pH 7.2. The PD10 elutes are pooled and sterile filtered with a 0.45 µm filter followed by a 0.22 µm filter and then samples are frozen and stored at -80°C. Prior to freezing, the final concentration of the oligomeric streptavidin mutein reagent is measured and the size of the oligomeric streptavidin mutein reagent is determined by dynamic light scattering (DLS).

[0592] In some embodiments, stimulatory agents such as an anti-CD3 antibody and an anti-CD28 Fab antibody are multimerized by reversible binding to the oligomeric streptavidin mutein reagent. In some embodiments, the stimulatory agents, *e.g.*, anti-CD3 and anti-CD28 Fab fragments, are reversibly bound to the streptavidin mutein oligomer via a streptavidin peptide-binding partner fused to each stimulatory agent, *e.g.*, each Fab fragment. In some embodiments, the anti-CD3 Fab fragment is derived from the CD3 binding monoclonal antibody produced by the hybridoma cell line OKT3 (ATCC® CRL-8001™; see also U.S. Patent No. 4,361,549), and contains the heavy chain variable domain and light chain variable domain of the anti-CD3 antibody OKT3 described in Arakawa et al. J. Biochem. 120, 657-662 (1996). These sequences are set forth in SEQ ID NOS: 60 and 61, respectively. In some embodiments, the anti-CD28 Fab fragment is derived from antibody CD28.3 (deposited as a synthetic single chain Fv construct under GenBank Accession No. AF451974.1; see also Vanhove et al., BLOOD, 15 July 2003, Vol. 102, No. 2, pages 564-570) and contains the heavy and light chain variable domains of the anti-CD28 antibody CD28.3 set forth in SEQ ID NOS: 62 and 63, respectively. For exemplary peptide-tagged Fab fragments, see International Patent App. Pub. Nos. WO 2013/011011 and WO 2013/124474.

[0593] In some embodiments, provided herein is an oligomeric particle reagent that is composed of and/or contains a plurality of streptavidin or streptavidin mutein tetramers. In certain embodiments, the oligomeric particle reagent provided herein contains a plurality of binding sites that reversibly bind or are capable of reversibly binding to one or more agents, *e.g.*, a stimulatory agent and/or a selection agent. In some embodiments, the oligomeric particle has a

radius, *e.g.*, an average radius, of between 80 nm and 120 nm, inclusive; a molecular weight, *e.g.*, an average molecular weight of between 7.5×10^6 g/mol and 2×10^8 g/mol, inclusive; and/or an amount, *e.g.*, an average amount, of between 500 and 10,000 streptavidin or streptavidin mutein tetramers, inclusive. In some embodiments, the oligomeric particle reagent is bound, *e.g.*, reversibly bound, to one or more agents, such as an agent that binds to a molecule, *e.g.*, receptor, on the surface of a cell. In some embodiments, the agent comprises one or more agents that bind to a cell surface receptor and/or an accessory molecule to stimulate the cell (*e.g.*, such as an antibody targeting the TCR complex or a component thereof, an antibody targeting a co-stimulatory molecule, anti-CD3 antibodies, anti-CD28 antibodies, or anti-CD3/anti-CD28 Fabs). In some embodiments, the agent is an anti-CD3 and/or an anti-CD28 Fab, such as a Fab that contains a binding partner, *e.g.*, a streptavidin binding peptide, *e.g.*, Strep-tag® II. In particular embodiments, the one or more agents is an anti-CD3 and/or an anti-CD28 Fab containing a binding partner, *e.g.*, a streptavidin binding peptide, *e.g.*, Strep-tag® II.

[0594] In some embodiments, the cells are stimulated or subjected to stimulation in the presence of, of about, or of at least 0.01 µg, 0.02 µg, 0.03 µg, 0.04 µg, 0.05 µg, 0.1 µg, 0.2 µg, 0.3 µg, 0.4 µg, 0.5 µg, 0.75 µg, 1 µg, 1.2 µg, 1.4 µg, 1.6 µg, 1.8 µg, 2 µg, 3 µg, 4 µg, 5 µg, 6 µg, 7 µg, 8 µg, 9 µg, or 10 µg of the oligomeric stimulatory reagent (*e.g.*, the streptavidin-based oligomer, such as a streptavidin mutein oligomer, conjugated to Strep-tagged anti-CD3 and Strep-tagged anti-CD28 Fabs) per 10^6 cells. In some embodiments, the cells are stimulated or subjected to stimulation in the presence of or of about 4 µg of the oligomeric stimulatory reagent (*e.g.*, the streptavidin-based oligomer, such as a such as a streptavidin mutein oligomer, conjugated to Strep-tagged anti-CD3 and Strep-tagged anti-CD28 Fabs) per 10^6 cells. In particular embodiments, the cells are stimulated or subjected to stimulation in the presence of or of about 1.2 µg of the oligomeric stimulatory reagent (*e.g.*, the streptavidin-based oligomer, such as a streptavidin mutein oligomer, conjugated to Strep-tagged anti-CD3 and Strep-tagged anti-CD28 Fabs) per 10^6 cells. In particular embodiments, the cells are stimulated or subjected to stimulation in the presence of or of about 0.8 µg of the oligomeric stimulatory reagent (*e.g.*, the streptavidin-based oligomer, such as a streptavidin mutein oligomer, conjugated to Strep-tagged anti-CD3 and Strep-tagged anti-CD28 Fabs) per 10^6 cells. In particular embodiments, the cells are stimulated or subjected to stimulation in the presence of or of about 1.8 µg of the oligomeric stimulatory reagent (*e.g.*, the streptavidin-based oligomer, such as a streptavidin mutein oligomer, conjugated to Strep-tagged anti-CD3 and Strep-tagged anti-CD28 Fabs) per 10^6 cells.

In certain aspects, within the oligomeric stimulatory reagent, the mass ratio between the oligomeric particles and the attached agents is about 3:1. In certain aspects, within the oligomeric stimulatory reagent, the mass ratio among the oligomeric particles, the attached anti-CD3 Fabs, and the attached anti-CD28 Fabs is about 3:0.5:0.5. In certain aspects, 4 μg of the oligomeric stimulatory reagent is or includes 3 μg of oligomeric particles and 1 μg of attached agents, *e.g.*, 0.5 μg of anti-CD3 Fabs and 0.5 μg of anti-CD28 Fabs. In other examples, 1.2 μg of the oligomeric stimulatory reagent per 10^6 cells is or includes 0.9 μg of oligomeric particles and 0.3 μg of attached agents, *e.g.*, 0.15 μg of anti-CD3 Fabs and 0.15 μg of anti-CD28 Fabs, per 10^6 cells. In some embodiments, the oligomeric stimulatory reagent is added to a serum-free medium and the stimulation is performed in the serum free medium, *e.g.*, as described in PCT/US2018/064627.

[0595] In particular embodiments, an amount of from 50×10^6 cells to 5000×10^6 cells (*e.g.*, 900×10^6 T cells), such as such number of CD3+ T cells, or CD4+ T cells and CD8+ T cells, of the input population are subjected to stimulation, *e.g.*, cultured under stimulating conditions, in the presence of the stimulatory reagent. In some embodiments, the stimulatory reagent is an oligomeric stimulatory reagent (*e.g.*, the streptavidin-based oligomer, such as a streptavidin mutein oligomer, conjugated to Strep-tagged anti-CD3 and Strep-tagged anti-CD28 Fabs). In certain embodiments, the cells, *e.g.*, cells of the input population, are stimulated or subjected to stimulation *e.g.*, cultured under stimulating conditions such as in the presence of a stimulatory reagent, at a density of, of about, or at least 0.01×10^6 cells/mL, 0.1×10^6 cells/mL, 0.5×10^6 cells/mL, 1.0×10^6 cells/mL, 1.5×10^6 cells/mL, 2.0×10^6 cells/mL, 2.5×10^6 cells/mL, 3.0×10^6 cells/mL, 4.0×10^6 cells/mL, 5.0×10^6 cells/mL, 10×10^6 cells/mL, or 50×10^6 cells/mL. In certain embodiments, the cells, *e.g.*, cells of the input population, are stimulated or subjected to stimulation *e.g.*, cultured under stimulating conditions such as in the presence of a stimulatory reagent, at a density of, of about, or at least 3.0×10^6 cells/mL.

[0596] In particular embodiments, the stimulating conditions include incubating, culturing, and/or cultivating the cells, *e.g.*, cells from an input composition, with and/or in the presence of one or more cytokines. In particular embodiments, the one or more cytokines are recombinant cytokines. In some embodiments, the one or more cytokines are human recombinant cytokines. In certain embodiments, the one or more cytokines bind to and/or are capable of binding to receptors that are expressed by and/or are endogenous to T cells. In particular embodiments, the one or more cytokines is or includes a member of the 4-alpha-helix bundle family of cytokines.

In some embodiments, members of the 4-alpha-helix bundle family of cytokines include, but are not limited to, interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-7 (IL-7), interleukin-9 (IL-9), interleukin 12 (IL-12), interleukin 15 (IL-15), granulocyte colony-stimulating factor (G-CSF), and granulocyte-macrophage colony-stimulating factor (GM-CSF). In some embodiments, the one or more cytokines is or includes IL-15. In particular embodiments, the one or more cytokines is or includes IL-7. In particular embodiments, the one or more cytokines is or includes IL-2.

[0597] In certain embodiments, the amount or concentration of the one or more cytokines are measured and/or quantified with International Units (IU). International units may be used to quantify vitamins, hormones, cytokines, vaccines, blood products, and similar biologically active substances. In some embodiments, IU are or include units of measure of the potency of biological preparations by comparison to an international reference standard of a specific weight and strength *e.g.*, W^{HO} 1st International Standard for Human IL-2, 86/504. International Units are the only recognized and standardized method to report biological activity units that are published and are derived from an international collaborative research effort. In particular embodiments, the IU for composition, sample, or source of a cytokine may be obtained through product comparison testing with an analogous WHO standard product. For example, in some embodiments, the IU/mg of a composition, sample, or source of human recombinant IL-2, IL-7, or IL-15 is compared to the WHO standard IL-2 product (NIBSC code: 86/500), the WHO standard IL-17 product (NIBSC code: 90/530) and the WHO standard IL-15 product (NIBSC code: 95/554), respectively.

[0598] In some embodiments, the biological activity in IU/mg is equivalent to (ED₅₀ in ng/mL)⁻¹ x 10⁶. In particular embodiments, the ED₅₀ of recombinant human IL-2 or IL-15 is equivalent to the concentration required for the half-maximal stimulation of cell proliferation (XTT cleavage) with CTLL-2 cells. In certain embodiments, the ED₅₀ of recombinant human IL-7 is equivalent to the concentration required for the half-maximal stimulation for proliferation of PHA-activated human peripheral blood lymphocytes. Details relating to assays and calculations of IU for IL-2 are discussed in Wadhwa et al., *Journal of Immunological Methods* (2013), 379 (1-2): 1-7; and Gearing and Thorpe, *Journal of Immunological Methods* (1988), 114 (1-2): 3-9; details relating to assays and calculations of IU for IL-15 are discussed in Soman et al. *Journal of Immunological Methods* (2009) 348 (1-2): 83-94.

[0599] In some embodiments, the cells, *e.g.*, the input cells, are incubated with a cytokine, *e.g.*, a recombinant human cytokine, at a concentration of between at or about 1 IU/mL and at or about 1,000 IU/mL, between at or about 10 IU/mL and at or about 50 IU/mL, between at or about 50 IU/mL and at or about 100 IU/mL, between at or about 100 IU/mL and at or about 200 IU/mL, between at or about 100 IU/mL and at or about 500 IU/mL, between at or about 250 IU/mL and at or about 500 IU/mL, or between at or about 500 IU/mL and at or about 1,000 IU/mL.

[0600] In some embodiments, the cells, *e.g.*, the input cells, are incubated with IL-2, *e.g.*, human recombinant IL-2, at a concentration between at or about 1 IU/mL and at or about 500 IU/mL, between at or about 10 IU/mL and at or about 250 IU/mL, between at or about 50 IU/mL and at or about 200 IU/mL, between at or about 50 IU/mL and at or about 150 IU/mL, between at or about 75 IU/mL and at or about 125 IU/mL, between at or about 100 IU/mL and at or about 200 IU/mL, or between at or about 10 IU/mL and at or about 100 IU/mL, *e.g.*, in a serum-free medium. In particular embodiments, cells, *e.g.*, cells of the input composition, are incubated with recombinant IL-2 at a concentration at or at about 50 IU/mL, 60 IU/mL, 70 IU/mL, 80 IU/mL, 90 IU/mL, 100 IU/mL, 110 IU/mL, 120 IU/mL, 130 IU/mL, 140 IU/mL, 150 IU/mL, 160 IU/mL, 170 IU/mL, 180 IU/mL, 190 IU/mL, or 100 IU/mL. In some embodiments, the cells, *e.g.*, the input cells, are incubated in the presence of or of about 100 IU/mL of recombinant IL-2, *e.g.*, human recombinant IL-2.

[0601] In some embodiments, the cells, *e.g.*, the input cells, are incubated with recombinant IL-7, *e.g.*, human recombinant IL-7, at a concentration between at or about 100 IU/mL and at or about 2,000 IU/mL, between at or about 500 IU/mL and at or about 1,000 IU/mL, between at or about 100 IU/mL and at or about 500 IU/mL, between at or about 500 IU/mL and at or about 750 IU/mL, between at or about 750 IU/mL and at or about 1,000 IU/mL, or between at or about 550 IU/mL and at or about 650 IU/mL, *e.g.*, in a serum-free medium. In particular embodiments, the cells, *e.g.*, the input cells, are incubated with IL-7 at a concentration at or at about 50 IU/mL, 100 IU/mL, 150 IU/mL, 200 IU/mL, 250 IU/mL, 300 IU/mL, 350 IU/mL, 400 IU/mL, 450 IU/mL, 500 IU/mL, 550 IU/mL, 600 IU/mL, 650 IU/mL, 700 IU/mL, 750 IU/mL, 800 IU/mL, 750 IU/mL, 750 IU/mL, 750 IU/mL, or 1,000 IU/mL. In particular embodiments, the cells, *e.g.*, the input cells, are incubated in the presence of or of about 600 IU/mL of IL-7, *e.g.*, human recombinant IL-7.

[0602] In some embodiments, the cells, *e.g.*, the input cells, are incubated with recombinant IL-15, *e.g.*, human recombinant IL-15, at a concentration between at or about 1 IU/mL and at or about 500 IU/mL, between at or about 10 IU/mL and at or about 250 IU/mL, between at or about 50 IU/mL and at or about 200 IU/mL, between at or about 50 IU/mL and at or about 150 IU/mL, between at or about 75 IU/mL and at or about 125 IU/mL, between at or about 100 IU/mL and at or about 200 IU/mL, or between at or about 10 IU/mL and at or about 100 IU/mL, *e.g.*, in a serum-free medium. In particular embodiments, cells, *e.g.*, a cell of the input composition, are incubated with recombinant IL-15 at a concentration at or at about 50 IU/mL, 60 IU/mL, 70 IU/mL, 80 IU/mL, 90 IU/mL, 100 IU/mL, 110 IU/mL, 120 IU/mL, 130 IU/mL, 140 IU/mL, 150 IU/mL, 160 IU/mL, 170 IU/mL, 180 IU/mL, 190 IU/mL, or 200 IU/mL. In some embodiments, the cells, *e.g.*, the input cells, are incubated in the presence of or of about 100 IU/mL of recombinant IL-15, *e.g.*, human recombinant IL-15.

[0603] In particular embodiments, the cells, *e.g.*, cells from the input composition, are incubated under stimulating conditions in the presence of IL-2, IL-7, and/or IL-15, *e.g.*, in a serum-free medium. In some embodiments, the IL-2, IL-7, and/or IL-15 are recombinant. In certain embodiments, the IL-2, IL-7, and/or IL-15 are human. In particular embodiments, the one or more cytokines are or include human recombinant IL-2, IL-7, and/or IL-15. In certain embodiments, the cells are incubated under stimulating conditions in the presence of recombinant IL-2, IL-7, and IL-15, *e.g.*, in a serum-free medium.

[0604] The conditions can include one or more of particular media, temperature, oxygen content, carbon dioxide content, time, agents, *e.g.*, nutrients, amino acids, antibiotics, ions, and/or stimulatory factors, such as cytokines, chemokines, antigens, binding partners, fusion proteins, recombinant soluble receptors, and any other agents designed to activate the cells.

[0605] In some aspects, incubation is carried out in accordance with techniques such as those described in US Patent No. 6,040,177 to Riddell et al., Klebanoff et al. (2012) J Immunother. 35(9): 651–660, Terakura et al. (2012) Blood. 117:72–82, and/or Wang et al. (2012) J Immunother. 35(9):689–701.

[0606] In some embodiments, the incubation is performed in serum free media. In some embodiments, the serum free media is a defined and/or well-defined cell culture media. In certain embodiments, the serum free media is a controlled culture media that has been processed, *e.g.*, filtered to remove inhibitors and/or growth factors. In some embodiments, the serum free media contains proteins. In certain embodiments, the serum-free media may contain

serum albumin, hydrolysates, growth factors, hormones, carrier proteins, and/or attachment factors.

3. Vectors and Methods for Genetic Engineering (e.g., Transduction)

[0607] In some embodiments, engineered cells, such as T cells, used in connection with the provided methods, uses, articles of manufacture or compositions are cells have been genetically engineered to express a recombinant receptor, *e.g.*, a CAR described herein. In some embodiments, the cells are engineered by introduction, delivery or transfer of nucleic acid sequences that encode the recombinant receptor and/or other molecules.

[0608] In some embodiments, methods for producing engineered cells includes the introduction of a polynucleotide encoding a recombinant receptor (*e.g.*, anti-CD19 CAR) into a cell, *e.g.*, such as a stimulated or activated cell. In particular embodiments, the recombinant receptor is an anti-CD19 CAR, such as any described in Section II.A. A polynucleotide encoding the CAR and vectors comprising the same may include any as described in Section II.B. Introduction of the nucleic acid molecules encoding the recombinant receptor in the cell may be carried out using any of a number of known vectors. Such vectors include viral and non-viral systems, including lentiviral and gammaretroviral systems, as well as transposon-based systems such as PiggyBac or Sleeping Beauty-based gene transfer systems. Exemplary methods include those for transfer of nucleic acids encoding the receptors, including via viral, *e.g.*, retroviral or lentiviral, transduction, transposons, and electroporation. In some embodiments, the engineering produces one or more engineered compositions of enriched T cells.

[0609] Any method of introducing a heterologous or recombinant polynucleotide that would result in integration of the polynucleotide encoding the recombinant receptor into the genome of a cell such as a T cell may be used, including viral and non-viral methods of genetic engineering. Introduction of the polynucleotides, *e.g.*, heterologous or recombinant polynucleotides, encoding the recombinant protein into the cell may be carried out using any of a number of known vectors. Such vectors include viral, including lentiviral and gammaretroviral, systems. Exemplary methods include those for transfer of heterologous polynucleotides encoding the receptors, including via viral, *e.g.*, retroviral or lentiviral, transduction. In some embodiments, a population of stimulated cells is genetically engineered, such as to introduce a heterologous or recombinant polynucleotide encoding a recombinant

receptor, thereby generating a population of transformed cells (also referred to herein as a transformed population of cells).

[0610] In some embodiments, the provided methods include genetically engineering the cells, *e.g.*, introducing a heterologous or recombinant polynucleotide encoding a recombinant protein, using a non-viral method, such as electroporation, calcium phosphate transfection, protoplast fusion, cationic liposome-mediated transfection, nanoparticles such as lipid nanoparticles, tungsten particle-facilitated microparticle bombardment, strontium phosphate DNA co-precipitation, and other approaches described in, *e.g.*, WO 2014055668, and U.S. Patent No. 7,446,190. Transposon-based systems also are contemplated.

[0611] In particular embodiments, the cells are genetically engineered, transformed, or transduced after the cells have been stimulated, activated, and/or incubated under stimulating conditions, such as by any of the methods provided herein, *e.g.*, in Section II. In particular embodiments, the one or more stimulated populations have been previously cryoprotected and stored, and are thawed and optionally washed prior to genetically engineering, transforming, transfecting, or transducing the cells.

[0612] In particular embodiments, the cells are genetically engineered, transformed, or transduced after the cells are stimulated or subjected to stimulation or cultured under stimulatory conditions. In particular embodiments, the cells are genetically engineered, transformed, or transduced at, at about, or within 72 hours, 60 hours, 48 hours, 36 hours, 24 hours, or 12 hours, inclusive, from the initiation of the stimulation. In particular embodiments, the cells are genetically engineered, transformed, or transduced at, at about, or within 3 days, two days, or one day, inclusive, from the initiation of the stimulation. In certain embodiments, the cells are genetically engineered, transformed, or transduced between or between about 12 hours and 48 hours, 16 hours and 36 hours, or 18 hours and 30 hours after the initiation of the stimulation. In particular embodiments, the cells are genetically engineered, transformed, or transduced between or between about 18 hours and 30 hours after the initiation of the stimulation. In particular embodiments, the cells are genetically engineered, transformed, or transduced at or at about 16 hours, 18 hours, 20 hours, 22 hours, or 24 hours after the initiation of the stimulation.

[0613] In certain embodiments, methods for genetic engineering are carried out by contacting or introducing one or more cells of a population with a nucleic acid molecule or polynucleotide encoding the recombinant protein, *e.g.*, a recombinant receptor. In certain embodiments, the nucleic acid molecule or polynucleotide is heterologous to the cells. In

particular embodiments, heterologous nucleic acid molecule or heterologous polynucleotide is not native to the cells. In certain embodiments, the heterologous nucleic acid molecule or heterologous polynucleotide encodes a protein, *e.g.*, a recombinant protein, that is not natively expressed by the cell. In particular embodiments, the heterologous nucleic acid molecule or polynucleotide is or contains a nucleic acid sequence that is not found in the cell prior to the contact or introduction.

[0614] In some embodiments, the cells, *e.g.*, stimulated cells, are engineered, *e.g.*, transduced or in the presence of a transduction adjuvant. Exemplary transduction adjuvants include, but are not limited to, polycations, fibronectin or fibronectin-derived fragments or variants, and RetroNectin. In certain embodiments, the cells are engineered in the presence of polycations, fibronectin or fibronectin-derived fragments or variants, and/or RetroNectin. In particular embodiments, the cells are engineered in the presence of a polycation that is polybrene, DEAE-dextran, protamine sulfate, poly-L-lysine, or a cationic liposome. In particular embodiments, the cells are engineered in the presence of protamine sulfate. In some embodiments, the presence of an oligomeric stimulatory reagent, *e.g.*, as described in Section II-C-2 can act as a transduction adjuvant, see, *e.g.*, WO/2017/068419 which is incorporated herein by reference.

[0615] In some embodiments, the genetic engineering, *e.g.*, transduction, is carried out in serum free media, *e.g.*, as described herein or in PCT/US2018/064627. In some embodiments, the serum free media is a defined or well-defined cell culture media. In certain embodiments, the serum free media is a controlled culture media that has been processed, *e.g.*, filtered to remove inhibitors and/or growth factors. In some embodiments, the serum free media contains proteins. In certain embodiments, the serum-free media may contain serum albumin, hydrolysates, growth factors, hormones, carrier proteins, and/or attachment factors.

[0616] In particular embodiments, the cells are engineered in the presence of one or more cytokines. In certain embodiments, the one or more cytokines are recombinant cytokines. In particular embodiments, the one or more cytokines are human recombinant cytokines. In certain embodiments, the one or more cytokines bind to and/or are capable of binding to receptors that are expressed by and/or are endogenous to T cells. In particular embodiments, the one or more cytokines is or includes a member of the 4-alpha-helix bundle family of cytokines. In some embodiments, members of the 4-alpha-helix bundle family of cytokines include, but are not limited to, interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-7 (IL-7), interleukin-9 (IL-9),

interleukin 12 (IL-12), interleukin 15 (IL-15), granulocyte colony-stimulating factor (G-CSF), and granulocyte-macrophage colony-stimulating factor (GM-CSF). In some embodiments, the one or more cytokines is or includes IL-15. In particular embodiments, the one or more cytokines is or includes IL-7. In particular embodiments, the one or more cytokines is or includes recombinant IL-2.

[0617] In particular embodiments, cells, *e.g.*, stimulated cells are engineered under stimulating conditions in the presence of IL-2, IL-7, and/or IL-15. In certain embodiments, the IL-2, IL-7, and/or IL-15 are recombinant. In certain embodiments, the IL-2, IL-7, and/or IL-15 are human. In particular embodiments, the one or more cytokines are or include human recombinant IL-2, IL-7, and/or IL-15. In certain embodiments, the cells are engineered, *e.g.*, transduced or under stimulating conditions in the presence of recombinant IL-2, IL-7, and IL-15, such as recombinant human IL-2 (*e.g.*, 100 IU/mL), recombinant human IL-7 (*e.g.*, 600 IU/mL), and/or recombinant human IL-15 (*e.g.*, 100 IU/mL).

[0618] In some embodiments, the cells are genetically engineered, transformed, or transduced in the presence of the same or similar media as was present during the stimulation. In some embodiments, the cells are genetically engineered, transformed, or transduced in media having the same cytokines as the media present during stimulation. In certain embodiments, the cells are genetically engineered, transformed, or transduced, in media having the same cytokines at the same concentrations as the media present during stimulation.

[0619] In some embodiments, genetically engineering the cells is or includes introducing the polynucleotide, *e.g.*, the heterologous or recombinant polynucleotide, into the cells by transduction. In some embodiments, the cells are transduced or subjected to transduction with a viral vector. In particular embodiments, the cells are transduced or subjected to transduction with a viral vector. In some embodiments, the virus is a retroviral vector, such as a gammaretroviral vector or a lentiviral vector. Methods of lentiviral transduction are known. Exemplary methods are described in, *e.g.*, Wang et al. (2012) *J. Immunother.* 35(9): 689-701; Cooper et al. (2003) *Blood.* 101:1637-1644; Verhoeven et al. (2009) *Methods Mol Biol.* 506: 97-114; and Cavalieri et al. (2003) *Blood.* 102(2): 497-505.

[0620] In some embodiments, the transduction is carried out by contacting one or more cells of a population with a nucleic acid molecule encoding the recombinant protein, *e.g.*, recombinant receptor. In some embodiments, the contacting can be effected with centrifugation, such as spinoculation (*e.g.*, centrifugal inoculation). Such methods include any of those as

described in International Publication Number WO2016/073602. Exemplary centrifugal chambers include those produced and sold by Biosafe SA, including those for use with the Sepax® and Sepax® 2 system, including an A-200/F and A-200 centrifugal chambers and various kits for use with such systems. Exemplary chambers, systems, and processing instrumentation and cabinets are described, for example, in US Patent No. 6,123,655, US Patent No. 6,733,433 and Published U.S. Patent Application, Publication No.: US 2008/0171951, and published international patent application, publication no. WO 00/38762, the contents of each of which are incorporated herein by reference in their entirety. Exemplary kits for use with such systems include, but are not limited to, single-use kits sold by BioSafe SA under product names CS-430.1, CS-490.1, CS-600.1 or CS-900.2.

[0621] In particular embodiments, an amount of, of about, or of at least 50×10^6 , 100×10^6 , 150×10^6 , 200×10^6 , 250×10^6 , 300×10^6 , 350×10^6 , 400×10^6 , 450×10^6 , 500×10^6 , 550×10^6 , 600×10^6 , 700×10^6 , 800×10^6 , 900×10^6 , or $1,000 \times 10^6$ cells of the composition that has been subjected to stimulation, *e.g.*, cultured under stimulating conditions, are subjected to genetic engineering, *e.g.*, transduction. In particular embodiments, the total number of cells, *e.g.*, viable T cells comprising both CD4+ T cells and CD8+ T cells, that have been subjected to stimulation and are subsequently subjected to transduction is at or about 50×10^6 cells, at or about 100×10^6 cells, at or about 150×10^6 cells, at or about 200×10^6 cells, at or about 250×10^6 cells, at or about 300×10^6 cells, at or about 350×10^6 cells, at or about 400×10^6 cells, at or about 450×10^6 cells, at or about 500×10^6 cells, at or about 550×10^6 cells, at or about 600×10^6 cells, at or about 700×10^6 cells, at or about 800×10^6 cells, at or about 900×10^6 cells, or at or about $1,000 \times 10^6$ cells, or any value between any of the foregoing. In particular embodiments, up to 900×10^6 cells of the input population are subjected to stimulation, and an amount of, of about, or up to 600×10^6 cells of the cells that have been subjected to stimulation are subjected to genetic engineering, *e.g.*, transduction. In particular embodiments, the cell composition subjected to genetic engineering, *e.g.*, transduction, comprises viable CD4+ T cells and viable CD8+ T cells, at a ratio of between 1:10 and 10:1, between 1:5 and 5:1, between 4:1 and 1:4, between 1:3 and 3:1, between 2:1 and 1:2, between 1.5:1 and 1:1.5, between 1.25:1 and 1:1.25, between 1.2:1 and 1:1.2, between 1.1:1 and 1:1.1, or about 1:1, or 1:1 viable CD4+ T cells to viable CD8+ T cells.

[0622] In some embodiments, the provided methods are used in connection with transducing a viral vector containing a polynucleotide encoding a recombinant receptor into, into about, or

into less than 300×10^6 cells, *e.g.*, viable T cells of a stimulated cell population. In certain embodiments, at or about 100×10^6 cells, *e.g.*, viable T cells of a stimulated cell population are transduced or subjected to transduction.

[0623] In some embodiments, the provided methods are used in connection with transducing a viral vector containing a polynucleotide encoding a recombinant receptor into, into about, or into less than 600×10^6 cells, *e.g.*, viable T cells of a stimulated cell population. In certain embodiments, at or about 600×10^6 cells, *e.g.*, viable T cells of a stimulated cell population are transduced or subjected to transduction. In some embodiments, up to 900×10^6 cells (*e.g.*, viable CD3+ cells or mixed viable CD4+ and viable CD8+ cells (*e.g.*, mixed at or at about a 1:1 ratio)) are subjected to stimulation, and an amount of, of about, or up to 600×10^6 cells of the cells that have been subjected to stimulation are subjected to transduction.

[0624] In some embodiments, the transduction is performed in serum free media. In some embodiments, the transduction is performed in the presence of IL-2, IL-7, and IL-15. In some embodiments, the viral vector for transduction is frozen and thawed prior to use, and the thawed viral vector is diluted with serum free media. In some embodiments, the serum free media for diluting the viral vector and for transduction are as described herein or in PCT/US2018/064627.

[0625] In some embodiments, the serum-free medium comprises a basal medium (*e.g.* OpTmizer™ T-Cell Expansion Basal Medium (ThermoFisher)), supplemented with one or more supplement. In some embodiments, the one or more supplement is serum-free. In some embodiments, the serum-free medium comprises a basal medium supplemented with one or more additional components for the maintenance, expansion, and/or activation of a cell (*e.g.*, a T cell), such as provided by an additional supplement (*e.g.*, OpTmizer™ T-Cell Expansion Supplement (ThermoFisher)). In some embodiments, the serum-free medium further comprises a serum replacement supplement, for example, an immune cell serum replacement, *e.g.*, ThermoFisher, #A2596101, the CTS™ Immune Cell Serum Replacement, or the immune cell serum replacement described in Smith *et al.* *Clin Transl Immunology*. 2015 Jan; 4(1): e31. In some embodiments, the serum-free medium further comprises a free form of an amino acid such as L-glutamine. In some embodiments, the serum-free medium further comprises a dipeptide form of L-glutamine (*e.g.*, L-alanyl-L-glutamine), such as the dipeptide in *Glutamax*™ (ThermoFisher). In some embodiments, the serum-free medium further comprises one or more recombinant cytokines, such as recombinant human IL-2, recombinant human IL-7, and/or recombinant human IL-15.

[0626] In particular embodiments, the cells, *e.g.*, the cells of the stimulated cell population contain at least 80%, at least 85%, at least 90%, or at least 95% cells that are CD4+ T cells or CD8+ T cells. In some embodiments, the transduction, including post-transduction incubation, is performed for between 24 and 48 hours, between 36 and 12 hours, between 18 and 30 hours, or for or for about 24 hours. In some embodiments, the transduction, including post-transduction incubation, is performed for or for about 24 hours, 48 hours, or 72 hours, or for or for about 1 day, 2 days, or 3 days, respectively. In particular embodiments, the transduction, including post-transduction incubation, is performed for or for about 24 hours $\pm$ 6 hours, 48 hours $\pm$ 6 hours, or 72 hours $\pm$ 6 hours. In particular embodiments, the transduction, including post-transduction incubation, is performed for or for about 72 hours, 72 $\pm$ 4 hours, or for or for about 3 days.

[0627] In certain embodiments, the transduction step is initiated within two days, within 36 hours, within 30 hours, within 24 hours, within 18 hours, within 16 hours, within 14 hours, or within 12 hours of the start or initiation of the incubation, *e.g.*, the incubation under stimulating conditions. In certain embodiments, the transduction step is initiated at about 20 hours of the start or initiation of the incubation, *e.g.*, the incubation under stimulating conditions. In certain embodiments, the transduction step is initiated at 20 $\pm$ 4 hours of the start or initiation of the incubation, *e.g.*, the incubation under stimulating conditions.

[0628] In some embodiments, the system is included with and/or placed into association with other instrumentation, including instrumentation to operate, automate, control and/or monitor aspects of the transduction step and one or more various other processing steps performed in the system, *e.g.*, one or more processing steps that can be carried out with or in connection with the centrifugal chamber system as described herein or in International Publication Number WO2016/073602. This instrumentation in some embodiments is contained within a cabinet. In some embodiments, the instrumentation includes a cabinet, which includes a housing containing control circuitry, a centrifuge, a cover, motors, pumps, sensors, displays, and a user interface. An exemplary device is described in US Patent No. 6,123,655, US Patent No. 6,733,433 and US 2008/0171951.

[0629] In some embodiments, the system comprises a series of containers, *e.g.*, bags, tubing, stopcocks, clamps, connectors, and a centrifuge chamber. In some embodiments, the containers, such as bags, include one or more containers, such as bags, containing the cells to be transduced and the viral vector particles, in the same container or separate containers, such as the same bag or separate bags. In some embodiments, the system further includes one or more containers,

such as bags, containing medium, such as diluent and/or wash solution, which is pulled into the chamber and/or other components to dilute, resuspend, and/or wash components and/or populations during the methods. The containers can be connected at one or more positions in the system, such as at a position corresponding to an input line, diluent line, wash line, waste line and/or output line.

[0630] In some embodiments, the chamber is associated with a centrifuge, which is capable of effecting rotation of the chamber, such as around its axis of rotation. Rotation may occur before, during, and/or after the incubation in connection with transduction of the cells and/or in one or more of the other processing steps. Thus, in some embodiments, one or more of the various processing steps is carried out under rotation, *e.g.*, at a particular force. The chamber is typically capable of vertical or generally vertical rotation, such that the chamber sits vertically during centrifugation and the side wall and axis are vertical or generally vertical, with the end wall(s) horizontal or generally horizontal.

[0631] In some embodiments, the population containing cells and population containing viral vector particles, and optionally air, can be combined or mixed prior to providing the populations to the cavity. In some embodiments, the population containing cells and population containing viral vector particles, and optionally air, are provided separately and combined and mixed in the cavity. In some embodiments, a population containing cells, a population containing viral vector particles, and optionally air, can be provided to the internal cavity in any order. In any of such some embodiments, a population containing cells and viral vector particles is the input population once combined or mixed together, whether such is combined or mixed inside or outside the centrifugal chamber and/or whether cells and viral vector particles are provided to the centrifugal chamber together or separately, such as simultaneously or sequentially.

[0632] In some embodiments, intake of the volume of gas, such as air, occurs prior to the incubating the cells and viral vector particles, such as rotation, in the transduction method. In some embodiments, intake of the volume of gas, such as air, occurs during the incubation of the cells and viral vector particles, such as rotation, in the transduction method.

[0633] In some embodiments, the liquid volume of the cells or viral vector particles that make up the transduction population, and optionally the volume of air, can be a predetermined volume. The volume can be a volume that is programmed into and/or controlled by circuitry associated with the system.

[0634] In some embodiments, intake of the transduction population, and optionally gas, such as air, is controlled manually, semi-automatically and/or automatically until a desired or predetermined volume has been taken into the internal cavity of the chamber. In some embodiments, a sensor associated with the system can detect liquid and/or gas flowing to and from the centrifuge chamber, such as via its color, flow rate and/or density, and can communicate with associated circuitry to stop or continue the intake as necessary until intake of such desired or predetermined volume has been achieved. In some aspects, a sensor that is programmed or able only to detect liquid in the system, but not gas (*e.g.*, air), can be made able to permit passage of gas, such as air, into the system without stopping intake. In some such embodiments, a non-clear piece of tubing can be placed in the line near the sensor while intake of gas, such as air, is desired. In some embodiments, intake of gas, such as air, can be controlled manually.

[0635] In aspects of the provided methods, the internal cavity of the centrifuge chamber is subjected to high speed rotation. In some embodiments, rotation is affected prior to, simultaneously, subsequently or intermittently with intake of the liquid input population, and optionally air. In some embodiments, rotation is affected subsequent to intake of the liquid input population, and optionally air. In some embodiments, rotation is by centrifugation of the centrifugal chamber at a relative centrifugal force at the inner surface of side wall of the internal cavity and/or at a surface layer of the cells of at or about or at least at or about 200 g, 300 g, 400 g, 500 g, 600 g, 700 g, 800 g, 1000 g, 1100 g, 1500, 1600 g, 1800 g, 2000 g, 2200 g, 2500 g, 3000 g, 3200 g, 3500 g or 4000 g. In some embodiments, rotation is by centrifugation at a force that is greater than or about 1100 g, such as by greater than or about 1200 g, greater than or about 1400 g, greater than or about 1600 g, greater than or about 1800 g, greater than or about 2000 g, greater than or about 2400 g, greater than or about 2800 g, greater than or about 3000 g or greater than or about 3200 g. In particular embodiments, the rotation by centrifugation is at a force between 600 g and 800 g. In particular embodiments, the rotation by centrifugation is at a force of or of about 693 g. In some embodiments, rotation is by centrifugation at a force that is or is about 1600g.

[0636] In some embodiments, the gas, such as air, in the cavity of the chamber is expelled from the chamber. In some embodiments, the gas, such as air, is expelled to a container that is operably linked as part of the closed system with the centrifugal chamber. In some embodiments, the container is a free or empty container. In some embodiments, the air, such as

gas, in the cavity of the chamber is expelled through a filter that is operably connected to the internal cavity of the chamber via a sterile tubing line. In some embodiments, the air is expelled using manual, semi-automatic or automatic processes. In some embodiments, air is expelled from the chamber prior to, simultaneously, intermittently or subsequently with expressing the output population containing incubated cells and viral vector particles, such as cells in which transduction has been initiated or cells have been transduced with a viral vector, from the cavity of the chamber.

[0637] In some embodiments, the transduction and/or other incubation is performed as or as part of a continuous or semi-continuous process. In some embodiments, a continuous process involves the continuous intake of the cells and viral vector particles, *e.g.*, the transduction composition (either as a single pre-existing composition or by continuously pulling into the same vessel, *e.g.*, cavity, and thereby mixing, its parts), and/or the continuous expression or expulsion of liquid, and optionally expelling of gas (*e.g.*, air), from the vessel, during at least a portion of the incubation, *e.g.*, while centrifuging. In some embodiments, the continuous intake and continuous expression are carried out at least in part simultaneously. In some embodiments, the continuous intake occurs during part of the incubation, *e.g.*, during part of the centrifugation, and the continuous expression occurs during a separate part of the incubation. The two may alternate. Thus, the continuous intake and expression, while carrying out the incubation, can allow for a greater overall volume of sample to be processed, *e.g.*, transduced.

[0638] In some embodiments, the incubation is part of a continuous process, the method including, during at least a portion of the incubation, effecting continuous intake of said transduction composition into the cavity during rotation of the chamber and during a portion of the incubation, effecting continuous expression of liquid and, optionally expelling of gas (*e.g.*, air), from the cavity through the at least one opening during rotation of the chamber.

[0639] In some embodiments, the semi-continuous incubation is carried out by alternating between effecting intake of the composition into the cavity, incubation, expression of liquid from the cavity and, optionally expelling of gas (*e.g.*, air) from the cavity, such as to an output container, and then intake of a subsequent (*e.g.*, second, third, etc.) composition containing more cells and other reagents for processing, *e.g.*, viral vector particles, and repeating the process. For example, in some embodiments, the incubation is part of a semi-continuous process, the method including, prior to the incubation, effecting intake of the transduction composition into the cavity through said at least one opening, and subsequent to the incubation, effecting expression

of fluid from the cavity; effecting intake of another transduction composition comprising cells and the viral vector particles into said internal cavity; and incubating the another transduction composition in said internal cavity under conditions whereby said cells in said another transduction composition are transduced or subjected to transduction with said vector. The process may be continued in an iterative fashion for a number of additional rounds. In this respect, the semi-continuous or continuous methods may permit production of even greater volume and/or number of cells.

[0640] In some embodiments, a portion of the transduction incubation is performed in the centrifugal chamber, which is performed under conditions that include rotation or centrifugation.

[0641] In particular embodiments, transduction of the cells with the viral vector is or includes spinoculation, *e.g.*, centrifugation of a mixture containing the cells and the viral particles. In some embodiments, the composition containing cells and viral particles can be rotated, generally at relatively low force or speed, such as speed lower than that used to pellet the cells, such as from or from about 600 rpm to 1700 rpm (*e.g.*, at or about or at least 600 rpm, 1000 rpm, or 1500 rpm or 1700 rpm). In some embodiments, the rotation is carried at a force, *e.g.*, a relative centrifugal force, of from or from about 100 g to 4000 g (*e.g.*, at or about or at least at or about 100 g, 200 g, 300 g, 400 g, 500 g, 600 g, 700 g, 800 g, 900 g, 1000 g, 1500 g, 2000 g, 2500 g, 3000 g or 3500 g), as measured for example at an internal or external wall of the chamber or cavity.

[0642] In some embodiments, the cells are spinoculated with the viral vector at a force, *e.g.*, a relative centrifugal force, of between or between about 100 g and 4000 g, 200 g and 1,000 g, 500 g and 1200 g, 1000 g and 2000 g, 600 g and 800 g, 1200 g and 1800 g, or 1500 g and 1800 g. In certain embodiments, the cells are spinoculated with the viral vector particle for, for at least, or for about 100 g, 200 g, 300 g, 400 g, 500 g, 600 g, 700 g, 800 g, 900 g, 1000 g, 1200g, 1500 g, 1600g, 2000 g, 2500 g, 3000 g, 3200 g, or 3500 g. In some embodiments, the cells are transduced or subjected to transduction with the viral vector at a force of or of about 692 g or 693 g. In particular embodiments, the cells are transduced or subjected to transduction with the viral vector at a force of or of about 1600 g. In some embodiments, the force is the force at the internal surface of the side wall of the internal cavity and/or at a surface layer of the cells.

[0643] In certain embodiments, the cells are spinoculated, *e.g.*, the cell composition containing cells and viral vector is rotated, for greater than or about 5 minutes, such as greater than or about 10 minutes, greater than or about 15 minutes, greater than or about 20 minutes,

greater than or about 30 minutes, greater than or about 45 minutes, greater than or about 60 minutes, greater than or about 90 minutes or greater than or about 120 minutes; or between or between about 5 minutes and 120 minutes, 30 minutes and 90 minutes, 15 minutes and 60 minutes, 15 minutes and 45 minutes, 30 minutes and 60 minutes or 45 minutes and 60 minutes, each inclusive. In some embodiments, the cells are spinoculated with the viral vector for or for about 30 minutes. In certain embodiments, the cells are spinoculated with the viral vector for or for about 60 minutes.

[0644] In some embodiments, the method of transduction includes a spinoculation, *e.g.*, a rotation or centrifugation of the transduction composition, and optionally air, in the centrifugal chamber for greater than or about 5 minutes, such as greater than or about 10 minutes, greater than or about 15 minutes, greater than or about 20 minutes, greater than or about 30 minutes, greater than or about 45 minutes, greater than or about 60 minutes, greater than or about 90 minutes or greater than or about 120 minutes. In some embodiments, the transduction composition, and optionally air, is rotated or centrifuged in the centrifugal chamber for greater than 5 minutes, but for no more than 60 minutes, no more than 45 minutes, no more than 30 minutes or no more than 15 minutes. In particular embodiments, the transduction includes rotation or centrifugation for or for about 60 minutes.

[0645] In some embodiments, the method of transduction includes rotation or centrifugation of the transduction composition, and optionally air, in the centrifugal chamber for between or between about 10 minutes and 60 minutes, 15 minutes and 60 minutes, 15 minutes and 45 minutes, 30 minutes and 60 minutes or 45 minutes and 60 minutes, each inclusive, and at a force at the internal surface of the side wall of the internal cavity and/or at a surface layer of the cells of, of about, or at 1000 g, 1100 g, 1200 g, 1400 g, 1500 g, 1600 g, 1800 g, 2000 g, 2200 g, 2400 g, 2800 g, 3200 g or 3600 g. In particular embodiments, the method of transduction includes rotation or centrifugation of the transduction composition, *e.g.*, the cells and the viral vector particles, at or at about 1600 g for or for about 60 minutes.

[0646] In some embodiments, genomic integration of transgene sequences, such as transgene sequences encoding a recombinant receptor, *e.g.*, a CAR, can be assessed in cells produced in connection with any of the provided processes for engineering cells. In some embodiments, the integrated copy number is assessed, which is the copy number of the transgene sequence integrated into the chromosomal DNA or genomic DNA of cells.

[0647] In some embodiments, methods for assessing genomic integration of a transgene sequence involve separating a high molecular weight fraction of deoxyribonucleic acid (DNA), such as DNA species that are greater than or greater than about 10 kilobases (kb), from DNA isolated from one or more cell. In some aspects, such separation can be carried out by methods such as pulse field gel electrophoresis (PFGE). In some aspects, the one or more cell contains, or is suspected to contain, at least one engineered cell comprising a transgene sequence encoding a recombinant protein. In some aspects, the methods involve determining the presence, absence or amount of the transgene sequence integrated into the genome of the one or more cell, for example, by quantitative methods such as quantitative polymerase chain reaction (qPCR), digital PCR (dPCR) or droplet digital PCR (ddPCR).

[0648] In some embodiments, the high molecular weight fraction primarily contains large DNA molecules such as chromosomal or genomic DNA, and contain low or almost no molecules that are smaller than the threshold value for size, such as plasmids, non-integrated DNA fragments, linear complementary DNA (cDNA), autointegrants, long terminal repeat (LTR) circles or other residual species or molecules that have not been integrated into the genome. In some embodiments, by determining the presence, absence or amount of the transgene sequences in the high molecular weight fraction, the detected transgene sequences represent those that have been integrated into the genome of the engineered cell, and minimizes the detection of non-integrated transgene sequences.

[0649] In some embodiments, the high molecular weight fraction comprises DNA molecules that are greater than or greater than about 10 kilobases (kb) in size. In some embodiments, the high molecular weight fraction comprises DNA molecules that are greater than or greater than about 10, 11, 12, 12.5, 13, 14, 15, 16, 17, 17.5, 18, 19, 20, 25 or 30 kilobases (kb) or more in size. In some embodiments, the high molecular weight fraction comprises DNA molecules that are greater than or greater than about 10, 12.5, 15, 17.5 or 20 kilobases (kb) or more in size. In some aspects, the high molecular weight fraction contains genomic DNA or genomic DNA fragments, and excludes or separates non-integrated or residual nucleic acid species that can be present in the DNA sample. In some aspects, the high molecular weight fraction, *e.g.*, DNA samples that are above a threshold value such as about 10, 11, 12, 12.5, 13, 14, 15, 16, 17, 17.5, 18, 19, 20, 25 or 30 kilobases (kb) or more. In some embodiments, the threshold value is greater than or greater than about 10, 12.5, 15, 17.5 or 20 kilobases (kb) or more.

[0650] In some embodiments, the high molecular weight fraction is separated or isolated using an electrophoresis-based method. In some aspects, electrophoresis separates biomolecules by charge and/or size via mobility through a separating matrix in the presence of an electric field. In some embodiments, electrophoresis systems can be used to fractionate, analyze, and collect particular analytes, including nucleic acid molecules, based on size or molecular weight. In some aspects, a fraction is or includes a subset of the plurality of molecules. In some aspects, a fraction can be defined or determined by size or molecular weight, or in some aspects, by any physical property that causes it to migrate at a faster or slower rate than other molecules or fractions of a plurality when driven to migrate through a buffer composition of the disclosure by the force of an electric field (i.e., electrophoretic mobility).

[0651] In some embodiments, the high molecular weight fraction is separated or isolated using pulse field gel electrophoresis (PFGE). In some aspects, PFGE involves introducing an alternating voltage gradient in an electrophoresis system to improve the resolution of larger nucleic acid molecules, such as chromosomal or genomic DNA. In some aspects, the voltage of the electrophoresis system is periodically switched among three directions: one that runs through the central axis of the gel and two that run at an angle of 60 degrees either side. In some aspects, exemplary systems and methods for separating or isolating nucleic acid molecules by PFGE include those described in, *e.g.*, US 9599590; US 2017/0240882; or US 2017/0254774.

[0652] In some aspects, the electrophoresis, such as PFGE, can be performed using an apparatus or system. In some aspects, the apparatus or system is an automated system or high-throughput system. Exemplary systems for performing PFGE, include, those described in, *e.g.*, US 9599590; US 2017/0240882; or US 2017/0254774, or commercially available apparatus or system, such as Pippin Prep, Blue Pippin or Pippin HT (Sage Science); CHEF Mapper® XA System, CHEF-DR® III Variable Angle System, CHEF-DR II System (Bio-Rad); and Biometra Rotaphor 8 System (Analytik Jena AG).

[0653] In some aspects, exemplary samples for assessment include a nucleic acid, an oligonucleotide, a DNA molecule, a RNA molecule, or any combination thereof. In some aspects, the sample can include, an amino acid, a peptide, a protein, or any combination thereof. In some aspects, the sample can be a whole cell lysate, or the DNA or protein fraction of a cell lysate, such as lysate of cells engineered for adoptive cell therapy.

[0654] In some embodiments, nucleic acids from the samples can include genomic DNA, double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), coding DNA (or cDNA),

messenger RNA (mRNA), short interfering RNA (siRNA), short-hairpin RNA (shRNA), microRNA (miRNA), single-stranded RNA, double-stranded RNA (dsRNA), a morpholino, RNA interference (RNAi) molecule, mitochondrial nucleic acid, chloroplast nucleic acid, viral DNA, viral RNA, and other organelles with separate genetic material. In some aspects, the nucleic acids from the sample can also include nucleic acid analogs that contain modified, synthetic, or non-naturally occurring nucleotides or structural elements or other alternative/modified nucleic acid chemistries, such as base analogs such as inosine, intercalators (U.S. Pat. No. 4,835,263) and minor groove binders (U.S. Pat. No. 5,801,115).

[0655] In some embodiments, prior to isolating or separating a high- or low-molecular weight fraction, the samples can be combined with a reagent that imparts a net negative charge, denatures a peptide or protein, or digests a DNA or RNA molecule prior to assessment in an electrophoresis system. In some aspects, samples can be combined with agents that impart fluorescent, magnetic, or radioactive properties to the sample or fractions thereof for the purpose of detection. In some examples, a dsDNA sample is mixed with ethidium bromide, applied to the electrophoresis cassette, and fractions of the sample are detected using an ultrabright green LED.

[0656] In some aspects, a system for separating or isolating the nucleic acid samples, such as an electrophoresis system, can be automated and/or high-throughput. In some aspects, the electrophoresis system can utilize disposable consumables or reagents, such as an electrophoresis cassette.

[0657] In some aspects, determining the presence, absence or amount of the transgene sequence can be performed using methods for determining the presence, absence or amount of a nucleic acid sequence. In particular, methods used to quantitate nucleic acid sequences, such as quantitative polymerase chain reaction (qPCR) or related methods, can be employed in determining the copy number of the transgene sequence in a sample containing DNA, or in a particular fraction, such as the high molecular weight fraction, that is separated or isolated from samples containing DNA. In some embodiments, the determining the presence, absence or amount of the transgene sequence comprises determining the copy number, for example, using any one of the exemplary assays below to quantitate nucleic acid molecules.

[0658] In some aspects, the presence, absence and/or amount of a particular sequence can be detected using a probe or a primer, that can specifically bind or recognize all or a portion of the transgene sequence. In some embodiments, copy number can be determined using probes that

can specifically detect a portion of the transgene sequence, or primer sequences that can specifically amplify a portion of the transgene sequence. In some aspects, the probe or primer sequences can specifically detect, bind or recognize a portion of the transgene sequence, such as a portion of the transgene sequence that is heterologous, exogenous or transgenic to the cell. In some embodiments, the primers or probe used for qPCR or other nucleic acid-based methods are specific for binding, recognizing and/or amplifying nucleic acids encoding the recombinant protein, and/or other components or elements of the plasmid and/or vector, including regulatory elements, *e.g.*, promoters, transcriptional and/or post-transcriptional regulatory elements or response elements, or markers, *e.g.*, surrogate markers. In some aspects, the probes or primers can be used for exemplary methods to determine the presence, absence and/or amount of transgene sequences, such as quantitative PCR (qPCR), digital PCR (dPCR) or droplet digital PCR (ddPCR).

[0659] In some aspects, the determining of the presence, absence or amount comprises determining the amount of the transgene sequence, such as determining the mass, weight, concentration or copy number of the transgene sequences, in one or more cells or in a biological sample containing one or more cells. In some aspects, the determining of the presence, absence or amount of a nucleic acid sequence, or assessing the mass, weight, concentration or copy number of the transgene sequences can be performed in a portion of a population of cells or a portion of a biological sample, and can be normalized, averaged, and/or extrapolated to determine the presence, absence or amount in the entire sample or entire population of cells.

[0660] In some embodiments, the determining the presence, absence or amount of the transgene sequence comprises determining the mass, weight, concentration or copy number of the transgene sequence per diploid genome or per cell in the one or more cells. In some embodiments, the one or more cell comprises a population of cells in which a plurality of cells of the population comprise the transgene sequence encoding the recombinant protein. In some embodiments, the copy number is an average or mean copy number per diploid genome or per cell among the population of cells.

[0661] In some aspects, determining the copy number comprises determining the number of copies of the transgene sequences present in one or more cells, or in a biological sample. In some aspects, the copy number can be expressed as an average or mean copy number. In some aspects, the copy number of a particular integrated transgene includes the number of integrants (containing transgene sequences) per cell. In some aspects, the copy number of a particular

integrated transgene includes the number of integrants (containing transgene sequences) per diploid genome. In some aspects, the copy number of transgene sequence is expressed as the number of integrated transgene sequences per cell. In some aspects, the copy number of transgene sequence is expressed as the number of integrated transgene sequences per diploid genome. In some aspects, the one or more cell comprises a population of cells in which a plurality of cells of the population comprise the transgene sequence encoding the recombinant protein. In some embodiments, the copy number is an average or mean copy number per diploid genome or per cell among the population of cells.

[0662] In some embodiments, the determining the amount of the transgene sequence comprises assessing the mass, weight, concentration or copy number of the transgene sequence per the one or more cells, optionally per CD3+, CD4+ and/or CD8+ cell, and/or per cell expressing the recombinant protein. In some aspects, surface markers or phenotypes expressed on the cell can be determined using cell-based methods, such as by flow cytometry or immunostaining. In some aspects, the cells expressing the recombinant protein can be determined using cell-based methods, such as by flow cytometry or immunostaining, for example with an anti-idiotypic antibody or staining for a surrogate marker. In some aspects, the amount of transgene sequences can be normalized to the number of particular cells, such as CD3+, CD4+ and/or CD8+ cell, and/or per cell expressing the recombinant protein, or per total number of cells, such as per total number of cells in the sample or per total number of cells undergoing an engineering process.

[0663] In some embodiments, the determined copy number is expressed as a normalized value. In some embodiments, the determined copy number is quantified as a number of copy of the transgene sequence per genome or per cell. In some aspects, the per genome value is expressed as copy of the transgene sequence per diploid genome, as a typical somatic cell, such as a T cell, contains a diploid genome. In some aspects, the determined copy number can be normalized against the copy number of a known reference gene in the genome of the cell. In some aspects, the reference gene is RRP30 (encoding ribonuclease P protein subunit p30), or 18S rRNA (18S ribosomal RNA), 28S rRNA (28S ribosomal RNA), TUBA (α -tubulin), ACTB (β -actin), β 2M (β 2-microglobulin), ALB (albumin), RPL32 (ribosomal protein L32), TBP (TATA sequence binding protein), CYCC (cyclophilin C), EF1A (elongation factor 1 α), GAPDH (glyceraldehyde-3-phosphate dehydrogenase), HPRT (hypoxanthine phosphoribosyl

transferase) or RPII (RNA polymerase II). In some embodiments, the determined copy number is quantified as copy of the transgene sequence per microgram of DNA.

[0664] In some aspects, the copy number is an average, mean, or median copy number from a plurality or population of cells, such as a plurality or population of engineered cells. In some aspects, the copy number is an average or mean copy number from a plurality or population of cells, such as a plurality or population of engineered cells. In some aspects, the average or mean copy number is determined from a plurality or population of cells, such as a plurality or population of cells undergoing one or more steps of the engineering or manufacturing process, or in a cell composition, such as a cell composition for administration to a subject. In some aspects, a normalized average copy number is determined, for example, as an average or mean copy number of the transgene sequences normalized to a reference gene, such as a known gene that is present in two copies in a diploid genome. In some aspects, normalization to a reference gene that is typically present in two copies per diploid genome, can correspond to the copy number in a cell, such as a diploid cell. Thus, in some aspects, the normalized average or mean copy number can correspond to the average or mean copy number of the detected transgene sequences among a plurality or a population of cells, for example, T cells that typically have a diploid genome.

[0665] In some embodiments, the determining the presence, absence or amount of the transgene sequence is carried out by polymerase chain reaction (PCR). In some embodiments, the PCR is quantitative polymerase chain reaction (qPCR), digital PCR or droplet digital PCR, such as any described below. In some embodiments, the presence, absence or amount of the transgene sequence is determined by droplet digital PCR. In some embodiments, the PCR is carried out using one or more primers that is complementary to or is capable of specifically amplifying at least a portion of the transgene sequence, and in some cases, one or more primers that is complementary to or is capable of specifically amplifying at least a portion of a reference gene.

[0666] In some aspects, qPCR can be used to detect the accumulation of amplification product measured as the reaction progresses, in real time, with product quantification after each cycle. Thus, in some aspects, qPCR can be used to determine the copy number of a particular nucleic acid sequence, such as the transgene sequence, in a sample. In some aspects, qPCR employs fluorescent reporter molecule in each reaction well that yields increased fluorescence with an increasing amount of product DNA. In some aspects, fluorescence chemistries employed

include DNA-binding dyes and fluorescently labeled sequence-specific primers or probes. In some aspects, qPCR employs a specialized thermal cycler with the capacity to illuminate each sample at a specified wavelength and detect the fluorescence emitted by the excited fluorophore. In some aspects, the measured fluorescence is proportional to the total amount of amplicon; the change in fluorescence over time is used to calculate the amount of amplicon produced in each cycle.

[0667] In some embodiments, dPCR is a method for detecting and quantifying nucleic acids, and permits accurate quantitative analysis and the highly sensitive detection of a target nucleic acid molecule. In some aspects, dPCR involves a limiting dilution of DNA into a succession of individual PCR reactions (or partitions). In some aspects, limiting dilution can employ the principles of partitioning with nanofluidics and emulsion chemistries, based on random distribution of the template nucleic acid to be assessed, *e.g.*, transgene sequences, and Poisson statistics to measure the quantities of DNA present for a given proportion of positive partitions. In some aspects, dPCR is generally linear and are sensitive, capable of detecting or quantifying very small amounts of DNA. In some aspects, dPCR permits absolute quantification of a DNA sample using a single molecule counting method without a standard curve, and absolute quantification can be obtained from PCR for a single partition per well (see Pohl et al., (2004) *Expert Rev. Mol. Diagn.* 4(1), 41-47).

[0668] Exemplary commercially available apparatuses or systems for dPCR include Raindrop™ Digital PCR System (Raindance™ Technologies); QX200™ Droplet Digital™ PCR System (Bio-Rad); BioMark™ HD System and qdPCR 37K™ IFC (Fluidigm Corporation) and QuantStudio™ 3D Digital PCR System (Life Technologies™) (see, *e.g.*, Huggett et al. (2013) *Clinical Chemistry* 59: 1691–1693; Shuga, et al. (2013) *Nucleic Acids Research* 41(16): e159; Whale et al. (2013) *PLoS One* 3: e58177).

[0669] In some embodiments, the presence, absence or amount of the transgene sequences, such as transgene sequences encoding a recombinant protein, for integration into the genome of the engineered cell, is determined using droplet digital polymerase chain reaction (ddPCR). ddPCR is a type of digital PCR, in which the PCR solution is divided or partitioned into smaller reactions through a water-oil emulsion chemistry, to generate numerous droplets. In some aspects, particular surfactants can be used to generate the water-in-oil droplets. (see, *e.g.*, Hindson et al., (2011) *Anal Chem* 83(22): 8604–8610; Pinheiro et al., (2012) *Anal Chem* 84, 1003–1011). In some aspects, each individual droplet is subsequently run as individual reaction.

In some aspects, the PCR sample is partitioned into nanoliter-size samples and encapsulated into oil droplets. In some aspects, the oil droplets are made using a droplet generator that applies a vacuum to each of the wells. In an exemplary case, approximately 20,000 oil droplets for individual reactions can be made from a 20 μ L sample volume.

[0670] In some aspects, methods assessing integrated copy number can be performed at various time points to determine and compare the timing, extent or progress of genetic engineering, such as integration of the introduced transgene sequences into the genome of the cell into which the transgene sequences are introduced. In some aspects, the methods can be carried out at various stages of an engineering or manufacturing process for engineered cell compositions, such as any of the processes described. For example, the provided methods can be performed at various stages of an expanded engineering process or a non-expanded engineering process.

[0671] In some aspects, cells engineered by the provided methods are assessed for genomic integration of a transgene sequence, such as encoding a recombinant receptor, *e.g.*, CAR, using the assays for vector copy number described above. In some embodiments, the methods involve separating a high molecular weight fraction of greater than or greater than about 10 kilobases (kb) from deoxyribonucleic acid (DNA) isolated from a cell, wherein prior to the separating, the cell has been introduced with a polynucleotide comprising the transgene sequence under conditions for integration of the transgene sequence into a genome of the cell, such as by viral transduction; and determining the presence, absence or amount of the transgene sequence in the high molecular weight fraction.

4. Incubation

[0672] In some embodiments, the methods for generating the engineered cells, *e.g.*, for cell therapy in accord with any of provided methods, uses, articles of manufacture or compositions, include one or more steps for incubating cells under conditions that do not promote proliferation and/or expansion. In some embodiments, cells are incubated under conditions that do not promote proliferation and/or expansion subsequent to a step of genetically engineering, *e.g.*, introducing a recombinant polypeptide to the cells by transduction or transfection. In particular embodiments, the cells are incubated after the cells have been incubated under stimulating conditions and transduced or transfected with a recombinant polynucleotide, *e.g.*, a polynucleotide encoding a recombinant receptor. Thus, in some embodiments, a composition of

CAR-positive T cells that has been engineered by transduction or transfection with a recombinant polynucleotide encoding the CAR, is incubated under conditions that do not promote proliferation and/or expansion.

[0673] In particular embodiments, genetic engineering, such as by transforming (*e.g.*, transducing) the cells with a viral vector, further includes one or more steps of incubating the cells after the introducing or contacting of the cells with the viral vector. In some embodiments, cells, *e.g.*, cells of the transformed cell population (also called “transformed cells”), are incubated subsequent to processes for genetically engineering, transforming, transducing, or transfecting the cells to introduce the viral vector into the cells. In particular embodiments, the incubation results in a population of incubated cells (also referred to herein as an incubated cell population).

[0674] In some embodiments, the cells, *e.g.*, transformed cells, are incubated after the introducing of the heterologous or recombinant polynucleotide, *e.g.*, viral vector particles is carried out without further processing of the cells. In particular embodiments, prior to the incubating, the cells are washed, such as to remove or substantially remove exogenous or remaining polynucleotides encoding the heterologous or recombinant polynucleotide, *e.g.*, viral vector particles, such as those remaining in the media after the genetic engineering process following the spinoculation.

[0675] In some such embodiments, the further incubation is effected under conditions to result in integration of the viral vector into a host genome of one or more of the cells. For example, the further incubation provides time for the viral vector that may be bound to the T cells following transduction, *e.g.*, via spinoculation, to integrate within the genome of the cell to deliver the gene of interest. In some aspects, the further incubation is carried out under conditions to allow the cells, *e.g.*, transformed cells, to rest or recover in which the culture of the cells during the incubation supports or maintains the health of the cells. In particular embodiments, the cells are incubated under static conditions, such as conditions that do not involve centrifugation, shaking, rotating, rocking, or perfusion, *e.g.*, continuous or semi-continuous perfusion of the media.

[0676] It is within the level of a skilled artisan to assess or determine if the incubation has resulted in integration of viral vector particles into a host genome, and hence to empirically determine the conditions for a further incubation. In some embodiments, integration of a viral vector into a host genome can be assessed by measuring the level of expression of a recombinant

protein, such as a heterologous protein, encoded by a nucleic acid contained in the genome of the viral vector particle following incubation. A number of well-known methods for assessing expression level of recombinant molecules may be used, such as detection by affinity-based methods, *e.g.*, immunoaffinity-based methods, *e.g.*, in the context of cell surface proteins, such as by flow cytometry. In some examples, the expression is measured by detection of a transduction marker and/or reporter construct. In some embodiments, nucleic acid encoding a truncated surface protein is included within the vector and used as a marker of expression and/or enhancement thereof.

[0677] In certain embodiments, the incubation is performed under static conditions, such as conditions that do not involve centrifugation, shaking, rotating, rocking, or perfusion, *e.g.*, continuous or semi-continuous perfusion of the media. In some embodiments, either prior to or shortly after, *e.g.*, within 5, 15, or 30 minutes, the initiation of the incubation, the cells are transferred (*e.g.*, transferred under sterile conditions) to a container such as a bag or vial, and placed in an incubator.

[0678] In some embodiments, at least a portion of the incubation is carried out in the internal cavity of a centrifugal chamber, such as described in International Publication Number WO2016/073602.

[0679] In some embodiments, the cells that have been introduced with a polynucleotide encoding the heterologous or recombinant polypeptide, *e.g.*, the viral vectors, are transferred into a container for the incubation. In some embodiments, the container is a vial. In particular embodiments, the container is a bag. In some embodiments, the cells, and optionally the heterologous or recombinant polypeptide, are transferred into the container under closed or sterile conditions. In some embodiments, the container, *e.g.*, the vial or bag, is then placed into an incubator for all or a portion of the incubation. In particular embodiments, incubator is set at, at about, or at least 16°C, 24°C, or 35°C. In some embodiments, the incubator is set at 37°C, at about 37°C, or at 37°C $\pm$ 2°C, $\pm$ 1°C, $\pm$ 0.5°C, or $\pm$ 0.1°C.

[0680] In some aspects, the conditions for the incubation can include one or more of particular media, temperature, oxygen content, carbon dioxide content, time, agents, *e.g.*, nutrients, amino acids, antibiotics, ions, and/or stimulatory factors, such as cytokines, chemokines, antigens, binding partners, fusion proteins, recombinant soluble receptors, and any other agents designed to activate the cells.

[0681] In some embodiments, the incubation is performed in serum free media. In some embodiments, the serum free media is a defined and/or well-defined cell culture media. In certain embodiments, the serum free media is a controlled culture media that has been processed, *e.g.*, filtered to remove inhibitors and/or growth factors. In some embodiments, the serum free media contains proteins. In certain embodiments, the serum-free media may contain serum albumin, hydrolysates, growth factors, hormones, carrier proteins, and/or attachment factors.

[0682] In particular embodiments, the cells are incubated in the presence of one or more cytokines. In certain embodiments, the one or more cytokines are recombinant cytokines. In particular embodiments, the one or more cytokines are human recombinant cytokines. In certain embodiments, the one or more cytokines bind to and/or are capable of binding to receptors that are expressed by and/or are endogenous to T cells. In particular embodiments, the one or more cytokines is or includes a member of the 4-alpha-helix bundle family of cytokines. In some embodiments, members of the 4-alpha-helix bundle family of cytokines include, but are not limited to, interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-7 (IL-7), interleukin-9 (IL-9), interleukin 12 (IL-12), interleukin 15 (IL-15), granulocyte colony-stimulating factor (G-CSF), and granulocyte-macrophage colony-stimulating factor (GM-CSF). In some embodiments, the one or more cytokines is or includes IL-15. In particular embodiments, the one or more cytokines is or includes IL-7. In particular embodiments, the one or more cytokines is or includes recombinant IL-2.

[0683] In particular embodiments, the cells are incubated in the presence of IL-2, IL-7, and/or IL-15. In certain embodiments, the IL-2, IL-7, and/or IL-15 are recombinant. In certain embodiments, the IL-2, IL-7, and/or IL-15 are human. In particular embodiments, the one or more cytokines are or include human recombinant IL-2, IL-7, and/or IL-15. In certain embodiments, the cells are incubated in the presence of recombinant IL-2, IL-7, and IL-15.

[0684] In some embodiments, the cells, *e.g.*, the transformed cells, are incubated with a cytokine, *e.g.*, a recombinant human cytokine, at a concentration of between 1 IU/mL and 1,000 IU/mL, between 10 IU/mL and 50 IU/mL, between 50 IU/mL and 100 IU/mL, between 100 IU/mL and 200 IU/mL, between 100 IU/mL and 500 IU/mL, between 250 IU/mL and 500 IU/mL, or between 500 IU/mL and 1,000 IU/mL.

[0685] In some embodiments, the cells, *e.g.*, the transformed cells, are incubated with IL-2, *e.g.*, human recombinant IL-2, at a concentration between 1 IU/mL and 500 IU/mL, between 10

IU/mL and 250 IU/mL, between 50 IU/mL and 200 IU/mL, between 50 IU/mL and 150 IU/mL, between 75 IU/mL and 125 IU/mL, between 100 IU/mL and 200 IU/mL, or between 10 IU/mL and 100 IU/mL. In particular embodiments, cells, *e.g.*, transformed cells, are incubated with recombinant IL-2 at a concentration at or at about 50 IU/mL, 60 IU/mL, 70 IU/mL, 80 IU/mL, 90 IU/mL, 100 IU/mL, 110 IU/mL, 120 IU/mL, 130 IU/mL, 140 IU/mL, 150 IU/mL, 160 IU/mL, 170 IU/mL, 180 IU/mL, 190 IU/mL, or 100 IU/mL. In some embodiments, the cells, *e.g.*, the transformed cells, are incubated in the presence of or of about 100 IU/mL of recombinant IL-2, *e.g.*, human recombinant IL-2.

[0686] In some embodiments, the cells, *e.g.*, the transformed cells, are incubated with recombinant IL-7, *e.g.*, human recombinant IL-7, at a concentration between 100 IU/mL and 2,000 IU/mL, between 500 IU/mL and 1,000 IU/mL, between 100 IU/mL and 500 IU/mL, between 500 IU/mL and 750 IU/mL, between 750 IU/mL and 1,000 IU/mL, or between 550 IU/mL and 650 IU/mL. In particular embodiments, the cells, *e.g.*, the transformed cells, are incubated with IL-7 at a concentration at or at about 50 IU/mL, 100 IU/mL, 150 IU/mL, 200 IU/mL, 250 IU/mL, 300 IU/mL, 350 IU/mL, 400 IU/mL, 450 IU/mL, 500 IU/mL, 550 IU/mL, 600 IU/mL, 650 IU/mL, 700 IU/mL, 750 IU/mL, 800 IU/mL, 750 IU/mL, 750 IU/mL, 750 IU/mL, or 1,000 IU/mL. In particular embodiments, the cells, *e.g.*, the transformed cells, are incubated in the presence of or of about 600 IU/mL of IL-7.

[0687] In some embodiments, the cells, *e.g.*, the transformed cells, are incubated with recombinant IL-15, *e.g.*, human recombinant IL-15, at a concentration between 1 IU/mL and 500 IU/mL, between 10 IU/mL and 250 IU/mL, between 50 IU/mL and 200 IU/mL, between 50 IU/mL and 150 IU/mL, between 75 IU/mL and 125 IU/mL, between 100 IU/mL and 200 IU/mL, or between 10 IU/mL and 100 IU/mL. In particular embodiments, cells, *e.g.*, transformed cells, are incubated with recombinant IL-15 at a concentration at or at about 50 IU/mL, 60 IU/mL, 70 IU/mL, 80 IU/mL, 90 IU/mL, 100 IU/mL, 110 IU/mL, 120 IU/mL, 130 IU/mL, 140 IU/mL, 150 IU/mL, 160 IU/mL, 170 IU/mL, 180 IU/mL, 190 IU/mL, or 200 IU/mL. In some embodiments, the cells, *e.g.*, the transformed cells, are incubated in the presence of or of about 100 IU/mL of recombinant IL-15, *e.g.*, human recombinant IL-15.

[0688] In particular embodiments, the cells, *e.g.*, transformed cells, are incubated in the presence of IL-2, IL-7, and/or IL-15. In some embodiments, the IL-2, IL-7, and/or IL-15 are recombinant. In certain embodiments, the IL-2, IL-7, and/or IL-15 are human. In particular embodiments, the one or more cytokines are or include human recombinant IL-2, IL-7, and/or

IL-15. In certain embodiments, the cells are incubated in the presence of recombinant IL-2, IL-7, and IL-15.

[0689] In some embodiments, all or a portion of the incubation, *e.g.*, of the non-expanded process, is performed in a media comprising a basal medium (*e.g.*, a CTS OpTmizer basal media (Thermofisher)), glutamine, and one or more recombinant cytokines, such as recombinant IL-2, IL-7, and/or IL-15. In some embodiments, the media can contain one or more additional components. In some embodiments, the one or more additional components may include a dipeptide form of L-glutamine (*e.g.*, L-alanyl-L-glutamine). In some embodiments, the one or more additional components are provided by an additional supplement, *e.g.*, OpTmizer® supplement (Thermofisher). In some embodiments, the media is a serum-free media and does not contain any serum component. In some aspects, the media can contain one or more serum-substituting proteins, such as as one or more of albumin, insulin or transferrin (*e.g.*, CTS™ Immune Cell Serum Replacement).

[0690] In some embodiments, the cells are incubated in the presence of the same or similar media as was present during the stimulation of the cells, such as carried out in connection with methods or processes of stimulation described above. In some embodiments, the cells are incubated in media having the same cytokines as the media present during stimulation of the cells, such as carried out in connection with methods or processes of stimulation described above. In certain embodiments, the cells are incubated in media having the same cytokines at the same concentrations as the media present during stimulation of the cells, such as carried out in connection with methods or processes of stimulation described above. In some embodiments, the cells are incubated in the absence of recombinant cytokines. In some embodiments, the cells are incubated in the absence of one or more cytokines as described herein. In some embodiments, the cells are incubated in the absence of all the cytokines described herein.

[0691] In some aspects, the further incubation is carried out under conditions to allow the cells to rest or recover that does not include the presence of a stimulating condition, *e.g.*, in the form of recombinant cytokines or other stimulating agents. For example, the incubating is carried out in the presence of a lean media sufficient to support or maintain the culture of health of the cells during the incubation.

[0692] In some embodiments, all or a portion of the incubation is performed in basal media, such as a basal media without one or more recombinant cytokines or without any recombinant cytokine. In some embodiments, the medium does not comprise one or more recombinant

cytokines, such as recombinant human IL-2, recombinant human IL-7, and/or recombinant human IL-15. In some aspects, the incubation is carried out without any recombinant cytokines. In certain embodiments, the basal media is supplemented with additional additives. In some embodiments, the basal media is not supplemented with any additional additives. Additives to cell culture media may include, but is not limited to nutrients, sugars, *e.g.*, glucose, amino acids, vitamins, or additives such as ATP and NADH. Other additives also can be added but in general the specific additives and amounts are such that the incubation of the media with the cells facilitates maintenance of the cells but minimizes, limits and/or does not induce the metabolic activity of the cells during the incubation.

[0693] In particular embodiments, the media is a basal media that does not contain one or more recombinant cytokines and that does not contain a serum component, *i.e.* is a serum-free media, but may contain one or more additional components. In particular embodiments, use of such a serum-free media in all or a portion of the incubation, *e.g.*, of the non-expanded process, provides for a lean media that provides for maintenance of the cells but does not include certain factors that may activate or render the cells metabolically active thereby fostering the cells in a state that is or is likely to be a resting or a quiescent state. In some aspects, incubation in the presence of such a serum-free media allows the cells to recover or rest after the stimulation and genetic engineering (*e.g.*, transduction). In some aspects, incubation in the presence of such a serum-free media results in an output composition containing cells that are less susceptible to damage or loss of viability, *e.g.*, during or following the manufacturing process and when the harvested/formulated cells are cryopreserved and then thawed immediately prior to use. In some embodiments, cells in the output composition when thawed have lower levels of caspase or other marker of apoptosis than cells that have been incubated in a similar media but containing one or more recombinant cytokines, serum, or other factors that may make the cells more metabolically active at cryopreservation of the output composition.

[0694] In some embodiments, the basal medium contains a mixture of inorganic salts, sugars, amino acids, and, optionally, vitamins, organic acids and/or buffers or other well known cell culture nutrients. In addition to nutrients, the medium also helps maintain pH and osmolality. In some aspects, the reagents of the basal media support cell growth, proliferation and/or expansion. A wide variety of commercially available basal media are well known to those skilled in the art, and include Dulb'ccos' Modified Eagles Medium (DMEM), Roswell Park Memorial Institute Medium (RPMI), Iscove modified Dulb'ccos' medium and Hams medium. In

some embodiments, the basal medium is Iscove's Modified Dulbecco's Medium, RPMI-1640, or α -MEM.

[0695] In some embodiments, the basal media is a balanced salt solution (*e.g.*, PBS, DPBS, HBSS, EBSS). In some embodiments, the basal media is selected from Dul'ecco's Modified 'agle's Medium (DMEM), Minimal Essential Medium (MEM), Basal Medium Eagle (BME), F-10, F-12, RPMI 1640, Gl'sgow's Minimal Essential Medium (GMEM), alpha Minimal Essential Medium (alpha MEM), I'cove's Modified Dul'ecco's Medium, and M199. In some embodiments, the basal media is a complex medium (*e.g.*, RPMI-1640, IMDM). In some embodiments, the basal medium is OpTmizer™ CTS™ T-Cell Expansion Basal Medium (ThermoFisher).

[0696] In some embodiments, the basal medium is free of a protein. In some embodiments, the basal medium is free of a human protein (*e.g.*, a human serum protein). In some embodiments, the basal medium is serum-free. In some embodiments, the basal medium is free of serum derived from human. In some embodiments, the basal medium is free of a recombinant protein. In some embodiments, the basal medium is free of a human protein and a recombinant protein. In some embodiments, the basal medium is free of one or more or all cytokines as described herein. In some embodiments, all or a portion of the incubation, *e.g.*, of the non-expanded process, is performed in sbasal medium without any additional additives or recombinant cytokines. In some embodiments, the basal media is a CTS OpTmizer basal media (Thermofisher) without any additional additives or recombinant cytokines.

[0697] In some embodiments, all or a portion of the incubation, *e.g.*, of the non-expanded process, is performed in a media comprising a basal medium and glutamine, *e.g.*, a CTS OpTmizer basal media (Thermofisher) with glutamine.

[0698] In some embodiments, all or a portion of the incubation, *e.g.*, of the non-expanded process, is performed in a media comprising a basal medium (*e.g.*, a CTS OpTmizer basal media (Thermofisher)) without one or more recombinant cytokines, such as recombinant human IL-2, recombinant human IL-7, and/or recombinant human IL-15. In some embodiments, the medium is supplemented with one or more additional non-serum component. In some embodiments, the one or more supplement is serum-free. In some embodiments, the serum-free medium further comprises a free form of an amino acid such as L-glutamine. In some embodiments, the serum-free medium does not comprise a serum replacement supplement. In some embodiments, the serum-free medium does not comprise a dipeptide form of L-glutamine (*e.g.*, L-alanyl-L-

glutamine). In some embodiments, the serum-free medium does not comprise any recombinant cytokine. In some embodiments, the serum-free medium comprises a basal medium supplemented with a T cell supplement and a free form of L-glutamine, and does not contain any immune cell serum replacement, any dipeptide form of L-glutamine, or any recombinant cytokine. In some embodiments, the serum-free medium comprises a basal medium (*e.g.*, OpTmizer™ T-Cell Expansion Basal Medium), L-glutamine and one or more additional components such as provided by a supplement (*e.g.*, OpTmizer™ T-Cell Expansion Supplement).

[0699] In particular embodiments, the cells are incubated in the serum free medium at a concentration of or of about 0.25×10^6 cells/mL, 0.5×10^6 cells/mL, 0.75×10^6 cells/mL, 1.0×10^6 cells/mL, 1.25×10^6 cells/mL, 1.5×10^6 cells/mL, 1.75×10^6 cells/mL, or 2.0×10^6 cells/mL. In particular embodiments, the cells are incubated in the serum free medium at a concentration of or of about 0.75×10^6 cells/mL. In some embodiments, the incubating is for or for about between 18 hours and 30 hours. In particular embodiments, the incubating is for or for about 24 hours or for for for about one day. In some embodiments, the incubating is for or for about 48 hours or 72 hours, or for or for about 2 days or 3 days, respectively. In particular embodiments, the incubating is for or for about 24 hours $\pm$ 6 hours, 48 hours $\pm$ 6 hours, or 72 hours $\pm$ 6 hours. In particular embodiments, the incubating is for or for about 72 hours, 72 ± 4 hours, or for or for about 3 days, *e.g.*, during which time the cells are incubated in the serum free medium at a concentration of or of about 0.75×10^6 cells/mL. In some embodiments, all or a portion of the incubation is performed in a serum free media comprising a basal medium (*e.g.*, a CTS OpTmizer basal media (Thermofisher)) without one or more recombinant cytokines, such as recombinant human IL-2, recombinant human IL-7, and/or recombinant human IL-15. In some embodiments, the serum-free media is supplemented with L-glutamine and/or one or more cell supplement, *e.g.*, OpTmizer™ T-Cell Expansion Supplement, but does not contain any immune cell serum replacement, any dipeptide form of L-glutamine, or any recombinant cytokine.

[0700] In particular embodiments, the cells are incubated in the absence of cytokines. In particular embodiments, the cells are incubated in the absence of any recombinant cytokine. In particular embodiments, the cells are incubated in the absence of one or more recombinant cytokine, such as recombinant IL-2, IL-7, and/or IL-15.

[0701] In some embodiments, the basal medium further comprises glutamine, such as L-glutamine. In some aspects, the glutamine is a free form of glutamine, such as L-glutamine. In

some embodiments, the concentration of the glutamine, such as L-glutamine, in the basal medium is about or less than about about 0.5mM-1mM, 0.5mM-1.5mM, 0.5mM-2mM, 0.5mM-2.5mM, 0.5mM-3mM, 0.5mM-3.5mM, 0.5mM-4mM, 0.5mM-4.5mM, 0.5mM-5mM, 1mM-1.5mM, 1mM-2mM, 1mM-2.5mM, 1mM-3mM, 1mM-3.5mM, 1mM-4mM, 1mM-4.5mM, 1mM-5mM, 1.5mM-2mM, 1.5mM-2.5mM, 1.5mM-3mM, 1.5mM-3.5mM, 1.5mM-4mM, 1.5mM-4.5mM, 1.5mM-5mM, 2mM-2.5mM, 2mM-3mM, 2mM-3.5mM, 2mM-4mM, 2mM-4.5mM, 2mM-5mM, 2.5mM-3mM, 2.5mM-3.5mM, 2.5mM-4mM, 2.5mM-4.5mM, 2.5mM-5mM, 3mM-3.5mM, 3mM-4mM, 3mM-4.5mM, 3mM-5mM, 3.5mM-4mM, 3.5mM-4.5mM, 3.5mM-5mM, 4mM-4.5mM, 4mM-5mM, or 4.5mM-5mM, each inclusive. In some embodiments, the concentration of glutamin, such as L-glutamine, in the basal medium is at least about 0.5mM, 1mM, 1.5mM, 2mM, 2.5mM, 3mM, 3.5mM, 4mM, 4.5mM, or 5mM. In some embodiments, the concentration of glutamine, such as L-glutamine, in the basal medium is at most about 2mM, 2.5mM, 3mM, 3.5mM, 4mM, 4.5mM, 5mM. In some embodiments, the concentration of glutamine, such as L-glutamine, in the basal medium is about 2 mM. In some embodiments, the basal medium further may comprises a protein or a peptide. In some embodiments, the at least one protein is not of non-mammalian origin. In some embodiments, the at least one protein is human or derived from human. In some embodiments, the at least one protein is recombinant. In some embodiments, the at least one protein includes albumin, transferrin, insulin, fibronectin, aprotinin or fetuin. In some embodiments, the protein comprises one or more of albumin, insulin or transferrin, optionally one or more of a human or recombinant albumin, insulin or transferrin.

[0702] In some embodiments, the protein is an albumin or albumin substitute. In some embodiments, the albumin is a human derived albumin. In some embodiments, the albumin is a recombinant albumin. In some embodiments, the albumin is a natural human serum albumin. In some embodiments, the albumin is a recombinant human serum albumin. In some embodiments, the albumin is a recombinant albumin from a non-human source. Albumin substitutes may be any protein or polypeptide source. Examples of such protein or polypeptide samples include but are not limited to bovine pituitary extract, plant hydrolysate (*e.g.*, rice hydrolysate), fetal calf albumin (fetuin), egg albumin, human serum albumin (HSA), or another animal-derived albumins, chick extract, bovine embryo extract, AlbuMAX® I, and AlbuMAX® II. In some embodiments, the protein or peptide comprises a transferrin. In some embodiments, the protein

or peptide comprises a fibronectin. In some embodiments, the protein or peptide comprises aprotinin. In some embodiments, the protein comprises fetuin.

[0703] In some embodiments, the one or more additional protein is part of a serum replacement supplement that is added to the basal medium. Examples of serum replacement supplements include, for example, Immune Cell Serum Replacement (ThermoFisher, #A2598101) or those described in Smith *et al.* *Clin Transl Immunology*. 2015 Jan; 4(1): e31.

[0704] In certain embodiments, the cells are incubated after the introducing of the polynucleotide encoding the heterologous or recombinant protein, *e.g.*, viral vector, for, for about, or for at least 18 hours, 24 hours, 30 hours, 36 hours, 40 hours, 48 hours, 54 hours, 60 hours, 72 hours, 84 hours, 96 hours, or more than 96 hours. In certain embodiments, the cells are incubated after the introducing of the polynucleotide encoding the heterologous or recombinant protein, *e.g.*, viral vector, for, for about, or for at least one day, 2 days, 3 days, 4 days, or more than 4 days. In some embodiments, the incubating is performed for an amount of time between 30 minutes and 2 hours, between 1 hour and 8 hours, between 6 hours and 12 hours, between 12 hours and 18 hours, between 16 hours and 24 hours, between 18 hours and 30 hours, between 24 hours and 48 hours, between 24 hours and 72 hours, between 42 hours and 54 hours, between 60 hours and 120 hours between 96 hours and 120 hours, between 90 hours and between 1 days and 7 days, between 3 days and 8 days, between 1 day and 3 days, between 4 days and 6 days, or between 4 days and 5 days prior to the genetic engineering. In some embodiments, the incubating is for or for about between 18 hours and 30 hours. In particular embodiments, the incubating is for or for about 24 hours or for for for about one day.

[0705] In certain embodiments, the total duration of the incubation is, is about, or is at least 12 hours, 18 hours, 24 hours, 30 hours, 36 hours, 42 hours, 48 hours, 54 hours, 60 hours, 72 hours, 84 hours, 96 hours, 108 hours, or 120 hours. In certain embodiments, the total duration of the incubation is, is about, or is at least one day, 2 days, 3 days, 4 days, or 5 days. In particular embodiments, the incubation is completed at, at about, or within 120 hours, 108 hours, 96 hours, 84 hours, 72 hours, 60 hours, 54 hours, 48 hours, 42 hours, 36 hours, 30 hours, 24 hours, 18 hours, or 12 hours. In particular embodiments, the incubation is completed at, at about, or within one day, 2 days, 3 days, 4 days, or 5 days. In some embodiments, the total duration of the incubation is between or between about 12 hour and 120 hours, 18 hour and 96 hours, 24 hours and 72 hours, or 24 hours and 48 hours, inclusive. In some embodiments, the total duration of the incubation is between or about between 1 hour and 48 hours, 4 hours and 36

hours, 8 hours and 30 hours or 12 hours and 24 hours, inclusive. In particular embodiments, the incubation is performed for or for about 24 hours, 48 hours, or 72 hours, or for or for about 1 day, 2 days, or 3 days, respectively. In particular embodiments, the incubation is performed for 24 hours $\pm$ 6 hours, 48 hours $\pm$ 6 hours, or 72 hours $\pm$ 6 hours. In particular embodiments, the incubation is performed for or for about 72 hours or for or for about 3 days.

[0706] In particular embodiments, the incubation is initiated at, at about, or is at least 12 hours, 18 hours, 24 hours, 30 hours, 36 hours, 42 hours, 48 hours after the initiation of the stimulation. In particular embodiments, the incubation is initiated at, at about, or is at least 0.5 days, one day, 1.5 days, or 2 days after the initiation of the stimulation. In particular embodiments, the incubation is initiated at, at about, or within 120 hours, 108 hours, 96 hours, 84 hours, 72 hours, 60 hours, 54 hours, 48 hours, 42 hours, 36 hours, 30 hours, 24 hours, 18 hours, or 12 hours of the initiation of the stimulation. In particular embodiments, the incubation is initiated at, at about, or within 5 days, 4 days, 3 days, 2 days, one day, or 0.5 days of the initiation of the stimulation.

[0707] In some embodiments, the incubation is completed between or between about 24 hour and 120 hours, 36 hour and 108 hours, 48 hours and 96 hours, or 48 hours and 72 hours, inclusive, after the initiation of the stimulation. In some embodiments, the incubation is completed at, about, or within 120 hours, 108 hours, 96 hours, 72 hours, 48 hours, or 36 hours from the initiation of the stimulation. In some embodiments, the incubation is completed at, about, or within 5 days, 4.5 days, 4 days, 3 days, 2 days, or 1.5 days from the initiation of the stimulation. In particular embodiments, the incubation is completed after hours 24 hours $\pm$ 6 hours, 48 hours $\pm$ 6 hours, or 72 hours $\pm$ 6 hours after the initiation of the stimulation. In some embodiments, the incubation is completed after or after about 72 hours or after or after about 3 days.

[0708] In some embodiments, the incubation is carried out for an amount of time sufficient for the heterologous or recombinant polynucleotide to be integrated into the genome. In particular embodiments, the incubation is performed for an amount of time sufficient for at least an integrated viral copy number (iVCN) of, of about, or of at least 0.1, 0.5, 1, 2, 3, 4, 5, or greater than 5 per diploid genome. In particular embodiments, the incubation is performed for an amount of time sufficient for at least an iVCN of, of about, or of at least 0.5 or 1. In particular embodiments, the incubation is carried out for an amount of time sufficient for the heterologous or recombinant polynucleotide to be stably integrated into the genome. In particular

embodiments, the heterologous or recombinant polynucleotide is considered to be stably integrated when the iVCN per diploid genome does not change by more than 20%, 15%, 10%, 5%, 1%, or 0.1% over a period of time, *e.g.*, at least 12, 24, or 48 hours. In particular embodiments, the incubation is completed prior to the stable integration.

[0709] In certain embodiments, the incubation is performed or carried out at least until the integrated vector is detected in the genome. In some embodiments, the incubation is completed prior to achieving stable integrated vector copy number (iVCN) per diploid genome. In particular embodiments, the incubation is performed or carried out at least until the integrated vector is detected in the genome but prior to achieving a stable iVCN per diploid genome. In certain embodiments, a stable iVCN per diploid genome is achieved when the iVCN peaks and/or remains unchanged, or unchanged within a tolerated error, for a period of time. In some embodiments, the tolerated error is, is within, or is about $\pm 40\%$, $\pm 35\%$, $\pm 30\%$, $\pm 25\%$, $\pm 20\%$, $\pm 15\%$, $\pm 10\%$, $\pm 5\%$, $\pm 2\%$, $\pm 1\%$, or less than $\pm 1\%$. In certain embodiments, the period of time is, is about, or is at least 2 hours, 4 hours, 6 hours, 8 hours, 12 hours, 16 hours, 18 hours, 24 hours, 36 hours, 48 hours, 60 hours, or 72 hours. In certain embodiments, the period of time is, is about, or is at least one day, 2 days, or 3 days. In certain embodiments, the stable iVCN per diploid genome is achieved when the iVCN peaks and remains unchanged, or unchanged within a tolerated error, *e.g.*, $\pm 25\%$, for a period of time that is, is about, or is at least 24 hours or one day. In some embodiments, a stable iVCN per diploid genome is achieved when the fraction of iVCN to total vector copy number (VCN) in the diploid genome of the population of transformed cells, on average, is, is at least or is about 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, or 1.5, or is within a tolerated error thereof, *e.g.*, $\pm 25\%$, $\pm 20\%$, $\pm 15\%$, $\pm 10\%$, $\pm 5\%$, or $\pm 1\%$. In certain embodiments, a stable iVCN per diploid genome is achieved when the fraction of iVCN to total vector copy number (VCN) in the diploid genome of the population of transformed cells, on average, is or is about 0.8, or is within a tolerated error thereof. In some embodiments, a stable iVCN per diploid genome is achieved when the fraction of iVCN to total vector copy number (VCN) in the diploid genome of the population of transformed cells, on average, is or is about 1.0 or is within a tolerated error thereof.

[0710] In some embodiments, the incubation is completed before the iVCN reaches, reaches about, or reaches at least 5.0, 4.0, 3.0, 2.5, 2.0, 1.75, 1.5, 1.25, 1.2, 1.1, 1.0, 0.9, 0.8, 0.75, 0.7, 0.6, 0.5, 0.4, 0.3, or 0.25 copies per diploid genome. In certain embodiments, the incubation is completed before the iVCN reaches or about 1.0 copy per diploid genome. In

particular embodiments, the incubation is completed before the iVCN reaches or about 0.5 copies per diploid genome.

[0711] In certain embodiments, the cells are harvested prior to, prior to about, or prior to at least one, two, three, four, five, six, eight, ten, twenty, or more cell doublings of the cell population, *e.g.*, doublings that occur during the incubating. In particular embodiments, the amount of cell doublings may be calculated by measuring the number of viable cells in a population at different time points, such as at different times or stages of an engineering process. In particular embodiment, the cell doubling can be calculated by comparing the total amount of viable cells at one time point to the total number of viable cells present at an earlier time point. In certain embodiments, the incubation is completed prior to, to about, or to at least one, two, three, four, five, six, eight, ten, twenty, or more cell doublings of the cell population, *e.g.*, doublings that occur during the incubating. In certain aspects, the cell doubling is calculated by determining the total nucleated cell number (TNC) when the incubation is initiated and when the incubation completed, and then determining the natural log of the product of the TNC at the completion divided by the TNC at the initiation, and then dividing said natural log of the product by the natural log of 2.

[0712] In some aspects, the number of doublings of that occurs in a population, *e.g.*, during an engineering process, is determined using the following formula:

$$1) \text{ Cell doublings} = \frac{\ln \left(\frac{\text{TNC at harvest}}{\text{TNC 3 days post - activation}} \right)}{\ln 2}$$

[0713] In some aspects, the number of doublings of that occurs in a population, *e.g.*, during an engineering process, using the following formula:

$$2) \text{ Cell doublings} = \frac{\ln \left(\frac{\text{TNC at harvest}}{\text{TNC at initiation of the stimulating}} \right)}{\ln 2}$$

[0714] In certain embodiments, the number of doublings that occurs in a population, *e.g.*, during the engineering process, is determined using the following formula:

$$3) \text{ Cell doublings} = \frac{\ln \left(\frac{\text{TNC at harvest}}{\text{TNC following stimulation}} \right)}{\ln 2}$$

[0715] In various embodiments, the number of doublings that occurs in a population, *e.g.*, during the engineering process, is determined using the following formula:

$$4) \text{ Cell doublings} = \frac{\ln \left(\frac{\text{TNC at harvest}}{\text{TNC at transduction}} \right)}{\ln 2}$$

[0716] In particular embodiments, the number of doublings that occurs in a population, *e.g.*, during the engineering process, is determined using the following formula:

$$5) \text{ Cell doublings} = \frac{\ln \left(\frac{\text{TNC at harvest}}{\text{TNC at the beginning of the incubation}} \right)}{\ln 2}$$

[0717] In certain embodiments, the incubation is completed before the total number cells, *e.g.*, total number of incubated cells or cells undergoing the incubation, is greater than or than about one, two, three, four, five, six, eight, ten, twenty, or more than twenty times the number of cells of the input population, *e.g.*, the total number of cells that were contacted with the stimulatory reagent. In various embodiments, the incubation is completed before the total number of incubated cells is greater than or than about one, two, three, four, five, six, eight, ten, twenty, or more than twenty times the total number of cells that were transformed, transduced, or spinoculated, *e.g.*, the total number of cells that were contacted with a viral vector. In certain embodiments, the cells are T cells, viable T cells, CD3+ T cells, CD4+ T cells, CD8+ T cells, CAR expressing T cells, or a combination of any of the foregoing. In some embodiments, the incubation is completed before the total number of cells is greater than the total number of cells of the input population. In some embodiments, the incubation is completed before the total

number of viable CD3+ T cells is greater than the total number of viable CD3+ cells of the input population. In certain embodiments, the incubation is completed before the total number of cells is greater than the total number of cells of the transformed, transduced, or spinoculated cells. In some embodiments, the incubation is completed before the total number of viable CD3+ T cells is greater than the total number of viable CD3+ of the transformed, transduced, or spinoculated cells.

[0718] In some embodiments, the total cell number or total viable cell number of the cell population remains similar, the same, or essentially the same during the incubation. In particular embodiments, the total cell number or total viable cell number of the cell population does not change during the incubation. In some aspects, the total cell number or total viable cell number decreases during the incubation. In particular aspects, the total viable cell number is, is about, or is less than 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, or 50% of the total cell number or total viable cell number of the input population prior to, *e.g.*, immediately prior to, or at the initiation of the stimulation.

5. Removal of Stimulatory Reagents

[0719] In some embodiments, the population of incubated T cells was produced or generated in accord with any of the methods provided herein in which a substance, such as a competition agent, was added to T cells to disrupt, such as to lessen and/or terminate, the signaling of the stimulatory agent or agents. In some embodiments, the population of the incubated T cells contains the presence of a substance, such as a competition agent, *e.g.*, biotin or a biotin analog, *e.g.*, D-Biotin. In some embodiments, the substance, such as a competition agent, *e.g.*, biotin or a biotin analog, *e.g.*, D-Biotin, is present in an amount that is at least 1.5-fold greater, at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 10-fold, at least 100-fold, at least 1000-fold or more greater than the amount of the substance in a reference population or preparation of cultured T cells in which the substance was not added exogenously during the incubation. In some embodiments, the amount of the substance, such as a competition agent, *e.g.*, biotin or a biotin analog, *e.g.*, D-Biotin, in the population of cultured T cells is from or from about 10 μ M to 100 μ M, 100 μ M to 1 mM, 100 μ M to 500 μ M or 10 μ M to 100 μ M. In some embodiments, 10 μ M or about 10 μ M of biotin or a biotin analog, *e.g.*, D-biotin, is added to the cells or the cell population to separate or remove the oligomeric stimulatory reagent from the cells or cell population.

[0720] In certain embodiments, the one or more agents (*e.g.*, agents that stimulate or activate a TCR and/or a co-receptor) associate with, such as are reversibly bound to, the oligomeric reagent, such as via the plurality of the particular binding sites (*e.g.*, binding sites Z) present on the oligomeric reagent. In some cases, this results in the agents being closely arranged to each other such that an avidity effect can take place if a target cell having (at least two copies of) a cell surface molecule that is bound by or recognized by the agent is brought into contact with the agent. In some aspects, the receptor binding reagent has a low affinity towards the receptor molecule of the cell at binding site B, such that the receptor binding reagent dissociates from the cell in the presence of the competition reagent. Thus, in some embodiments, the agents are removed from the cells in the presence of the competition reagent.

[0721] In some embodiments, the oligomeric stimulatory reagent is a streptavidin mutein oligomer with reversibly attached anti-CD3 and anti-CD28 Fabs. In some embodiments, the Fabs are attached contain streptavidin binding domains, *e.g.*, that allow for the reversible attachment to the streptavidin mutein oligomer. In some cases, anti-CD3 and anti-CD28 Fabs are closely arranged to each other such that an avidity effect can take place if a T cell expressing CD3 and/or CD28 is brought into contact with the oligomeric stimulatory reagent with the reversibly attached Fabs. In some aspects, the Fabs have a low affinity towards CD3 and CD28, such that the Fabs dissociate from the cell in the presence of the competition reagent, *e.g.*, biotin or a biotin variant or analogue. Thus, in some embodiments, the Fabs are removed or dissociated from the cells in the presence of the competition reagent, *e.g.*, D-biotin.

[0722] In some embodiments, the oligomeric stimulatory reagent, *e.g.*, the oligomeric stimulatory streptavidin mutein reagent, is removed or separated from the cells or cell populations prior to collecting, harvesting, or formulating the cells. In some embodiments, oligomeric stimulatory reagent, *e.g.*, the oligomeric stimulatory streptavidin mutein reagent, is removed or separated from the cells or cell populations by contact or exposure to a competition reagent, *e.g.*, biotin or a biotin analog such as D-biotin, after or during the incubation, *e.g.*, an incubation described herein such as in Section II-C-5. In certain embodiments, the cells or cell population are contacted or exposed to a competition reagent, *e.g.*, biotin or a biotin analog such as D-biotin, to remove oligomeric stimulatory reagent, *e.g.*, the oligomeric stimulatory streptavidin mutein reagent, after the incubation but prior to steps for collecting, harvesting, or formulating the cells. In particular embodiments, the cells or cell population are contacted or exposed to a competition reagent, *e.g.*, biotin or a biotin analog such as D-biotin, to remove the

oligomeric stimulatory reagent, *e.g.*, the oligomeric stimulatory streptavidin mutein reagent, after the incubation. In some aspects, when oligomeric stimulatory reagent, *e.g.*, the oligomeric stimulatory streptavidin mutein reagent, is separated or removed from the cells during the incubation, *e.g.*, by contact or exposure to a competition reagent, *e.g.*, biotin or a biotin analog such as D-biotin, the cells are returned to the same incubation conditions as prior to the separation or removal for the remaining duration of the incubation.

[0723] In some embodiments, the cells are contacted with, with about, or with at least 0.01 μ M, 0.05 μ M, 0.1 μ M, 0.5 μ M, 1 μ M, 2 μ M, 3 μ M, 4 μ M, 5 μ M, 10 μ M, 100 μ M, 500 μ M, 0.01 μ M, 1 mM, or 10 mM of the competition reagent to remove or separate the oligomeric stimulatory reagent from the cells. In various embodiments, the cells are contacted with, with about, or with at least 0.01 μ M, 0.05 μ M, 0.1 μ M, 0.5 μ M, 1 μ M, 2 μ M, 3 μ M, 4 μ M, 5 μ M, 10 μ M, 100 μ M, 500 μ M, 0.01 μ M, 1 mM, or 10 mM of biotin or a biotin analog such as D-biotin, to remove or separate the stimulatory streptavidin mutein oligomers with reversibly attached anti-CD3 and anti-CD28 Fabs from the cells. In various embodiments, the cells are contacted with between or between about 100 μ M and 10 mM, *e.g.*, 1 mM, of biotin or a biotin analog such as D-biotin, to remove or separate the oligomeric stimulatory reagent, such as streptavidin mutein oligomers with reversibly attached anti-CD3 and anti-CD28 Fabs from the cells. In various embodiments, the cells are contacted with between or between about 100 μ M and 10 mM, *e.g.*, 1 mM, of biotin or a biotin analog such as D-biotin for or for about 2 hours, 6 hours, 12 hours, 18 hours, 24 hours, 30 hours, 36 hours, 42 hours, or 48 hours post contact or exposure to D-biotin.

[0724] In particular embodiments, the oligomeric stimulatory reagent, *e.g.*, the oligomeric stimulatory streptavidin mutein reagent, is removed or separated from the cells within or within about 120 hours, 108 hours, 96 hours, 84 hours, 72 hours, 60 hours, 48 hours, 36 hours, 24 hours, or 12 hours, inclusive, of the initiation of the stimulation. In particular embodiments, the oligomeric stimulatory reagent, *e.g.*, the oligomeric stimulatory streptavidin mutein reagent, is removed or separated from the cells within or within about 5 days, 4 days, 3 days, 2 days, one day or 0.5 days, inclusive, of the initiation of the stimulation. In particular embodiments, the oligomeric stimulatory reagent, *e.g.*, the oligomeric stimulatory streptavidin mutein reagent, is removed or separated from the cells at or at about 48 hours or at or at about 2 days after the stimulation is initiated. In certain embodiments, the oligomeric stimulatory reagent, *e.g.*, the oligomeric stimulatory streptavidin mutein reagent, is removed or separated from the cells at or

at about 72 hours or at or at about 3 days after the stimulation is initiated. In some embodiments, the oligomeric stimulatory reagent, *e.g.*, the oligomeric stimulatory streptavidin mutein reagent is removed or separated from the cells at or at about 96 hours or at or at about 4 days after the stimulation is initiated.

[0725] In certain embodiments, the cells or cell population are contacted or exposed to a competition reagent, *e.g.*, biotin or a biotin analog such as D-biotin, to remove oligomeric stimulatory reagent, *e.g.*, the oligomeric stimulatory streptavidin mutein reagent, at or at about 48 hours or at or at about 2 days after the stimulation is initiated, *e.g.*, during or after the incubation described herein such as in Section II-C-4. In some aspects, when oligomeric stimulatory reagent, *e.g.*, the oligomeric stimulatory streptavidin mutein reagent, is separated or removed from the cells during the incubation, *e.g.*, by contact or exposure to a competition reagent, *e.g.*, biotin or a biotin analog such as D-biotin, the cells are returned to the same incubation conditions as prior to the separation or removal for the remaining duration of the incubation. In other aspects, when oligomeric stimulatory reagent, *e.g.*, the oligomeric stimulatory streptavidin mutein reagent, is separated or removed from the cells after the incubation, *e.g.*, by contact or exposure to a competition reagent, *e.g.*, biotin or a biotin analog such as D-biotin, the cells are further incubated for or for about 2 hours, 6 hours, 12 hours, 18 hours, 24 hours, 30 hours, 36 hours, 42 hours, or 48 hours post contact or exposure to the competition reagent. In some embodiments, the transduced cells with D-Biotin treatment are further incubated for or for about 48 hours post D-Biotin addition.

6. Harvesting, Collecting, or Formulating Cells

[0726] In some embodiments, the cells are harvested or collected. In particular embodiments, the cells are collected or harvested after the completion of the incubation. In certain embodiments, the collected or harvested cells are the cells of an output population. In some embodiments, the output population includes cells that are viable, CD3+, CD4+, CD8+, and/or positive for a recombinant receptor, *e.g.*, CAR+. In particular embodiments, the harvested CD4+ T cells and formulated CD8+ T cells are the output CD4+ and CD8+ T cells. In particular embodiments, a formulated cell population, *e.g.*, a formulated population of enriched CD4+ and CD8+ cells, is an output cell population, *e.g.*, an output population of enriched CD4+ and CD8+ cells.

[0727] In some embodiments, the cells or cell population that is harvested, collected, or formulated have not undergone any expansion, *e.g.*, any conditions where the cells were incubated or cultivated under conditions that increase the amount of viable cells during the incubation or cultivation. For example, in some aspects, the cells that are harvested have not undergone any incubation or cultivation where the amount of total viable cells is increased at the end of the incubation or cultivation as compared to the number of total viable cells at the beginning of the incubation or cultivation. In some embodiments, the cells that are harvested have not undergone any incubation or cultivation step explicitly for the purpose of increasing (*e.g.*, expanding) the total number of viable cells at the end of the incubation or cultivation process compared to the beginning of said incubation or cultivation process. In some embodiments, the cells are incubated under conditions that may result in expansion, but the incubating conditions are not carried out for purposes of expanding the cell population. In some embodiments, the cells that are harvested may have undergone expansion despite having been manufactured in a process that does not include an expansion step. In some embodiments, a manufacturing process that does not include an expansion step is referred to as a non-expanded or minimally expanded process. A “non-expanded” process may also be referred to as a “minimally expanded” process. In some embodiments, a non-expanded or minimally expanded process may result in cells having undergone expansion despite the process not including a step for expansion. In some embodiments, the cells that are harvested may have undergone an incubation or cultivating step that includes a media composition designed to reduce, suppress, minimize, or eliminate expansion of a cell population as a whole. In some embodiments, the collected, harvested, or formulated cells have not previously undergone an incubation or cultivation that was performed in a bioreactor, or under conditions where the cells were rocked, rotated, shaken, or perfused for all or a portion of the incubation or cultivation. Exemplary non-expanded processes of manufacturing and engineered cells produced by such processes are disclosed in PCT/US2019/046062, which is incorporated by reference in its entirety.

[0728] In some embodiments, a cell selection, isolation, separation, enrichment, and/or purification step is performed before the cells or cell population is harvested, collected, or formulated. In some embodiments, the cell selection, isolation, separation, enrichment, and/or purification step is carried out using chromatography as disclosed herein. In some embodiments, a T cell selection step by chromatography is performed after T cell transduction, but prior to harvesting, prior to collecting, and/or prior to formulating the cells. In some

embodiments, a T cell selection step by chromatography is performed immediately prior to harvesting the cells.

[0729] In certain embodiments, the amount of time from the initiation of the stimulation to collecting, harvesting, or formulating the cells is, is about, or is less than 36 hours, 42 hours, 48 hours, 54 hours, 60 hours, 72 hours, 84 hours, 96 hours, 108 hours, or 120 hours. In certain embodiments, the amount of time from the initiation of the stimulation to collecting, harvesting, or formulating the cells is, is about, or is less than 1.5 days, 2 days, 3 days, 4 days, or 5 days. In some embodiments, the amount of time from the initiation of the stimulation to collecting, harvesting, or formulating the cells for generating engineered cells, from the initiation of the stimulation to collecting, harvesting, or formulating the cells is between or between about 36 hours and 120 hours, 48 hours and 96 hours, or 48 hours and 72 hours, inclusive, or between or between about 1.5 days and 5 days, 2 days and 4 days, or 2 day and 3 days, inclusive,. In particular embodiments, the amount of time from the initiation of incubation to harvesting, collecting, or formulating the cells is, is about, or is less than 48 hours, 72 hours, or 96 hours. In particular embodiments, the amount of time from the initiation of incubation to harvesting, collecting, or formulating the cells is, is about, or is less than 2 days, 3 days, or 4 days. In particular embodiments, the amount of time from the initiation of incubation to harvesting, collecting, or formulating the cells is 48 hours $\pm$ 6 hours, 72 hours $\pm$ 6 hours, or 96 hours $\pm$ 6 hours. In particular embodiments, the amount of time from the initiation of incubation to harvesting, collecting, or formulating the cells is or is about 96 hours or four days.

[0730] In particular embodiments, the cells are harvested, collected, or formulated in a serum-free medium, such as one described herein or in PCT/US2018/064627, which is incorporated herein by reference. In some embodiments, the cells are harvested, collected, or formulated into the same serum-free medium as used during the incubation.

[0731] In particular embodiments, the cells are harvested, collected or formulated in a basal media that does not contain one or more recombinant cytokines and that does not contain a serum component, *i.e.* is a serum-free media, but may contain one or more additional components. In particular embodiments, use of such a serum-free media provides for a lean media that provides for maintenance of cells but does not include certain factors that may activate or render the cells metabolically active thereby fostering the cells in a state that is or is likely to be a resting or a quiescent state. In some aspects, incubation in the presence of such a serum-free media allows the cells to recover or rest after the stimulation and genetic engineering

(*e.g.*, transduction). In some aspects, harvesting, collecting or formulating cells in the presence of such a serum-free media results in a formulation of the output composition containing cells that are less susceptible to damage or loss of viability, *e.g.*, when the harvested/formulated cells are cryopreserved and then thawed immediately prior to use. In some embodiments, cells in the output composition when thawed have lower levels of caspase or other marker of apoptosis than cells that have been incubated in a similar media but containing one or more recombinant cytokines, serum, or other factors that may make the cells more metabolically active at cryopreservation of the output composition.

[0732] In certain embodiments, one or more populations of enriched T cells are formulated. In particular embodiments, one or more populations of enriched T cells are formulated after the one or more populations have been engineered and/or incubated. In particular embodiments, the one or more populations are input populations. In some embodiments, the one or more input populations have been previously cryoprotected and stored, and are thawed prior to the incubation.

[0733] In certain embodiments, the cells are harvested or collected at least when the integrated vector is detected in the genome. In some embodiments, the cells are harvested or collected prior to stable integrated vector copy number (iVCN) per diploid genome. In particular embodiments, the cells are harvested or collected after the integrated vector is detected in the genome but prior to when a stable iVCN per diploid genome is achieved.

[0734] In some embodiments, the cells are harvested or collected before the iVCN of reaches, reaches about, or reaches at least 5.0, 4.0, 3.0, 2.5, 2.0, 1.75, 1.5, 1.25, 1.2, 1.1, 1.0, 0.9, 0.8, 0.75, 0.7, 0.6, 0.5, 0.4, 0.3, or 0.25 copies per diploid genome. In particular embodiments, the cells are harvested or collected before the iVCN reaches or about 1.0 copy per diploid genome. In some embodiments, the cells are collected or harvested before the iVCN reaches or about 0.5 copies per diploid genome.

[0735] In certain embodiments, the cells are harvested or collected prior to, prior to about, or prior to at least one, two, three, four, five, six, eight, ten, twenty, or more cell doublings of the cell population, *e.g.*, doublings that occur during the incubating.

[0736] In particular embodiments, the cells are harvested or collected at a time before the total number cells, *e.g.*, total number of incubated cells or cells undergoing the incubation, is greater than or than about one, two, three, four, five, six, eight, ten, twenty, or more than twenty times the number of cells of the input population, *e.g.*, the total number of cells that were

contacted with the stimulatory reagent. In some embodiments, the cells are harvested or collected at a time before the total number of incubated cells is greater than or than about one, two, three, four, five, six, eight, ten, twenty, or more than twenty times the total number of cells that were transformed, transduced, or spinoculated, *e.g.*, the total number of cells that were contacted with a viral vector. In certain embodiments, the cells are T cells, viable T cells, CD3+ T cells, CD4+ T cells, CD8+ T cells, CAR expressing T cells, or a combination of any of the foregoing. In particular embodiments, the cells are harvested or collected at a time before the total number of cells is greater than the total number of cells of the input population. In various embodiments, the cells are harvested or collected at a time before the total number of viable CD3+ T cells is greater than the total number of viable CD3+ cells of the input population. In particular embodiments, the cells are harvested or collected at a time before the total number of cells is greater than the total number of cells of the transformed, transduced, or spinoculated cells. In various embodiments, the cells are harvested or collected at a time before the total number of viable CD3+ T cells is greater than the total number of viable CD3+ cells of the transformed, transduced, or spinoculated cells. In various embodiments, the cells are harvested or collected at a time before the total number of viable CD4+ cells and CD8+ cells is greater than the total number of viable CD4+ cells and CD8+ cells of the input population. In particular embodiments, the cells are harvested or collected at a time before the total number of cells is greater than the total number of cells of the transformed, transduced, or spinoculated cells. In various embodiments, the cells are harvested or collected at a time before the total number of viable CD4+ cells and CD8+ cells is greater than the total number of viable CD4+ cells and CD8+ cells of the transformed, transduced, or spinoculated cells.

[0737] In certain embodiments, the process comprises a step of filtering the cell composition during or after the harvesting or collecting, *e.g.*, using a filter (*e.g.*, a 40µm filter), for example, to remove large particulates. In certain embodiments, the filtering step is performed while the cells are being harvested or collected. For example, a filter may be in-line with between the cells being incubated after transduction and a harvesting/collection device such as the Sepax® or Sepax 2® cell processing systems. In certain embodiments, the cells are harvested or collected and then filtered before the filtered composition is optionally washed. In certain embodiments, the cells are harvested or collected, washed, and the washed cell composition is filtered.

[0738] In certain embodiments, the formulated cells are output cells. In some embodiments, a formulated population of enriched T cells is an output population of enriched T cells. In particular embodiments, the formulated CD4+ T cells and formulated CD8+ T cells are the output CD4+ and CD8+ T cells. In particular embodiments, a formulated cell population, *e.g.*, a formulated population of enriched CD4+ and CD8+ cells, is an output cell population, *e.g.*, an output population of enriched CD4+ and CD8+ cells.

[0739] In some embodiments, cells can be formulated into a container, such as a bag or vial. In some embodiments, the vial may be an infusion vial. In some embodiments, the vial is formulated with a single unit dose of the engineered cells, such as including the number of cells for administration in a given dose or fraction thereof.

[0740] In some embodiments, the cells are formulated in a pharmaceutically acceptable buffer, which may, in some aspects, include a pharmaceutically acceptable carrier or excipient. In some embodiments, the processing includes exchange of a medium into a medium or formulation buffer that is pharmaceutically acceptable or desired for administration to a subject. In some embodiments, the processing steps can involve washing the transduced and/or expanded cells to replace the cells in a pharmaceutically acceptable buffer that can include one or more optional pharmaceutically acceptable carriers or excipients. Exemplary of such pharmaceutical forms, including pharmaceutically acceptable carriers or excipients, can be any described below in conjunction with forms acceptable for administering the cells and compositions to a subject. The pharmaceutical composition in some embodiments contains the cells in amounts effective to treat or prevent the disease or condition, such as a therapeutically effective or prophylactically effective amount.

[0741] A “pharmaceutically acceptable carrier” refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[0742] In some aspects, the choice of carrier is determined in part by the particular cell and/or by the method of administration. Accordingly, there are a variety of suitable formulations. For example, the pharmaceutical composition can contain preservatives. Suitable preservatives may include, for example, methylparaben, propylparaben, sodium benzoate, and benzalkonium chloride. In some aspects, a mixture of two or more preservatives is used. The preservative or mixtures thereof are typically present in an amount of about 0.0001% to about 2% by weight of the total composition. Carriers are described, *e.g.*, by Remington’s

Pharmaceutical Science^{es} 16th edition, Osol, A. Ed. (1980). Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.*, Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG).

[0743] Buffering agents in some aspects are included in the compositions. Suitable buffering agents include, for example, citric acid, sodium citrate, phosphoric acid, potassium phosphate, and various other acids and salts. In some aspects, a mixture of two or more buffering agents is used. The buffering agent or mixtures thereof are typically present in an amount of about 0.001% to about 4% by weight of the total composition. Methods for preparing administrable pharmaceutical compositions are known. Exemplary methods are described in more detail in, for example, Remington: The Science and Practice of Pharmacy, Lippincott Williams & Wilkin^s; 21st ed. (May 1, 2005).

[0744] The formulations can include aqueous solutions. The formulation or composition may also contain more than one active ingredient useful for the particular indication, disease, or condition being treated with the cells, preferably those with activities complementary to the cells, where the respective activities do not adversely affect one another. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended. Thus, in some embodiments, the pharmaceutical composition further includes other pharmaceutically active agents or drugs, such as chemotherapeutic agents, *e.g.*, asparaginase, busulfan, carboplatin, cisplatin, daunorubicin, doxorubicin, fluorouracil, gemcitabine, hydroxyurea, methotrexate, paclitaxel, rituximab, vinblastine, and/or vincristine. In some embodiments, the agents or cells are administered in the form of a salt, *e.g.*, a pharmaceutically

acceptable salt. Suitable pharmaceutically acceptable acid addition salts include those derived from mineral acids, such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric, and sulphuric acids, and organic acids, such as tartaric, acetic, citric, malic, lactic, fumaric, benzoic, glycolic, gluconic, succinic, and arylsulphonic acids, for example, p-toluenesulphonic acid.

[0745] The pharmaceutical composition in some embodiments contains agents or cells in amounts effective to treat or prevent the disease or condition, such as a therapeutically effective or prophylactically effective amount. Therapeutic or prophylactic efficacy in some embodiments is monitored by periodic assessment of treated subjects. For repeated administrations over several days or longer, depending on the condition, the treatment is repeated until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful and can be determined. The desired dosage can be delivered by a single bolus administration of the composition, by multiple bolus administrations of the composition, or by continuous infusion administration of the composition.

[0746] The agents or cells can be administered by any suitable means, for example, by bolus infusion, by injection, *e.g.*, intravenous or subcutaneous injections, intraocular injection, periocular injection, subretinal injection, intravitreal injection, trans-septal injection, subcleral injection, intrachoroidal injection, intracameral injection, subconjunctival injection, altival injection, sub-Tenon's injection, retrobulbar injection, peribulbar injection, or posterior juxtasccleral delivery. In some embodiments, they are administered by parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. In some embodiments, a given dose is administered by a single bolus administration of the cells or agent. In some embodiments, it is administered by multiple bolus administrations of the cells or agent, for example, over a period of no more than 3 days, or by continuous infusion administration of the cells or agent.

[0747] For the prevention or treatment of disease, the appropriate dosage may depend on the type of disease to be treated, the type of agent or agents, the type of cells or recombinant receptors, the severity and course of the disease, whether the agent or cells are administered for preventive or therapeutic purposes, previous therapy, the subject's clinical history and response to the agent or the cells, and the discretion of the attending physician. The compositions are in some embodiments suitably administered to the subject at one time or over a series of treatments.

[0748] The cells or agents may be administered using standard administration techniques, formulations, and/or devices. Provided are formulations and devices, such as syringes and vials, for storage and administration of the compositions. With respect to cells, administration can be autologous or heterologous. For example, immunoresponsive cells or progenitors can be obtained from one subject, and administered to the same subject or a different, compatible subject. Peripheral blood derived immunoresponsive cells or their progeny (*e.g.*, in vivo, ex vivo or in vitro derived) can be administered via localized injection, including catheter administration, systemic injection, localized injection, intravenous injection, or parenteral administration. When administering a therapeutic composition (*e.g.*, a pharmaceutical composition containing a genetically modified immunoresponsive cell or an agent that treats or ameliorates symptoms of neurotoxicity), it will generally be formulated in a unit dosage injectable form (solution, suspension, emulsion).

[0749] Formulations include those for oral, intravenous, intraperitoneal, subcutaneous, pulmonary, transdermal, intramuscular, intranasal, buccal, sublingual, or suppository administration. In some embodiments, the agent or cell populations are administered parenterally. The term “parenteral,” as used herein, includes intravenous, intramuscular, subcutaneous, rectal, vaginal, and intraperitoneal administration. In some embodiments, the agent or cell populations are administered to a subject using peripheral systemic delivery by intravenous, intraperitoneal, or subcutaneous injection.

[0750] Compositions in some embodiments are provided as sterile liquid preparations, *e.g.*, isotonic aqueous solutions, suspensions, emulsions, dispersions, or viscous compositions, which may in some aspects be buffered to a selected pH. Liquid preparations are normally easier to prepare than gels, other viscous compositions, and solid compositions. Additionally, liquid compositions are somewhat more convenient to administer, especially by injection. Viscous compositions, on the other hand, can be formulated within the appropriate viscosity range to provide longer contact periods with specific tissues. Liquid or viscous compositions can comprise carriers, which can be a solvent or dispersing medium containing, for example, water, saline, phosphate buffered saline, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol) and suitable mixtures thereof.

[0751] Sterile injectable solutions can be prepared by incorporating the agent or cells in a solvent, such as in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, dextrose, or the like.

[0752] The formulations to be used for in vivo administration are generally sterile. Sterility may be readily accomplished, *e.g.*, by filtration through sterile filtration membranes.

[0753] In some embodiments, the dose of cells administered is in a cryopreserved composition. In some aspects, the composition is administered after thawing the cryopreserved composition. In some embodiments, the composition is administered within at or about 30 minutes, 45 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes or 180 minutes after thawing. In some embodiments, the composition is administered within at or about 120 minutes after thawing.

[0754] In some embodiments, the dose of cells is administered with a syringe. In some embodiments, the syringe has a volume of at or about 0.5, 1, 2, 2.5, 3, 4, 5, 7.5, 10, 20 or 25 mL, or a range defined by any of the foregoing.

[0755] Also provided are articles of manufacture and kits containing engineered cells expressing a recombinant receptor or compositions thereof, and optionally instructions for use, for example, instructions for administering, according to the provided methods. In some embodiments, the instructions specify the criteria for selection or identification of subjects for therapy in accord with any of the provided methods.

[0756] In some embodiments, provided are articles of manufacture and/or kits that include a composition comprising a therapeutically effective amount of any of the engineered cells described herein, and instructions for administering, to a subject for treating a disease or condition. In some embodiments, the instructions can specify some or all of the elements of the methods provided herein. In some embodiments, the instructions specify particular instructions for administration of the cells for cell therapy, *e.g.*, doses, timing, selection and/or identification of subjects for administration and conditions for administration. In some embodiments, the articles of manufacture and/or kits further include one or more additional agents for therapy, *e.g.*, lymphodepleting therapy and/or combination therapy, such as any described herein and optionally further includes instructions for administering the additional agent for therapy. In some embodiments, the articles of manufacture and/or kits further comprise an agent for lymphodepleting therapy, and optionally further includes instructions for administering the lymphodepleting therapy. In some embodiments, the instructions are included as a label or package insert accompanying the compositions for administration.

[0757] Various additives which enhance the stability and sterility of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added.

Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, and sorbic acid. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0758] In some embodiments, the formulation buffer contains a cryopreservative. In some embodiments, the cells are formulated with a cryopreservative solution that contains 1.0% to 30% DMSO solution, such as a 5% to 20% DMSO solution or a 5% to 10% DMSO solution. In some embodiments, the cryopreservation solution is or contains, for example, PBS containing 20% DMSO and 8% human serum albumin (HSA), or other suitable cell freezing media. In some embodiments, the cryopreservative solution is or contains, for example, at least or about 7.5% DMSO. In some embodiments, the processing steps can involve washing the transduced and/or expanded cells to replace the cells in a cryopreservative solution. In some embodiments, the cells are frozen, *e.g.*, cryoprotected or cryopreserved, in media and/or solution with a final concentration of or of about 12.5%, 12.0%, 11.5%, 11.0%, 10.5%, 10.0%, 9.5%, 9.0%, 8.5%, 8.0%, 7.5%, 7.0%, 6.5%, 6.0%, 5.5%, or 5.0% DMSO, or between 1% and 15%, between 6% and 12%, between 5% and 10%, or between 6% and 8% DMSO. In particular embodiments, the cells are frozen, *e.g.*, cryoprotected or cryopreserved, in media and/or solution with a final concentration of or of about 5.0%, 4.5%, 4.0%, 3.5%, 3.0%, 2.5%, 2.0%, 1.5%, 1.25%, , 1.2%, 1.0%, 0.75%, 0.5%, or 0.25% HSA, or between 0.1% and -5%, between 0.25% and 4%, between 0.5% and 2%, or between 1% and 2% HSA.

[0759] In particular embodiments, the composition of enriched T cells, *e.g.*, T cells that have been stimulated, engineered, and/or incubated, are formulated, cryoprotected, and then stored for an amount of time. In certain embodiments, the formulated, cryoprotected cells are stored until the cells are released for infusion. In particular embodiments, the formulated cryoprotected cells are stored for between 1 day and 6 months, between 1 month and 3 months, between 1 day and 14 days, between 1 day and 7 days, between 3 days and 6 days, between 6 months and 12 months, or longer than 12 months. In some embodiments, the cells are cryoprotected and stored for, for about, or for less than 1 days, 2 days, 3 days, 4 days, 5 days, 6 days, or 7 days. In certain embodiments, the cells are thawed and administered to a subject after the storage. In certain embodiments, the cells are stored for or for about 5 days. In some embodiments, the formulated cells are not cryopreserved.

[0760] In some embodiments, the formulation is carried out using one or more processing step including washing, diluting or concentrating the cells. In some embodiments, the processing can include dilution or concentration of the cells to a desired concentration or number, such as unit dose form compositions including the number of cells for administration in a given dose or fraction thereof. In some embodiments, the processing steps can include a volume-reduction to thereby increase the concentration of cells as desired. In some embodiments, the processing steps can include a volume-addition to thereby decrease the concentration of cells as desired. In some embodiments, the processing includes adding a volume of a formulation buffer to transduced and/or incubated cells. In some embodiments, the volume of formulation buffer is from or from about 10 mL to 1000 mL, such as at least or about at least or about or 50 mL, 100 mL, 200 mL, 300 mL, 400 mL, 500 mL, 600 mL, 700 mL, 800 mL, 900 mL or 1000 mL.

[0761] In some embodiments, such processing steps for formulating a cell composition are carried out in a closed system. Exemplary of such processing steps can be performed using a centrifugal chamber in conjunction with one or more systems or kits associated with a cell processing system, such as a centrifugal chamber produced and sold by Biosafe SA, including those for use with the Sepax® or Sepax 2® cell processing systems. An exemplary system and process is described in International Publication Number WO2016/073602. In some embodiments, the method includes effecting expression from the internal cavity of the centrifugal chamber a formulated composition, which is the resulting composition of cells formulated in a formulation buffer, such as pharmaceutically acceptable buffer, in any of the above embodiments as described. In some embodiments, the expression of the formulated composition is to a container, such as a bag that is operably linked as part of a closed system with the centrifugal chamber. In some embodiments, the container, such as bag, is connected to a system at an output line or output position.

[0762] In some embodiments, the closed system, such as associated with a centrifugal chamber or cell processing system, includes a multi-port output kit containing a multi-way tubing manifold associated at each end of a tubing line with a port to which one or a plurality of containers can be connected for expression of the formulated composition. In some aspects, a desired number or plurality of output containers, *e.g.*, bags, can be sterilely connected to one or more, generally two or more, such as at least 3, 4, 5, 6, 7, 8 or more of the ports of the multi-port output. For example, in some embodiments, one or more containers, *e.g.*, bags can be attached

to the ports, or to fewer than all of the ports. Thus, in some embodiments, the system can affect expression of the output composition into a plurality of output bags.

[0763] In some aspects, cells can be expressed to the one or more of the plurality of output bags in an amount for dosage administration, such as for a single unit dosage administration or multiple dosage administration. For example, in some embodiments, the output bags may each contain the number of cells for administration in a given dose or fraction thereof. Thus, each bag, in some aspects, may contain a single unit dose for administration or may contain a fraction of a desired dose such that more than one of the plurality of output bags, such as two of the output bags, or 3 of the output bags, together constitute a dose for administration.

[0764] Thus, the containers, *e.g.*, output bags, generally contain the cells to be administered, *e.g.*, one or more unit doses thereof. The unit dose may be an amount or number of the cells to be administered to the subject or twice the number (or more) of the cells to be administered. It may be the lowest dose or lowest possible dose of the cells that would be administered to the subject.

[0765] In some embodiments, each of the containers, *e.g.*, bags, individually comprises a unit dose of the cells. Thus in some embodiments, each of the containers comprises the same or approximately or substantially the same number of cells. In some embodiments, each unit dose contains at least or about at least 1×10^6 , 2×10^6 , 5×10^6 , 1×10^7 , 5×10^7 , or 1×10^8 engineered cells, total cells, T cells, or PBMCs. In some embodiments, the volume of the formulated cell composition in each bag is 10 mL to 100 mL, such as at least or about at least 20 mL, 30 mL, 40 mL, 50 mL, 60 mL, 70 mL, 80 mL, 90 mL or 100 mL.

[0766] In some embodiments, such cells produced by the method, or a composition comprising such cells, are administered to a subject for treating a disease or condition.

III. COMPOSITIONS AND FORMULATIONS

[0767] The provided methods and uses involve use or administration of a dose of engineered cells of a composition comprising engineered T cells expressing a chimeric antigen receptor (CAR), *e.g.*, an anti-CD19 CAR such as a CAR targeting human CD19. In certain embodiments, the composition is a therapeutic composition enriched in T cells, *e.g.*, composition enriched in CD3+ T cells or composition enriched in CD4+ and CD8+ T cells, manufactured using a process for generating or producing output engineered cells and/or output compositions comprising engineered T cells disclosed herein, *e.g.*, in Section II-C. In some

embodiments, the engineered T cells are provided as a composition, formulation, or dose, such as a pharmaceutical composition, formulation, or dose. Such compositions, formulations, or doses can be used in accord with the provided methods or uses, and/or with the provided articles of manufacture or compositions, such as in the prevention or treatment of diseases, conditions, and disorders, or in detection, diagnostic, and prognostic methods.

[0768] In particular embodiments, the composition comprising engineered T cells expressing an anti-CD19 CAR is enriched in CD3+ T cells. In some embodiments, at least or about 50%, at least or about 60%, at least or about 65%, at least or about 70%, at least or about 75%, at least or about 80%, at least or about 85%, at least or about 90%, at least or about 95%, at least or about 96%, at least or about 98%, at least or about 98.5%, at least or about 99%, at least or about 99.5%, at least or about 99.9%, 100%, or about 100% of the total cells, the total viable cells, the total live cell, the total T cells, the total viable T cells, the total live T cell, the total live CD45+ cells, or CAR-expressing cells thereof in the composition, are CD3+, *e.g.*, CD3+ T cells or CAR+CD3+ T cells. In some embodiments, between at or about 75% and at or about 80%, between at or about 80% and at or about 85%, between at or about 85% and at or about 90%, between at or about 90% and at or about 95%, between at or about 95% and at or about 99% of the total cells, the total viable cells, the total live cell, the total T cells, the total viable T cells, the total live T cell, the total live CD45+ cells, or CAR-expressing cells thereof in the composition, are CD3+, *e.g.*, CD3+ T cells or CAR+CD3+ T cells. In some embodiments, at or about 80%, at or about 81%, at or about 82%, at or about 83%, at or about 84%, at or about 85%, at or about 86%, at or about 87%, at or about 88%, at or about 89%, at or about 90%, at or about 91%, at or about 92%, at or about 93%, at or about 94%, at or about 95%, at or about 96%, at or about 97%, at or about 98%, at or about 99% of the total live CD45+ cells, or CAR-expressing cells thereof in the composition, are CD3+, *e.g.*, CD3+ T cells or CAR+CD3+ T cells. In some embodiments, between about 80% and about 100%, between about 85% and about 99%, between about 88% and about 98%, between about 96% and about 99%, or between about 97% and about 99% of the total live CD45+ cells, or CAR-expressing cells thereof in the composition, are CD3+, *e.g.*, CD3+ T cells or CAR+CD3+ T cells. In some embodiments, the composition consists of or consists essentially of CD3+ T cells. In some embodiments, at least or about 80% of the total cells in the composition are CD3+ T cells and at least or about 30%, at least or about 40%, at least or about 50%, at least or about 60%, at least or about 70%, at least or about 80%, at least or about 90%, or at least or about 95% of the total cells in the composition

express the anti-CD19 CAR. In some embodiments, at least or about 80%, at least or about 85%, at least or about 90%, at least or about 95%, at least or about 96%, at least or about 97%, at least or about 98%, or at least or about 99% of the total live CD45+ cells in the composition are CD3+ and at least or about 40% or at least or about 50% of the total cells in the composition express the anti-CD19 CAR.

[0769] In some embodiments, less than or less than about 2.5%, less than or less than about 2%, less than or less than about 1.5%, less than or less than about 1%, less than or less than about 0.5%, less than or less than about 0.4%, less than or less than about 0.3%, less than or less than about 0.2%, less than or less than about 0.1%, less than or less than about 0.05%, or at or about 0% of the total cells, the total viable cells, the total live cell, the total T cells, the total viable T cells, the total live T cell, the total live CD45+ cells, or CAR-expressing cells thereof in the composition, are positive for expression of an NK cell marker. In some embodiments, between at or about 2.5% and at or about 2%, between at or about 2% and at or about 1.5%, between at or about 1.5% and at or about 1%, between at or about 1% and at or about 0.5%, between at or about 0.5% and at or about 0.4%, between at or about 0.4% and at or about 0.3%, between at or about 0.3% and at or about 0.2%, between at or about 0.2% and at or about 0.1%, between at or about 0.1% and at or about 0.05%, less than at or about 0.05%, or at or about 0% of the total cells, the total viable cells, the total live cell, the total T cells, the total viable T cells, the total live T cell, the total live CD45+ cells, or CAR-expressing cells thereof in the composition, are NK cells. In some embodiments, between at or about 1.5% and at or about 0%, or between at or about 0.5% and at or about 0%, of the total live CD45+ cells in the composition are NK cells. In some embodiments, the composition is free of or essentially free of NK cells or cells positive for expression of an NK cell marker.

[0770] In some embodiments, less than or less than about 0.2%, less than or less than about 0.15%, less than or less than about 0.1%, less than or less than about 0.05%, less than or less than about 0.01%, or at or about 0% of the total cells, the total viable cells, the total live cell, the total T cells, the total viable T cells, the total live T cell, the total live CD45+ cells, or CAR-expressing cells thereof in the composition, are CD19+. In some embodiments, between at or about 0.2% and at or about 0.15%, between at or about 0.15% and at or about 0.1%, between at or about 0.1% and at or about 0.05%, between at or about 0.05% and at or about 0.01%, less than at or about 0.01%, or at or about 0% of the total cells, the total viable cells, the total live cell, the total T cells, the total viable T cells, the total live T cell, the total live CD45+ cells, or

CAR-expressing cells thereof in the composition, are CD19+. In some embodiments, between at or about 0.1% and at or about 0%, or between at or about 0.05% and at or about 0%, of the total live CD45+ cells in the composition are CD19+. In some embodiments, the composition is free of or essentially free of CD19+ cells.

[0771] In some embodiments, at least or about 80% of the total live CD45+ cells in the composition are CD3+, at least or about 40% of the total cells in the composition express the anti-CD19 CAR, less than about 1.5% of the total live CD45+ cells in the composition are NK cells or cells positive for expression of an NK cell marker, and less than about 0.1% of the total live CD45+ cells in the composition are CD19+. In some embodiments, at least or about 96% of the total live CD45+ cells in the composition are CD3+, at least or about 50% of the total cells in the composition express the anti-CD19 CAR, less than about 0.5% of the total live CD45+ cells in the composition are NK cells or cells positive for expression of an NK cell marker, and less than about 0.05% of the total live CD45+ cells in the composition are CD19+.

[0772] In particular embodiments, the composition comprising engineered T cells expressing an anti-CD19 CAR is enriched in CD4+ and CD8+ T cells. In some embodiments, at least or about 50%, at least or about 60%, at least or about 65%, at least or about 70%, at least or about 75%, at least or about 80%, at least or about 85%, at least or about 90%, at least or about 95%, at least or about 96%, at least or about 98%, at least or about 98.5%, at least or about 99%, at least or about 99.5%, at least or about 99.9%, 100%, or about 100% of the total cells, the total viable cells, the total live cell, the total T cells, the total viable T cells, the total live T cell, the total live CD45+ cells, or CAR-expressing cells thereof in the composition, are CD4+ or CD8+. In some embodiments, between at or about 75% and at or about 80%, between at or about 80% and at or about 85%, between at or about 85% and at or about 90%, between at or about 90% and at or about 95%, between at or about 95% and at or about 99% of the total cells, the total viable cells, the total live cell, the total T cells, the total viable T cells, the total live T cell, the total live CD45+ cells, or CAR-expressing cells thereof in the composition, are CD4+ or CD8+. In some embodiments, at or about 80%, at or about 81%, at or about 82%, at or about 83%, at or about 84%, at or about 85%, at or about 86%, at or about 87%, at or about 88%, at or about 89%, at or about 90%, at or about 91%, at or about 92%, at or about 93%, at or about 94%, at or about 95%, at or about 96%, at or about 97%, at or about 98%, at or about 99% of the total live CD45+ cells, or CAR-expressing cells thereof in the composition, are CD4+ or CD8+. In some embodiments, between about 80% and about 100%, between about 85% and about 99%,

between about 88% and about 98%, between about 96% and about 99%, or between about 97% and about 99% of the total live CD45+ cells, or CAR-expressing cells thereof in the composition, are CD4+ or CD8+. In some embodiments, the composition consists of or consists essentially of CD4+ T cells and CD8+ T cells. In some embodiments, at least or about 80% of the total cells in the composition are CD4+ T cells and CD8+ T cells and at least or about 30%, at least or about 40%, at least or about 50%, at least or about 60%, at least or about 70%, at least or about 80%, at least or about 90%, or at least or about 95% of the total cells in the composition express the anti-CD19 CAR. In some embodiments, at least or about 80%, at least or about 85%, at least or about 90%, at least or about 95%, at least or about 96%, at least or about 97%, at least or about 98%, or at least or about 99% of the total live CD45+ cells in the composition are CD4+ T cells and CD8+ T cells and at least or about 40% or at least or about 50% of the total cells in the composition express the anti-CD19 CAR.

[0773] In particular embodiments, CD3+CD4+ cells account for at least or about 50%, at least or about 60%, at least or about 65%, at least or about 70%, at least or about 75%, at least or about 80%, at least or about 85%, at least or about 90%, at least or about 95%, at least or about 96%, at least or about 98%, at least or about 98.5%, at least or about 99%, at least or about 99.5%, at least or about 99.9%, 100%, or about 100% of the total cells, the total viable cells, the total live cell, the total T cells, the total viable T cells, the total live T cell, the total live CD45+ cells, or CAR-expressing cells thereof in the composition. In particular embodiments, CD3+CD4+ cells account for between at or about 50% and at or about 70%, between at or about 50% and at or about 55%, between at or about 55% and at or about 60%, between at or about 60% and at or about 65%, or between at or about 65% and at or about 70% of the total live CD45+ cells in the composition.

[0774] In particular embodiments, CD3+CD8+ cells account for at least or about 30%, at least or about 35%, at least or about 40%, at least or about 45%, at least or about 50%, at least or about 55%, at least or about 60%, at least or about 65%, at least or about 70%, at least or about 75%, at least or about 80%, at least or about 85%, at least or about 90%, at least or about 95%, at least or about 96%, at least or about 98%, at least or about 98.5%, at least or about 99%, at least or about 99.5%, at least or about 99.9%, 100%, or about 100% of the total cells, the total viable cells, the total live cell, the total T cells, the total viable T cells, the total live T cell, the total live CD45+ cells, or CAR-expressing cells thereof in the composition. In particular embodiments, CD3+CD8+ cells account for between at or about 30% and at or about 50%,

between at or about 30% and at or about 35%, between at or about 35% and at or about 40%, between at or about 40% and at or about 45%, or between at or about 45% and at or about 50% of the total live CD45+ cells in the composition.

[0775] In particular embodiments, CD3+CD4+ cells account for between about 55% and about 65% of the total live CD45+ cells in the composition, while CD3+CD8+ cells account for between about 35% and about 45% of the total live CD45+ cells in the composition. In particular embodiments, CD3+CD4+ cells account for about 60% while CD3+CD8+ cells account for about 40% of the total live CD45+ cells in the composition.

[0776] In particular embodiments, CAR+CD3+ cells (*e.g.*, CD3+ cells expressing the anti-CD19 CAR) account for at least or about 20%, at least or about 30%, at least or about 40%, at least or about 50%, at least or about 60%, at least or about 65%, at least or about 70%, at least or about 75%, at least or about 80%, at least or about 85%, at least or about 90%, at least or about 95%, at least or about 96%, at least or about 98%, at least or about 98.5%, at least or about 99%, at least or about 99.5%, at least or about 99.9%, 100%, or about 100% of the total cells, the total viable cells, the total live cell, the total T cells, the total viable T cells, the total live T cell, the total live CD45+ cells, or CAR-expressing cells thereof in the composition. In particular embodiments, CAR+CD3+ cells (*e.g.*, CD3+ cells expressing the anti-CD19 CAR) account for between at or about 40% and at or about 100%, between at or about 40% and at or about 45%, between at or about 45% and at or about 50%, between at or about 50% and at or about 55%, between at or about 55% and at or about 60%, between at or about 60% and at or about 65%, between at or about 65% and at or about 70%, between at or about 70% and at or about 75%, between at or about 75% and at or about 80%, between at or about 80% and at or about 85%, between at or about 85% and at or about 90%, between at or about 90% and at or about 95%, or between at or about 95% and at or about 99% of the total live CD45+ cells in the composition.

[0777] In particular embodiments, CAR+CD4+ cells (*e.g.*, CD4+ cells expressing the anti-CD19 CAR) account for at least or about 20%, at least or about 30%, at least or about 40%, at least or about 50%, at least or about 60%, at least or about 65%, at least or about 70%, at least or about 75%, at least or about 80%, at least or about 85%, at least or about 90%, at least or about 95%, at least or about 96%, at least or about 98%, at least or about 98.5%, at least or about 99%, at least or about 99.5%, at least or about 99.9%, 100%, or about 100% of the total cells, the total viable cells, the total live cell, the total T cells, the total viable T cells, the total live T cell, the total live CD45+ cells, or CAR-expressing cells thereof in the composition. In particular

embodiments, CAR+CD4+ cells (*e.g.*, CD4+ cells expressing the anti-CD19 CAR) account for between at or about 20% and at or about 60%, between at or about 20% and at or about 25%, between at or about 25% and at or about 30%, between at or about 30% and at or about 35%, between at or about 35% and at or about 40%, between at or about 40% and at or about 45%, between at or about 45% and at or about 50%, between at or about 50% and at or about 55%, or between at or about 55% and at or about 60% of the total live CD45+ cells in the composition.

[0778] In particular embodiments, CAR+CD8+ cells (*e.g.*, CD8+ cells expressing the anti-CD19 CAR) account for at least or about 10%, at least or about 20%, at least or about 30%, at least or about 35%, at least or about 40%, at least or about 45%, at least or about 50%, at least or about 55%, at least or about 60%, at least or about 65%, at least or about 70%, at least or about 75%, at least or about 80%, at least or about 85%, at least or about 90%, at least or about 95%, at least or about 96%, at least or about 98%, at least or about 98.5%, at least or about 99%, at least or about 99.5%, at least or about 99.9%, 100%, or about 100% of the total cells, the total viable cells, the total live cell, the total T cells, the total viable T cells, the total live T cell, the total live CD45+ cells, or CAR-expressing cells thereof in the composition. In particular embodiments, CAR+CD8+ cells (*e.g.*, CD8+ cells expressing the anti-CD19 CAR) account for between at or about 5% and at or about 35%, between at or about 5% and at or about 10%, between at or about 10% and at or about 15%, between at or about 15% and at or about 20%, between at or about 20% and at or about 25%, between at or about 25% and at or about 30%, between at or about 30% and at or about 35% of the total live CD45+ cells in the composition.

[0779] In particular embodiments, CAR+CD3+ cells (*e.g.*, CD3+ cells expressing the anti-CD19 CAR) account for between about 35% and about 65% of the total live CD45+ cells in the composition. In particular embodiments, CAR+CD4+ cells (*e.g.*, CD4+ cells expressing the anti-CD19 CAR) account for between about 25% and about 55% of the total live CD45+ cells in the composition, while CAR+CD8+ cells (*e.g.*, CD8+ cells expressing the anti-CD19 CAR) account for between about 10% and about 30% of the total live CD45+ cells in the composition. In particular embodiments, CD3+ cells expressing the anti-CD19 CAR account for about 50% of the total live CD45+ cells in the composition. In particular embodiments, CAR+CD4+ cells account for about 30% while CAR+CD8+ cells account for about 20% of the total live CD45+ cells in the composition. In particular embodiments, CD3+ cells expressing the anti-CD19 CAR account for about 60% of the total live CD45+ cells in the composition. In particular

embodiments, CAR+CD4+ cells account for about 40% while CAR+CD8+ cells account for about 20% of the total live CD45+ cells in the composition.

[0780] In particular embodiments, the composition contains a ratio of between 3:1 and 1:3, between 2.5:1 and 1:2.5, between 2:1 and 1:2, between 1.5:1 and 1:1.5, between 1.4:1 and 1:1.4, between 1.3:1 and 1:1.3, between 1.2:1 and 1:1.2, or between 1.1:1 and 1:1.1 CD4+ T cells to CD8+ T cells. In some embodiments, the composition of cells has a ratio of or of about 3:1, of or of about 2.8:1, of or of about 2.5:1, of or of about 2.25:1, of or of about 2:1, of or of about 1.8:1, of or of about 1.7:1, of or of about 1.6:1, of or of about 1.5:1, of or of about 1.4:1, of or of about 1.3:1, of or of about 1.2:1, of or of about 1.1:1, of or of about 1:1, of or of about 1:1.1, of or of about 1:1.2, of or of about 1:1.3, of or of about 1:1.4, of or of about 1:1.5, of or of about 1:1.6, of or of about 1:1.7, of or of about 1:1.8, of or of about 1:2, of or of about 1:2.25, of or of about 1:2.5, of or of about 1:2.8, or of or of about 1:3 CD4+ T cells to CD8+ T cells.

[0781] In some embodiments, the composition contains a ratio of between 3:1 and 1:3, between 2.5:1 and 1:2.5, between 2:1 and 1:2, between 1.5:1 and 1:1.5, between 1.4:1 and 1:1.4, between 1.3:1 and 1:1.3, between 1.2:1 and 1:1.2, or between 1.1:1 and 1:1.1 CD4+ T cells that express the recombinant receptor, *e.g.*, the anti-CD19 CAR, to CD8+ T cells that express the recombinant receptor, *e.g.*, the anti-CD19 CAR. In some embodiments, the ratio of CD4+ T cells that express the recombinant receptor (*e.g.*, the anti-CD19 CAR) to CD8+ T cells that express the recombinant receptor (*e.g.*, the anti-CD19 CAR) in the composition is of or of about 3:1, of or of about 2.8:1, of or of about 2.5:1, of or of about 2.25:1, of or of about 2:1, of or of about 1.8:1, of or of about 1.7:1, of or of about 1.6:1, of or of about 1.5:1, of or of about 1.4:1, of or of about 1.3:1, of or of about 1.2:1, of or of about 1.1:1, of or of about 1:1, of or of about 1:1.1, of or of about 1:1.2, of or of about 1:1.3, of or of about 1:1.4, of or of about 1:1.5, of or of about 1:1.6, of or of about 1:1.7, of or of about 1:1.8, of or of about 1:2, of or of about 1:2.25, of or of about 1:2.5, of or of about 1:2.8, or of or of about 1:3.

[0782] In particular embodiments, the composition contains a ratio of between about 2.5:1 and about 1:2 or between about 2:1 and about 1:1 CD4+ T cells to CD8+ T cells. In some embodiments, the composition of cells has a ratio of or of about 1.5:1 CD4+ T cells to CD8+ T cells. In particular embodiments, the composition contains a ratio of between about 3:1 and about 1:1 CAR+CD4+ cells to CAR+CD8+ cells. In particular embodiments, the composition contains a ratio of between about 2.5:1 and about 1.5:1 CAR+CD4+ cells to CAR+CD8+ cells. In some embodiments, the composition of cells has a ratio of or of about 2:1 CAR+CD4+ cells

to CAR+CD8+ cells. In some embodiments, the composition of cells has a ratio of or of about 1.5:1 CD4+ T cells to CD8+ T cells, and a ratio of or of about 2:1 CAR+CD4+ cells to CAR+CD8+ cells.

[0783] In particular embodiments, the composition contains at least at or about 50%, at least at or about 60%, at least at or about 70%, at least at or about 75%, at least at or about 80%, at least at or about 85%, at least at or about 90%, at least at or about 95%, at least at or about 99%, or at least at or about 99.9% viable cells. In some embodiments, the composition contains at least at or about 75% viable cells. In certain embodiments, the composition contains at least at or about 85%, at least at or about 90%, or at least at or about 95% viable cells. In some embodiments, at least 70% of the T cells in the composition are viable T cells. In some embodiments, at least 75% of the T cells in the composition are viable T cells. In some embodiments, at least 80% of the T cells in the composition are viable T cells. In some embodiments, at least 85% of the T cells in the composition are viable T cells. In some embodiments, at least 90% of the T cells in the composition are viable T cells. In some embodiments, the T cells are characterized as CD3+. In some embodiments, the composition contains at least at or about 50%, at least at or about 60%, at least at or about 70%, at least at or about 75%, at least at or about 80%, at least at or about 85%, at least at or about 90%, at least at or about 95%, at least at or about 99%, or at least at or about 99.9% viable CD3+ T cells. In particular embodiments, the composition contains at least at or about 75% viable CD3+ T cells. In certain embodiments, the composition contains at least at or about 85%, at least at or about 90%, or at least at or about 95% viable CD3+ T cells. In some embodiments, the composition contains at least at or about 50%, at least at or about 60%, at least at or about 70%, at least at or about 75%, at least at or about 80%, at least at or about 85%, at least at or about 90%, at least at or about 95%, at least at or about 99%, or at least at or about 99.9% viable CD4+ T cells. In certain embodiments, the composition contains at least at or about 75% viable CD4+ T cells. In particular embodiments, the composition contains at least at or about 85%, at least at or about 90%, or at least at or about 95% viable CD4+ T cells. In particular embodiments, the composition contains at least at or about 50%, at least at or about 60%, at least at or about 70%, at least at or about 75%, at least at or about 80%, at least at or about 85%, at least at or about 90%, at least at or about 95%, at least at or about 99%, or at least at or about 99.9% viable CD8+ T cells. In some embodiments, the composition contains at least at or about 75% viable CD8+ T

cells. In certain embodiments, the composition contains at least at or about 85%, at least at or about 90%, or at least at or about 95% viable CD8+ T cells.

[0784] In some of any embodiments, viability is determined by staining for acridine orange (AO) and propidium iodide (PI).

[0785] In particular embodiments, the composition has a low portion and/or frequency of cells that are undergoing and/or are prepared, primed, and/or entering apoptosis. In particular embodiments, the composition has a low portion and/or frequency of cells that are positive for an apoptotic marker. In some embodiments, less than at or about 40%, less than at or about 35%, less than at or about 30%, less than at or about 25%, less than at or about 20%, less than at or about 15%, less than at or about 10%, less than at or about 5%, or less than at or about 1% of the cells of the composition express, contain, and/or are positive for an apoptotic marker. In certain embodiments, less than at or about 25% of the cells of the composition express, contain, and/or are positive for a marker of apoptosis. In certain embodiments, less than at or about less than at or about 10% cells of the composition express, contain, and/or are positive for an apoptotic marker.

[0786] In particular embodiments, at least at or about 50%, at least at or about 60%, at least at or about 70%, at least at or about 75%, at least at or about 80%, at least at or about 85%, at least at or about 90%, at least at or about 95%, at least at or about 99%, or at least at or about 99.9% of anti-CD19 CAR-expressing cells of the composition are viable cells, *e.g.*, cells negative for an apoptotic marker, such as a caspase (*e.g.*, an activated caspase-3). In certain embodiments, at least at or about 85%, at least at or about 90%, or at least at or about 95% of anti-CD19 CAR-expressing cells of the composition are negative for an apoptotic marker, such as a caspase (*e.g.*, an activated caspase-3). In some embodiments, at least at or about 50%, at least at or about 60%, at least at or about 70%, at least at or about 75%, at least at or about 80%, at least at or about 85%, at least at or about 90%, at least at or about 95%, at least at or about 99%, or at least at or about 99.9% of CD3+ T cells of the composition are viable cells, *e.g.*, cells negative for an apoptotic marker, such as a caspase (*e.g.*, an activated caspase-3). In certain embodiments, at least at or about 85%, at least at or about 90%, or at least at or about 95% of CD3+ T cells of the composition are negative for an apoptotic marker, such as a caspase (*e.g.*, an activated caspase-3). In particular embodiments, at least at or about 90% of CD3+ T cells of the composition are viable cells, *e.g.*, cells negative for an apoptotic marker, such as a caspase (*e.g.*, an activated caspase-3). In some embodiments, at least at or about 50%, at least at or about

60%, at least at or about 70%, at least at or about 75%, at least at or about 80%, at least at or about 85%, at least at or about 90%, at least at or about 95%, at least at or about 99%, or at least at or about 99.9% of CAR+CD3+ T cells of the composition are viable cells, *e.g.*, cells negative for an apoptotic marker, such as a caspase (*e.g.*, an activated caspase-3). In particular embodiments, at least at or about 85%, at least at or about 90%, or at least at or about 95% of the anti-CD19 CAR-expressing CD3+ T cells of the composition are viable cells, *e.g.*, cells negative for an apoptotic marker, such as a caspase (*e.g.*, an activated caspase-3).

[0787] In some embodiments, less than or less than about 30%, less than or less than about 25%, less than or less than about 20%, less than or less than about 15%, less than or less than about 10%, or less than or less than about 5% of the total cells, the total T cells, the total CD45+ cells, the total CD3+ cells, the total CD4+ and CD8+ cells, or CAR-expressing cells thereof in the composition, express a marker of apoptosis, optionally Annexin V or active Caspase 3. In some embodiments, between at or about 30% and at or about 25%, between at or about 25% and at or about 20%, between at or about 20% and at or about 15%, between at or about 15% and at or about 10%, between at or about 10% and at or about 5% of the total cells, the total T cells, the total CD45+ cells, the total CD3+ cells, the total CD4+ and CD8+ cells, or CAR-expressing cells thereof in the composition, express a marker of apoptosis, optionally Annexin V or active Caspase 3. In some embodiments, at or about 6%, at or about 8%, at or about 10%, at or about 12%, at or about 14%, at or about 16%, at or about 18%, at or about 20%, at or about 22%, at or about 24%, at or about 26%, at or about 28%, at or about 30% of the CD3+ cells in the composition, express a marker of apoptosis, optionally Annexin V or active Caspase 3.

[0788] In some embodiments, expressing the anti-CD19 CAR recombinant receptor protein may include, but is not limited to, having the CAR recombinant receptor protein localized at the cell membrane and/or cell surface, having a detectable amount of the CAR recombinant receptor protein, having a detectable amount of mRNA encoding the CAR recombinant receptor, having or containing a recombinant polynucleotide that encodes the CAR recombinant receptor, and/or having or containing an mRNA or protein that is a surrogate marker for the CAR recombinant receptor expression.

[0789] In some embodiments, at least or about 5%, at least or about 10%, at least or about 20%, at least or about 30%, at least or about 40%, at least or about 45%, at least or about 50%, at least or about 55%, at least or about 60%, at least or about 65%, at least or about 70%, at least or about 75%, at least or about 80%, at least or about 85%, at least or about 90%, at least or

about 95%, at least or about 97%, at least or about 99%, or more than 99% of the cells of the composition express the recombinant receptor, *e.g.*, the anti-CD19 CAR. In certain embodiments, at least or about 50% of the cells of the composition express the anti-CD19 CAR. In certain embodiments, at least or about 5%, at least or about 10%, at least or about 20%, at least or about 30%, at least or about 40%, at least or about 45%, at least or about 50%, at least or about 55%, at least or about 60%, at least or about 65%, at least or about 70%, at least or about 75%, at least or about 80%, at least or about 85%, at least or about 90%, at least or about 95%, at least or about 97%, at least or about 99%, or more than 99% of the CD3+ T cells of the composition express the anti-CD19 CAR. In some embodiments, at least or about 50% of the CD3+ T cells of the composition express the anti-CD19 CAR. In certain embodiments, at least or about 5%, at least or about 10%, at least or about 20%, at least or about 30%, at least or about 40%, at least or about 45%, at least or about 50%, at least or about 55%, at least or about 60%, at least or about 65%, at least or about 70%, at least or about 75%, at least or about 80%, at least or about 85%, at least or about 90%, at least or about 95%, at least or about 97%, at least or about 99%, or more than 99% of the cells of the composition are CD3+ T cells that express the anti-CD19 CAR. In some embodiments, at least or about 50% of the cells of the composition are CD3+ T cells that express the anti-CD19 CAR.

[0790] In some embodiments, the composition includes at least or at least about 0.2×10^6 CD3+CAR+ cells/mL, 0.3×10^6 CD3+CAR+ cells/mL, 0.4×10^6 CD3+CAR+ cells/mL, 0.5×10^6 CD3+CAR+ cells/mL, 0.6×10^6 CD3+CAR+ cells/mL, 0.7×10^6 CD3+CAR+ cells/mL, 0.8×10^6 CD3+CAR+ cells/mL, 0.9×10^6 CD3+CAR+ cells/mL, 1×10^6 CD3+CAR+ cells/mL, 1.1×10^6 CD3+CAR+ cells/mL, 1.2×10^6 CD3+CAR+ cells/mL, 1.3×10^6 CD3+CAR+ cells/mL, 1.4×10^6 CD3+CAR+ cells/mL, 1.5×10^6 CD3+CAR+ cells/mL, 1.6×10^6 CD3+CAR+ cells/mL, 1.7×10^6 CD3+CAR+ cells/mL, 1.8×10^6 CD3+CAR+ cells/mL, 1.9×10^6 CD3+CAR+ cells/mL, 2×10^6 CD3+CAR+ cells/mL, 2.1×10^6 CD3+CAR+ cells/mL, 2.2×10^6 CD3+CAR+ cells/mL, 2.3×10^6 CD3+CAR+ cells/mL, 2.4×10^6 CD3+CAR+ cells/mL, 2.5×10^6 CD3+CAR+ cells/mL, 2.6×10^6 CD3+CAR+ cells/mL, 2.7×10^6 CD3+CAR+ cells/mL, 2.8×10^6 CD3+CAR+ cells/mL, 2.9×10^6 CD3+CAR+ cells/mL, 3×10^6 CD3+CAR+ cells/mL, 3.1×10^6 CD3+CAR+ cells/mL, 3.2×10^6 CD3+CAR+ cells/mL, 3.3×10^6 CD3+CAR+ cells/mL, 3.4×10^6 CD3+CAR+ cells/mL, 3.5×10^6 CD3+CAR+ cells/mL, 3.6×10^6 CD3+CAR+ cells/mL, 3.7×10^6 CD3+CAR+ cells/mL, 3.8×10^6 CD3+CAR+ cells/mL, 3.9×10^6 CD3+CAR+ cells/mL, 4×10^6 CD3+CAR+ cells/mL, 4.1×10^6 CD3+CAR+ cells/mL, 4.2×10^6

CD3+CAR+ cells/mL, 4.3×10^6 CD3+CAR+ cells/mL, 4.4×10^6 CD3+CAR+ cells/mL, 4.5×10^6 CD3+CAR+ cells/mL, 4.6×10^6 CD3+CAR+ cells/mL, 4.7×10^6 CD3+CAR+ cells/mL, 4.8×10^6 CD3+CAR+ cells/mL, 4.9×10^6 CD3+CAR+ cells/mL, 5×10^6 CD3+CAR+ cells/mL, 5.1×10^6 CD3+CAR+ cells/mL, 5.2×10^6 CD3+CAR+ cells/mL, 5.3×10^6 CD3+CAR+ cells/mL, 5.4×10^6 CD3+CAR+ cells/mL, 5.5×10^6 CD3+CAR+ cells/mL, 5.6×10^6 CD3+CAR+ cells/mL, 5.7×10^6 CD3+CAR+ cells/mL, 5.8×10^6 CD3+CAR+ cells/mL, 5.9×10^6 CD3+CAR+ cells/mL, or 6×10^6 CD3+CAR+ cells/mL, each inclusive. In some embodiments, the composition includes at least or at least about 0.2×10^6 viable CD3+CAR+ cells/mL, 0.3×10^6 viable CD3+CAR+ cells/mL, 0.4×10^6 viable CD3+CAR+ cells/mL, 0.5×10^6 viable CD3+CAR+ cells/mL, 0.6×10^6 viable CD3+CAR+ cells/mL, 0.7×10^6 viable CD3+CAR+ cells/mL, 0.8×10^6 viable CD3+CAR+ cells/mL, 0.9×10^6 viable CD3+CAR+ cells/mL, 1×10^6 viable CD3+CAR+ cells/mL, 1.1×10^6 viable CD3+CAR+ cells/mL, 1.2×10^6 viable CD3+CAR+ cells/mL, 1.3×10^6 viable CD3+CAR+ cells/mL, 1.4×10^6 viable CD3+CAR+ cells/mL, 1.5×10^6 viable CD3+CAR+ cells/mL, 1.6×10^6 viable CD3+CAR+ cells/mL, 1.7×10^6 viable CD3+CAR+ cells/mL, 1.8×10^6 viable CD3+CAR+ cells/mL, 1.9×10^6 viable CD3+CAR+ cells/mL, 2×10^6 viable CD3+CAR+ cells/mL, 2.1×10^6 viable CD3+CAR+ cells/mL, 2.2×10^6 viable CD3+CAR+ cells/mL, 2.3×10^6 viable CD3+CAR+ cells/mL, 2.4×10^6 viable CD3+CAR+ cells/mL, 2.5×10^6 viable CD3+CAR+ cells/mL, 2.6×10^6 viable CD3+CAR+ cells/mL, 2.7×10^6 viable CD3+CAR+ cells/mL, 2.8×10^6 viable CD3+CAR+ cells/mL, 2.9×10^6 viable CD3+CAR+ cells/mL, 3×10^6 viable CD3+CAR+ cells/mL, 3.1×10^6 viable CD3+CAR+ cells/mL, 3.2×10^6 viable CD3+CAR+ cells/mL, 3.3×10^6 viable CD3+CAR+ cells/mL, 3.4×10^6 viable CD3+CAR+ cells/mL, 3.5×10^6 viable CD3+CAR+ cells/mL, 3.6×10^6 viable CD3+CAR+ cells/mL, 3.7×10^6 viable CD3+CAR+ cells/mL, 3.8×10^6 viable CD3+CAR+ cells/mL, 3.9×10^6 viable CD3+CAR+ cells/mL, 4×10^6 viable CD3+CAR+ cells/mL, 4.1×10^6 viable CD3+CAR+ cells/mL, 4.2×10^6 viable CD3+CAR+ cells/mL, 4.3×10^6 viable CD3+CAR+ cells/mL, 4.4×10^6 viable CD3+CAR+ cells/mL, 4.5×10^6 viable CD3+CAR+ cells/mL, 4.6×10^6 viable CD3+CAR+ cells/mL, 4.7×10^6 viable CD3+CAR+ cells/mL, 4.8×10^6 viable CD3+CAR+ cells/mL, 4.9×10^6 viable CD3+CAR+ cells/mL, 5×10^6 viable CD3+CAR+ cells/mL, 5.1×10^6 viable CD3+CAR+ cells/mL, 5.2×10^6 viable CD3+CAR+ cells/mL, 5.3×10^6 viable CD3+CAR+ cells/mL, 5.4×10^6 viable CD3+CAR+ cells/mL, 5.5×10^6 viable CD3+CAR+ cells/mL, 5.6×10^6 viable CD3+CAR+

cells/mL, 5.7×10^6 viable CD3+CAR+ cells/mL, 5.8×10^6 viable CD3+CAR+ cells/mL, 5.9×10^6 viable CD3+CAR+ cells/mL, or 6×10^6 viable CD3+CAR+ cells/mL, each inclusive.

[0791] In particular embodiments, at least or about 30%, at least or about 40%, at least or about 45%, at least or about 50%, at least or about 55%, at least or about 60%, at least or about 65%, at least or about 70%, at least or about 75%, at least or about 80%, at least or about 85%, at least or about 90%, at least or about 95%, at least or about 97%, at least or about 99%, or more than 99% of the CD4+ T cells of the composition express the recombinant receptor, *e.g.*, the anti-CD19 CAR. In particular embodiments, at least or about 50% of the CD4+ T cells of the composition express the recombinant receptor, *e.g.*, the anti-CD19 CAR. In some embodiments, at least or about 30%, at least or about 40%, at least or about 45%, at least or about 50%, at least or about 55%, at least or about 60%, at least or about 65%, at least or about 70%, at least or about 75%, at least or about 80%, at least or about 85%, at least or about 90%, at least or about 95%, at least or about 97%, at least or about 99%, or more than 99% of the CD8+ T cells of the composition express the recombinant receptor, *e.g.*, the anti-CD19 CAR. In certain embodiments, at least or about 50% of the CD8+ T cells of the composition express the recombinant receptor, *e.g.*, the anti-CD19 CAR.

[0792] In some embodiments, at least or about 5%, at least or about 10%, at least or about 20%, at least or about 30%, at least or about 40%, at least or about 45%, at least or about 50%, at least or about 55%, at least or about 60%, at least or about 65%, at least or about 70%, at least or about 75%, at least or about 80%, at least or about 85%, at least or about 90%, at least or about 95%, at least or about 97%, at least or about 99%, or more than 99% of live CD45+ cells in the composition are CD3+CAR+ (*e.g.*, CD3+ T cells that express the anti-CD19 CAR), CD4+CAR+ (*e.g.*, CD4+ T cells that express the anti-CD19 CAR), and/or CD8+CAR+ (*e.g.*, CD8+ T cells that express the anti-CD19 CAR). In certain embodiments, at least or about 50% of live CD45+ cells in the composition are CD3+ T cells that express the anti-CD19 CAR. In certain embodiments, between at or about 60% and at or about 65% of live CD45+ cells in the composition are CD3+ T cells that express the anti-CD19 CAR. In certain embodiments, between at or about 35% and at or about 45%, between at or about 35% and at or about 40%, or between at or about 40% and at or about 45% of live CD45+ cells in the composition are CD4+ T cells that express the anti-CD19 CAR. In certain embodiments, between at or about 15% and at or about 25%, between at or about 15% and at or about 20%, or between at or about 20% and at or about 25% of live CD45+ cells in the composition are CD8+ T cells that express the anti-

CD19 CAR. In certain embodiments, of the live CD45+ cells in the composition, at least or about 60% are CD3+ T cells that express the anti-CD19 CAR, at least or about 40% are CD4+ T cells that express the anti-CD19 CAR, and at least or about 20% are CD8+ T cells that express the anti-CD19 CAR.

[0793] In any of the proceeding embodiments, the composition can comprise about or at least about 10×10^6 , about or at least about 20×10^6 , about or at least about 25×10^6 , about or at least about 50×10^6 , about or at least about 100×10^6 , about or at least about 200×10^6 , about or at least about 400×10^6 , about or at least about 600×10^6 , about or at least about 800×10^6 , about or at least about 1000×10^6 , about or at least about 1200×10^6 , about or at least about 1400×10^6 , about or at least about 1600×10^6 , about or at least about 1800×10^6 , about or at least about 2000×10^6 , about or at least about 2500×10^6 , about or at least about 3000×10^6 , or about or at least about 4000×10^6 total cells, *e.g.*, total viable cells, in one or more containers such as vials. In any of the proceeding embodiments, the volume of the composition can be 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. In some embodiments, the composition is contained in a plurality of containers, such as 2, 3, 4, 5, 6, 7, 8, 9, 10 or more vials. In any of the proceeding embodiments, the composition can comprise about or at least about 5×10^6 , about or at least about 10×10^6 , about or at least about 20×10^6 , about or at least about 25×10^6 , about or at least about 50×10^6 , about or at least about 100×10^6 , about or at least about 150×10^6 , about or at least about 200×10^6 , about or at least about 250×10^6 , about or at least about 300×10^6 , about or at least about 350×10^6 , about or at least about 400×10^6 , about or at least about 450×10^6 , about or at least about 500×10^6 , about or at least about 550×10^6 , or about or at least about 600×10^6 total cells, *e.g.*, total viable cells, per unit container such as per vial. In some embodiments, cells of the composition in the one or more containers are at a density of, of about, or at least 5×10^6 cells/mL, 10×10^6 cells/mL, 20×10^6 cells/mL, 30×10^6 cells/mL, 40×10^6 cells/mL, 50×10^6 cells/mL, 60×10^6 cells/mL, 70×10^6 cells/mL, 80×10^6 cells/mL, 90×10^6 cells/mL, 100×10^6 cells/mL, 110×10^6 cells/mL, 120×10^6 cells/mL, 130×10^6 cells/mL, 140×10^6 cells/mL, or 150×10^6 cells/mL in a solution or buffer, *e.g.*, in a cryopreservation solution or buffer. In some embodiments, about or up to about 900×10^6 cells (*e.g.*, viable CD4+ T cells and viable CD8+ T cells, or viable CD3+ T cells) are subjected to stimulation, where about or up to about 600×10^6 cells (*e.g.*, viable CD4+ T cells and viable

CD8+ T cells, or viable CD3+ T cells) of the stimulated composition are subjected to genetic engineering such as with a viral vector, *e.g.*, by transduction, or a non-viral method of genetic engineering followed by incubation in a serum-free basal media (*e.g.*, supplemented with one or more supplements) without any recombinant cytokine for about 72 hours or about three days. In some embodiments, the output composition produced comprises between about 100×10^6 and about 1400×10^6 total cells, *e.g.*, total viable cells, in one or more containers such as vials.

[0794] In certain embodiments, the cells of the composition have a high portion and/or frequency of naïve-like and/or central memory cells. In particular embodiments, a majority of the cells of the composition are naïve or naïve-like cells, central memory cells, and/or effector memory cells. In particular embodiments, a majority of the cells of the composition are naïve-like or central memory cells. In some embodiments, a majority of the cells of the composition are central memory cells. In some aspects, less differentiated cells, *e.g.*, central memory cells, are longer lived and exhaust less rapidly, thereby increasing persistence and durability. In some aspects, a responder to a cell therapy, such as a CAR-T cell therapy, has increased expression of central memory genes. *See, e.g.*, Fraietta et al. (2018) Nat Med. 24(5):563-571. In certain embodiments, the cells of the composition have a high portion and/or frequency of T cells in an early stage of differentiation, or T cells that are surface positive for a marker expressed on T cells in an early stage of differentiation. In certain embodiments, the cells of the compositions have a greater portion and/or frequency of T cells in an early stage of differentiation than compositions generated from alternative processes, such as processes that involve expansion (*e.g.*, processes that include an expansion unit operation and/or include steps intended to cause expansion of cells). In certain embodiments, T cells in an early stage of differentiation may be characterized by positive or high expression (*e.g.*, surface expression or intracellular expression) of certain cell markers and/or negative or low expression (*e.g.*, surface expression or intracellular expression) of other cell markers. In some aspects, T cells in an early stage of differentiation are characterized by positive or high expression of CCR7 and/or CD27. In certain embodiments, T cells in an early stage of differentiation or the T cells that are surface positive for a marker expressed on T cells in an early stage of differentiation are CCR7+CD27+.

[0795] In certain embodiments, the cells of the composition have a high portion and/or frequency of naïve-like T cells or T cells that are surface positive for a marker expressed on naïve-like T cells. In certain embodiments, the cells of the compositions have a greater portion and/or frequency of naïve-like cells than compositions generated from alternative processes,

such as processes that involve expansion (*e.g.*, processes that include an expansion unit operation and/or include steps intended to cause expansion of cells). In certain embodiments, naïve-like T cells may include cells in various differentiation states and may be characterized by positive or high expression (*e.g.*, surface expression or intracellular expression) of certain cell markers and/or negative or low expression (*e.g.*, surface expression or intracellular expression) of other cell markers. In some aspects, naïve-like T cells are characterized by positive or high expression of CCR7, CD45RA, CD28, and/or CD27. In some aspects, naïve-like T cells are characterized by negative expression of CD25, CD45RO, CD56, CD62L, and/or KLRG1. In some aspects, naïve-like T cells are characterized by low expression of CD95. In certain embodiments, naïve-like T cells or the T cells that are surface positive for a marker expressed on naïve-like T cells are CCR7+CD45RA+, where the cells are CD27+ or CD27-. In certain embodiments, naïve-like T cells or the T cells that are surface positive for a marker expressed on naïve-like T cells are CD27+CCR7+, where the cells are CD45RA+ or CD45RA-. In certain embodiments, naïve-like T cells or the T cells that are surface positive for a marker expressed on naïve-like T cells are CD62L-CCR7+.

[0796] In certain embodiments, the cells of the composition have a high portion and/or frequency of central memory T cells or T cells that are surface positive for a marker expressed on central memory T cells. In certain embodiments, central memory T cells may include cells in various differentiation states and may be characterized by positive or high expression (*e.g.*, surface expression) of certain cell markers and/or negative or low expression (*e.g.*, surface expression) of other cell markers. In some aspects, central memory T cells are characterized by positive or high expression of CD45RO, CD62L, CCR7, CD28, CD3, and/or CD127. In some aspects, central memory T cells are characterized by negative or low expression of CD45RA and/or granzyme B. In certain embodiments, central memory T cells or the T cells that are surface positive for a marker expressed on central memory T cells are CCR7+CD45RA-.

[0797] In some embodiments, the provided therapeutic T cell composition comprises and/or is enriched in CD3+ T cells expressing a recombinant receptor, wherein at least 50%, 60%, 70%, 80% or 90% of the total receptor⁺/CD3⁺ cells in the composition are naïve-like T cells or central memory T cells or are surface positive for a marker expressed on naïve-like T cells or central memory T cells. In some embodiments, 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 embodiments, at least or at least about 90% of the cells in the composition are CD3⁺ T cells, and at least or at least about 40%, 50%, 60%, 70%, 80% or 90% of the total receptor⁺/CD3⁺ cells in the composition are naïve-like T cells or central memory T cells or are surface positive for a marker expressed on naïve-like T cells or central memory T cells. In some embodiments, at least or at least about 95% of the cells in the composition are CD3⁺ T cells, and at least or at least about 50%, 60%, 70%, 80% or 90% of the total receptor⁺/CD3⁺ cells in the composition are naïve-like T cells or central memory T cells or are surface positive for a marker expressed on naïve-like T cells or central memory T cells. In some embodiments, at least or at least about 98% of the cells in the composition are CD3⁺ T cells, and at least or at least about 50%, 60%, 70%, 80% or 90% of the total receptor⁺/CD3⁺ cells in the composition are naïve-like T cells or central memory T cells or are surface positive for a marker expressed on naïve-like T cells or central memory T cells. In some embodiments, at least 50%, 60%, 70%, 80% or 90% of the cells in the composition are CD3⁺ T cells, at least 50% of the total receptor⁺/CD8⁺ cells in the composition are naïve-like T cells or central memory T cells or are surface positive for a marker expressed on naïve-like T cells or central memory T cells and at least 50% of the total receptor⁺/CD4⁺ cells in the composition are naïve-like T cells or central memory T cells or are surface positive for a marker expressed on naïve-like T cells or central memory T cells. In some embodiments, at least 90% of the cells in the composition are CD3⁺ T cells, at least 50%, 60%, 70%, 80% or 90% of the total receptor⁺/CD8⁺ cells in the composition are naïve-like T cells or central memory T cells or are surface positive for a marker expressed on naïve-like T cells or central memory T cells, and at least 50%, 60%, 70%, 80% or 90% of the total receptor⁺/CD4⁺ cells in the composition are naïve-like T cells or central memory T cells or are surface positive for a marker expressed on naïve-like T cells or central memory T cells.

[0798] In particular embodiments, the cells of the composition are enriched in CCR7⁺ cells. CCR7 is a chemokine receptor that is involved in T cell entry into lymph nodes. In particular aspects, CCR7 is expressed by naïve or naïve-like T cells (*e.g.*, CCR7⁺CD45RA⁺ or CCR7⁺CD27⁺) and central memory T cells (CCR7⁺CD45RA⁻). In some embodiments, provided compositions of engineered T cells produced by the provided methods include a population of T cells in which greater than at or about 50%, greater than at or about 55%, greater than or greater than at or about 60%, greater than or greater than at or about 65%, greater than or greater than at or about 70%, greater than or greater than at or about 75%, greater than or greater

than at or about 80%, greater than or greater than at or about 85%, or greater than or greater than at or about 90%, of the T cells of the population are central memory and naïve-like T cells. In some embodiments, provided compositions of engineered T cells produced by the provided methods include a population of T cells in which greater than at or about 50%, greater than at or about 55%, greater than or greater than at or about 60%, greater than or greater than at or about 65%, greater than or greater than at or about 70%, greater than or greater than at or about 75%, greater than or greater than at or about 80%, greater than or greater than at or about 85%, or greater than or greater than at or about 90%, of the T cells of the population are CCR7+ T cells.

[0799] In some embodiments, at least 70% of the CAR+ T cells in the composition are CCR7+. In some embodiments, at least 75% of the CAR+ T cells in the composition are CCR7+. In some embodiments, at least 80% of the CAR+ T cells in the composition are CCR7+. In some embodiments, at least 85% of the CAR+ T cells in the composition are CCR7+. In some embodiments, at least 90% of the CAR+ T cells in the composition are CCR7+. In some embodiments, at least 95% of the CAR+ T cells in the composition are CCR7+. In some embodiments, at least 85% of the CD8+CAR+ T cells in the composition are CCR7+ and at least 90% of the CD4+ CAR+ T cells in the composition are CCR7+. In some embodiments, 85% to 98% of the CD8+ CAR+ T cells in the composition are CCR7+ and 94% to 99% of the CD4+ CAR+ T cells in the composition are CCR7+.

[0800] In some embodiments, provided compositions of engineered T cells include a population of T cells in which greater than at or about 50%, greater than at or about 55%, greater than or greater than at or about 60%, greater than or greater than at or about 65%, greater than or greater than at or about 70%, greater than or greater than at or about 75%, greater than or greater than at or about 80%, greater than or greater than at or about 85%, or greater than or greater than at or about 90%, of the T cells of the population are CCR7+CD27+.

[0801] In some embodiments, the provided therapeutic T cell composition comprises and/or is enriched in CD4+ T cells and CD8+ T cells expressing a recombinant receptor, wherein at least 50%, 60%, 70%, 80% or 90% of the total receptor⁺/CD4⁺ and receptor⁺/CD8⁺ cells in the composition are CD27+CCR7+. In some embodiments, 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.

[0802] In some embodiments, provided compositions of engineered T cells include a population of T cells in which greater than 30%, greater than 40%, greater than at or about 50%, greater than at or about 55%, greater than or greater than at or about 60%, greater than or greater than at or about 65%, greater than or greater than at or about 70%, greater than or greater than at or about 75%, greater than or greater than at or about 80%, greater than or greater than at or about 85%, or greater than or greater than at or about 90%, of the T cells of the population are CCR7+CD45RA-. In some embodiments, at least 20% of the CAR+ T cells in the composition are CD45RA-CCR7+. In some embodiments, at least 30% of the CAR+ T cells in the composition are CD45RA-CCR7+. In some embodiments, at least 40% of the CAR+ T cells in the composition are CD45RA-CCR7+. In some embodiments, at least 50% of the CAR+ T cells in the composition are CD45RA-CCR7+. In some embodiments, at least 60% of the CAR+ T cells in the composition are CD45RA-CCR7+.

[0803] In some embodiments, provided compositions of engineered T cells include a population of T cells in which greater than 30%, greater than 40%, greater than at or about 50%, greater than at or about 55%, greater than or greater than at or about 60%, greater than or greater than at or about 65%, greater than or greater than at or about 70%, greater than or greater than at or about 75%, greater than or greater than at or about 80%, greater than or greater than at or about 85%, or greater than or greater than at or about 90%, of the T cells of the population are CCR7+CD45RA+. In some embodiments, least 40% of the CAR+ T cells in the composition are CD45RA+CCR7+. In some embodiments, at least 50% of the CAR+ T cells in the composition are CD45RA+CCR7+. In some embodiments, at least 60% of the CAR+ T cells in the composition are CD45RA+CCR7+. In some embodiments, at least 70% of the CAR+ T cells in the composition are CD45RA+CCR7+. In some embodiments, at least 80% of the CAR+ T cells in the composition are CD45RA+CCR7+.

[0804] In some embodiments, the method of producing the compositions is a non-expanded method, such as described herein. Any of such compositions can be used in the provided methods and uses for treating a systemic autoimmune disease or condition.

[0805] In certain embodiments, the cells of the compositions have a greater portion and/or frequency of naïve-like cells and central memory cells than output compositions generated from alternative processes, such as processes that involve expansion (*e.g.*, processes that include an expansion unit operation and/or include steps intended to cause expansion of cells).

[0806] In certain embodiments, the cells of the composition have a low portion and/or frequency of T cells in an intermediate stage of differentiation, or T cells that are surface positive for a marker expressed on T cells in an intermediate stage of differentiation. In certain embodiments, the cells of the compositions have a lower portion and/or frequency of T cells in an intermediate stage of differentiation than compositions generated from alternative processes, such as processes that involve expansion. In certain embodiments, T cells in an intermediate stage of differentiation may be characterized by positive or high expression (*e.g.*, surface expression or intracellular expression) of certain cell markers and/or negative or low expression (*e.g.*, surface expression or intracellular expression) of other cell markers. In certain embodiments, T cells in an intermediate stage of differentiation or the T cells that are surface positive for a marker expressed on T cells in an intermediate stage of differentiation are CCR7+CD27-. In certain embodiments, T cells in an intermediate stage of differentiation or the T cells that are surface positive for a marker expressed on T cells in an intermediate stage of differentiation are CCR7-CD27+. In certain embodiments, T cells in an intermediate stage of differentiation or the T cells that are surface positive for a marker expressed on T cells in an intermediate stage of differentiation include cells that are CCR7+CD27- and cells that are CCR7-CD27+.

[0807] In certain embodiments, the cells of the composition have a low portion and/or frequency of highly differentiated T cells, or T cells that are surface positive for a marker expressed on highly differentiated T cells. In certain embodiments, the cells of the compositions have a lower portion and/or frequency of highly differentiated T cells than compositions generated from alternative processes, such as processes that involve expansion. In certain embodiments, highly differentiated T cells may be characterized by positive or high expression (*e.g.*, surface expression or intracellular expression) of certain cell markers and/or negative or low expression (*e.g.*, surface expression or intracellular expression) of other cell markers. In some aspects, highly differentiated T cells are characterized by negative or low expression of CCR7 and/or CD27. In certain embodiments, highly differentiated T cells or the T cells that are surface positive for a marker expressed on highly differentiated T cells are CCR7-CD27-.

[0808] In certain embodiments, the cells of the composition have a low portion and/or frequency of effector memory and/or effector memory RA T cells or T cells that are surface positive for a marker expressed on effector memory and/or effector memory RA T cells. In certain embodiments, the cells of the output compositions have a lower portion and/or frequency

of effector memory and/or effector memory RA T cells than output compositions generated from alternative processes, such as processes that involve expansion (*e.g.*, processes that include an expansion unit operation and/or include steps intended to cause expansion of cells). In certain embodiments, effector memory and/or effector memory RA T cells may include cells in various differentiation states and may be characterized by positive or high expression (*e.g.*, surface expression or intracellular expression) of certain cell markers and/or negative or low expression (*e.g.*, surface expression or intracellular expression) of other cell markers. In certain embodiments, effector memory T cells or the T cells that are surface positive for a marker expressed on effector memory T cells are CCR7-CD45RA-. In certain embodiments, effector memory RA T cells or the T cells that are surface positive for a marker expressed on effector memory RA T cells are CCR7-CD45RA+.

[0809] In certain embodiments, the cells of the compositions have a lower portion and/or frequency of effector memory T cells than output compositions generated from alternative processes, such as processes that involve expansion (*e.g.*, processes that include an expansion unit operation and/or include steps intended to cause expansion of cells). In certain embodiments, the cells of the compositions have a lower portion and/or frequency of effector memory RA T cells than output compositions generated from alternative processes, such as processes that involve expansion (*e.g.*, processes that include an expansion unit operation and/or include steps intended to cause expansion of cells). In certain embodiments, the cells of the compositions have a greater portion and/or frequency of naïve-like cells and central memory cells and a lower portion and/or frequency of effector memory and effector memory RA T cells, than output compositions generated from alternative processes, such as processes that involve expansion (*e.g.*, processes that include an expansion unit operation and/or include steps intended to cause expansion of cells).

[0810] In some embodiments, the cells of the composition have a low portion and/or frequency of cells that are negative for CD27 and CD28 expression, *e.g.*, surface expression. In particular embodiments, the cells of the composition have a low portion and/or frequency of CD27-CD28- cells. In some embodiments, less than at or about 40%, less than at or about 35%, less than at or about 30%, less than at or about 25%, less than at or about 20%, less than at or about 15%, less than at or about 10%, less than at or about 5%, or less than at or about 1% of the cells of the composition are CD27-CD28- cells. In certain embodiments, less than at or about 25% of the cells of the composition are CD27-CD28- cells. In certain embodiments, less than at

or about less than at or about 10% of the cells of the composition are CD27-CD28- cells. In embodiments, less than at or about 5% of the cells of the composition are CD27-CD28- cells.

[0811] In certain embodiments, the cells of the composition have a high portion and/or frequency of cells that are positive for CD27 and CD28 expression, *e.g.*, surface expression. In some embodiments, the cells of the composition have a high portion and/or frequency of CD27+CD28+ cells. In some embodiments, at least at or about 50%, at least at or about 60%, at least at or about 70%, at least at or about 75%, at least at or about 80%, at least at or about 85%, at least at or about 90%, at least at or about 95%, or greater than at or about 95% of the cells of the composition are CD27+CD28+ cells. In certain embodiments, less than at or about 25% of the cells of the composition are CD27-CD28- cells. In certain embodiments, at least at or about 50% of the cells of the composition are CD27+CD28+ cells. In embodiments, at least at or about 75% of the cells of the composition are CD27+CD28+ cells.

[0812] In particular embodiments, the cells of the composition have a low portion and/or frequency of cells that are T_{EMRA} cells. In particular embodiments, the cells of the composition have a low portion and/or frequency of T_{EMRA} cells. In some embodiments, less than at or about 40%, less than at or about 35%, less than at or about 30%, less than at or about 25%, less than at or about 20%, less than at or about 15%, less than at or about 10%, less than at or about 5%, or less than at or about 1% of the cells of the composition are T_{EMRA} cells. In some embodiments, less than at or about 25% of the cells of the composition are T_{EMRA} cells. In some embodiments, less than at or about 10% of the cells of the composition are T_{EMRA} cells. In some embodiments, less than at or about 5% of the cells of the composition are T_{EMRA} cells.

[0813] In certain embodiments, the cells of the composition have a low portion and/or frequency of cells that are negative for CCR7 and positive for CD45RA expression, *e.g.*, surface expression. In some embodiments, the cells of the composition have a low portion and/or frequency of CCR7-CD45RA+ cells. In particular embodiments, less than at or about 40%, less than at or about 35%, less than at or about 30%, less than at or about 25%, less than at or about 20%, less than at or about 15%, less than at or about 10%, less than at or about 5%, or less than at or about 1% of the cells of the composition are CCR7-CD45RA+ cells. In some embodiments, less than at or about 25% of the cells of the composition are CCR7-CD45RA+ cells. In particular embodiments, less than at or about less than at or about 10% of the cells of the composition are CCR7-CD45RA+ cells. In certain embodiments, less than at or about 5% of the cells of the composition are CCR7-CD45RA+ cells.

[0814] In some embodiments, provided herein is a therapeutic T cell composition comprising and/or enriched in CD3⁺ T cells expressing a recombinant receptor, wherein at least 50%, 60%, 70%, 80% or 90% of the total receptor⁺/CD3⁺ cells in the composition are CD27+CCR7⁺. In some embodiments, 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 embodiments, at least or at least about 90% of the cells in the composition are CD3⁺ T cells, and at least or at least about 40%, 50%, 60%, 70%, 80% or 90% of the total receptor⁺/CD3⁺ cells in the composition are CD27+CCR7⁺. In some embodiments, at least or at least about 95% of the cells in the composition are CD3⁺ T cells, and at least or at least about 50%, 60%, 70%, 80% or 90% of the total receptor⁺/CD3⁺ cells in the composition are CD27+CCR7⁺. In some embodiments, at least or at least about 98% of the cells in the composition are CD3⁺ T cells, and at least or at least about 50%, 60%, 70%, 80% or 90% of the total receptor⁺/CD3⁺ cells in the composition are CD27+CCR7⁺. In some embodiments, at least 50%, 60%, 70%, 80% or 90% of the cells in the composition are CD3⁺ T cells, at least 50% of the total receptor⁺/CD8⁺ cells in the composition are CD27+CCR7⁺ and at least 50% of the total receptor⁺/CD4⁺ cells in the composition are CD27+CCR7⁺. In some embodiments, at least 90% of the cells in the composition are CD3⁺ T cells, at least 50%, 60%, 70%, 80% or 90% of the total receptor⁺/CD8⁺ cells in the composition are CD27+CCR7⁺, and at least 50%, 60%, 70%, 80% or 90% of the total receptor⁺/CD4⁺ cells in the composition are CD27+CCR7⁺.

[0815] In some embodiments, the composition is characterized by (i) at least 60%, at least 70%, at least 75%, at least 80%, at least 85% or at least 90% of the T cells in the composition are viable; (ii) at least 25%, at least 30%, at least 35%, at least 40%, at least 45% or at least 50% of the T cells in the composition are CAR⁺ T cells; (iii) at least 90%, 91%, 92%, 93%, 94%, 95%, 96% of the cells in the composition are T cells and (iv) at least 80%, at least 85%, at least 90% or at least 95% of the CAR⁺ T cells are CCR7⁺. In some embodiments, the composition is characterized by (i) 60%-95% of the T cells in the composition are viable; (ii) 25%-60% of the T cells in the composition are CAR⁺ T cells; (iii) 90%-99% of the cells in the composition are T cells; and (iv) 85%-99% of the CAR⁺ T cells in the composition are CCR7⁺ T cells. In some embodiments, the composition is characterized by (i) 80%-95% of the T cells in the composition are viable; (ii) 25%-60% of the T cells in the composition are CAR⁺ T cells; (iii) 90%-99% of

the cells in the composition are T cells; and (iv) 85%-99% of the CAR+ T cells in the composition are CCR7+ T cells. In some embodiments, the T cells are CD3+ T cells that include CD4+ and CD8+ T cells. In some embodiments, 85% to 98% of the CD8+ CAR+ T cells in the composition are CCR7+ and 94% to 99% of the CD4+ CAR+ T cells in the composition are CCR7+. In some embodiments, the CCR7+ T cells are CD45RA+CCR7+ and/or CD45RA-CCR7+. In some embodiments, the composition is characterized by 40-90% of the T cells, such as 40-85% of the T cells that are CD45RA+CCR7+. In some embodiments, the composition is characterized by 15-70% of the T cells, such as 15-60% of the T cells that are CD45RA-CCR7+. In some embodiments, 40-85% of the T cells are CD45RA+CCR7+ and 15-60% of the T cells that are CD45RA-CCR7+. In some embodiments, the composition is further characterized by (v) less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, or less than 4% of the cells of the composition are positive for an apoptotic marker. In some embodiments, the apoptotic marker is Annexin V or Caspase 3.

[0816] In some embodiments, at least 60% of the T cells in the composition are viable, at least 25% of the T cells of the composition are CAR+ T cells; less than 10% of the cells of the composition are positive for an apoptotic marker, optionally wherein the marker of apoptosis is Annexin V or active Caspase 3; at least 85% of the CD8+CAR+ T cells in the composition are CCR7+; and/or at least 90% of the CD4+ CAR+ T cells in the composition are CCR7+.

[0817] In some embodiments, at least 80% of the T cells in the composition are viable, at least 45% of the T cells of the composition are CAR+, less than 4% of the cells of the composition are positive for an apoptotic marker, optionally wherein the marker of apoptosis is Annexin V or active Caspase 3; at least 85% of the CD8+CAR+ T cells in the composition are CCR7+; and/or at least 90% of the CD4+ CAR+ T cells in the composition are CCR7+.

[0818] In some embodiments, at least 60% of the T cells in the composition are viable, at least 25% of the T cells of the composition are CAR+, less than 10% of the cells of the composition are positive for an apoptotic marker, optionally wherein the marker of apoptosis is Annexin V or active Caspase 3; and/or greater than at or about 40% of the CAR+ T cells in the composition are CCR7+CD45RA+.

[0819] In some embodiments, at least 80% of the T cells in the composition are viable; at least 45% of the T cells of the composition are CAR+; less than 4% of the cells of the composition are positive for an apoptotic marker, optionally wherein the marker of apoptosis is

Annexin V or active Caspase 3; and/or at least 40% of the CAR+ T cells in the composition are CCR7+CD45RA+.

[0820] In some embodiments, at least 60% of the T cells in the composition are viable; at least 25% of the T cells of the composition are CAR+; less than 10% of the cells of the composition are positive for an apoptotic marker, optionally wherein the marker of apoptosis is Annexin V or active Caspase 3; and/or greater than 20% of the CAR+ T cells in the composition are CCR7+CD45RA-.

[0821] In some embodiments, at least 80% of the T cells in the composition are viable; at least 45% of the T cells of the composition are CAR+; less than 4% of the cells of the composition are positive for an apoptotic marker, optionally wherein the marker of apoptosis is Annexin V or active Caspase 3; and/or at least 20% of the CAR+ T cells in the composition are CCR7+CD45RA-.

[0822] In some of any of the embodiments, the T cells are CD3+ T cells. In some embodiments, at least 90%, 91%, 92%, 93%, 94%, 95%, 96% of the cells in the composition are CD3+ T cells. In some embodiments, the CD3+ T cells include CD4+ T cells and CD8+ T cells. In some embodiments, the ratio of CD4+ to CD8+ T cells is 1:10 to 10:1. In some embodiments, the ratio of CD4+ to CD8+ T cells is 1:5 to 5:1. In some embodiments, the ratio of CD4+ to CD8+ T cells is 1:3 to 3:1. In some embodiments, the ratio of CD4+ to CD8+ T cells is 1:1 to 10:1. In some embodiments, the ratio of CD4+ to CD8+ T cells is 1:1 to 5:1. In some embodiments, the ratio of CD4+ to CD8+ T cells is 1:1 to 3:1. In some embodiments, the ratio of CD4+ to CD8+ T cells is 1:10 to 1:1. In some embodiments, the ratio of CD4+ to CD8+ T cells is 1:5 to 1:1. In some embodiments, the ratio of CD4+ to CD8+ T cells is 1:3 to 1:1.

[0823] In some embodiments, the composition comprises T cells having the heterologous or recombinant polynucleotide encoding the anti-CD19 CAR integrated into the T cell genomes. In particular embodiments, the integrated vector copy number (iVCN) of the cells in the composition, on average, is of about, or of at least 0.1, 0.5, 1, 2, 3, 4, 5, or greater than 5 per diploid genome. In particular embodiments, iVCN of the CAR+ cells in the composition, on average, is between or between about 0.4 copies per diploid genome and 3.0 copies per diploid genome, inclusive. In particular embodiments, iVCN of the CAR+ cells in the composition, on average, is about 0.4, about 0.5, about 0.6, about 0.7, about 0.8, about 0.9, about 1.0, about 1.1, about 1.2, about 1.3, about 1.4, about 1.5, about 1.6, about 1.7, about 1.8, about 1.9, about 2.0,

about 2.1, about 2.2, about 2.3, about 2.4, about 2.5, about 2.6, about 2.7, about 2.8, about 2.9, or about 3.0 copies per diploid genome, inclusive.

[0824] In certain embodiments, the fraction of iVCN to total vector copy number (VCN) in the diploid genome of the population of transformed cells, on average, is less than or less than about 0.9, for example, is at least or is about 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, or is within a tolerated error thereof, *e.g.*, $\pm 25\%$, $\pm 20\%$, $\pm 15\%$, $\pm 10\%$, $\pm 5\%$, or $\pm 1\%$. In certain embodiments, the fraction of iVCN to total vector copy number (VCN) in the diploid genome of the population of transformed cells, on average, is or is about 0.8, or is within a tolerated error thereof.

[0825] In some embodiments, the total vector copy number (VCN) of the cells in the composition is, on average, less than or less than about 20, 18, 16, 14, 12, 10, 9, 8, 7, 6, 5, 4, 3, or 2 copies, inclusive. In some embodiments, the total vector copy number (VCN) of the CD3+ cells in the composition is, on average, less than or less than about 20, 18, 16, 14, 12, 10, 9, 8, 7, 6, 5, 4, 3, or 2 copies, inclusive. In some embodiments, the total vector copy number (VCN) of the CD3+CAR+ cells in the composition is, on average, less than or less than about 20, 18, 16, 14, 12, 10, 9, 8, 7, 6, 5, 4, 3, or 2 copies, inclusive.

[0826] In some embodiments, the composition includes residual stimulatory reagents, *e.g.*, stimulatory reagents not removed following any of the methods described in Section II-C-6. In some embodiments, the residual stimulatory reagents include any of the oligomeric stimulatory reagents described in Section II-C-2. In some embodiments, the residual stimulatory reagents include any of the oligomeric streptavidin mutein reagents described in Section II-C-2. In some embodiments, the composition contains between or between about 50 and 2000 ng/mL of residual stimulatory reagent, such as between or between about 50 and 1900 ng/mL, 50 and 1800 ng/mL, 50 and 1700 ng/mL, 50 and 1600 ng/mL, 50 and 1500 ng/mL, 50 and 1400 ng/mL, 50 and 1300 ng/mL, 50 and 1200 ng/mL, 50 and 1100 ng/mL, 50 and 1000 ng/mL, 50 and 900 ng/mL, 50 and 800 ng/mL, 50 and 700 ng/mL, 50 and 600 ng/mL, 50 and 500 ng/mL, 50 and 400 ng/mL, 50 and 300 ng/mL, 50 and 200 ng/mL, 50 and 100 ng/mL, 100 and 2000 ng/mL, 100 and 1900 ng/mL, 100 and 1800 ng/mL, 100 and 1700 ng/mL, 100 and 1600 ng/mL, 100 and 1500 ng/mL, 100 and 1400 ng/mL, 100 and 1300 ng/mL, 100 and 1200 ng/mL, 100 and 1100 ng/mL, 100 and 1000 ng/mL, 100 and 900 ng/mL, 100 and 800 ng/mL, 100 and 700 ng/mL, 100 and 600 ng/mL, 100 and 500 ng/mL, 100 and 400 ng/mL, 100 and 300 ng/mL, 100 and 200 ng/mL, 200 and 2000 ng/mL, 200 and 1900 ng/mL, 200 and 1800 ng/mL, 200 and 1700 ng/mL, 200 and 1600 ng/mL, 200 and 1500 ng/mL, 200 and 1400 ng/mL, 200 and 1300 ng/mL, 200 and

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[0827] In some of any embodiments, the cells are formulated in an isotonic solution. In some of any embodiments, the isotonic solution comprises PlasmaLyte pH 7.4 and about 1-1.5% (v/v) human serum albumin. In some embodiments, the isotonic solution comprises PlasmaLyte pH 7.4 and about 1.2% (v/v) human serum albumin. In some embodiments, the cells may be further formulated with a cryoprotectant, such as 5-10% (v/v) DMSO (*e.g.*, Cryostor® CS-10 media). In some of any embodiments, the composition comprises Plasmalyte, 1.2% (v/v) human serum albumin, and 7.5% (v/v) DMSO.

IV. DEFINITIONS

[0828] Unless defined otherwise, all terms of art, notations and other technical and scientific terms or terminology used herein are intended to have the same meaning as is commonly understood by one of ordinary skill in the art to which the claimed subject matter pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art.

[0829] The terms “polypeptide” and “protein” are used interchangeably to refer to a polymer of amino acid residues and are not limited to a minimum length. Polypeptides, including the provided receptors and other polypeptides, *e.g.*, linkers or peptides, may include amino acid residues including natural and/or non-natural amino acid residues. The terms also include post-expression modifications of the polypeptide, for example, glycosylation, sialylation, acetylation, and phosphorylation. In some aspects, the polypeptides may contain modifications with respect to a native or natural sequence, as long as the protein maintains the desired activity. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental,

such as through mutations of hosts which produce the proteins or errors due to PCR amplification.

[0830] As used herein, a “subject” is a mammal, such as a human or other animal, and typically is human. In some embodiments, the subject, *e.g.*, patient, to whom the agent or agents, cells, cell populations, or compositions are administered, is a mammal, typically a primate, such as a human. In some embodiments, the primate is a monkey or an ape. The subject can be male or female and can be any suitable age, including infant, juvenile, adolescent, adult, and geriatric subjects. In some embodiments, the subject is a non-primate mammal, such as a rodent.

[0831] As used herein, “treatment” (and grammatical variations thereof such as “treat” or “treating”) refers to complete or partial amelioration or reduction of a disease or condition or disorder, or a symptom, adverse effect or outcome, or phenotype associated therewith. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. The terms do not imply complete curing of a disease or complete elimination of any symptom or effect(s) on all symptoms or outcomes.

[0832] As used herein, “delaying development of a disease” means to defer, hinder, slow, retard, stabilize, suppress and/or postpone development of the disease (such as severe refractory SLE). This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. In some embodiments, sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease.

[0833] “Preventing,” as used herein, includes providing prophylaxis with respect to the occurrence or recurrence of a disease in a subject that may be predisposed to the disease but has not yet been diagnosed with the disease. In some embodiments, the provided cells and compositions are used to delay development of a disease or to slow the progression of a disease.

[0834] As used herein, to “suppress” a function or activity is to reduce the function or activity when compared to otherwise same conditions except for a condition or parameter of interest, or alternatively, as compared to another condition. For example, cells that suppress or reduce immune activity compared to the absence of the cells.

[0835] An “effective amount” of an agent, *e.g.*, a pharmaceutical formulation, cells, or composition, in the context of administration, refers to an amount effective, at dosages/amounts and for periods of time necessary, to achieve a desired result, such as a therapeutic or prophylactic result.

[0836] A “therapeutically effective amount” of an agent, *e.g.*, a pharmaceutical formulation or cells, refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result, such as for treatment of a disease, condition, or disorder, and/or pharmacokinetic or pharmacodynamic effect of the treatment. The therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the subject, and the populations of cells administered. In some embodiments, the provided methods involve administering the cells and/or compositions at effective amounts, *e.g.*, therapeutically effective amounts.

[0837] A “prophylactically effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically but not necessarily, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

[0838] The term “about” as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to “about” a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter *per se*.

[0839] As used herein, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. For example, “a” or “an” means “at least one” or “one or more.”

[0840] Throughout this disclosure, various aspects of the claimed subject matter are presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the claimed subject matter. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, where a range of values is provided, it is understood that each intervening value, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the claimed subject matter. The upper and lower limits of these smaller ranges may independently be included in

the smaller ranges, and are also encompassed within the claimed subject matter, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the claimed subject matter. This applies regardless of the breadth of the range.

[0841] As used herein, a composition refers to any mixture of two or more products, substances, or compounds, including cells. It may be a solution, a suspension, liquid, powder, a paste, aqueous, non-aqueous or any combination thereof.

[0842] As used herein, “enriching” when referring to one or more particular cell type or cell population, refers to increasing the number or percentage of the cell type or population, *e.g.*, compared to the total number of cells in or volume of the composition, or relative to other cell types, such as by positive selection based on markers expressed by the population or cell, or by negative selection based on a marker not present on the cell population or cell to be depleted. The term does not require complete removal of other cells, cell type, or populations from the composition and does not require that the cells so enriched be present at or even near 100% in the enriched composition.

[0843] As used herein, a statement that a cell or population of cells is “positive” for a particular marker refers to the detectable presence on or in the cell of a particular marker, typically a surface marker. When referring to a surface marker, the term refers to the presence of surface expression as detected by flow cytometry, for example, by staining with an antibody that specifically binds to the marker and detecting said antibody, wherein the staining is detectable by flow cytometry at a level substantially above the staining detected carrying out the same procedure with an isotype-matched control or fluorescence minus one (FMO) gating control under otherwise identical conditions and/or at a level substantially similar to that for cell known to be positive for the marker, and/or at a level substantially higher than that for a cell known to be negative for the marker.

[0844] As used herein, a statement that a cell or population of cells is “negative” for a particular marker refers to the absence of substantial detectable presence on or in the cell of a particular marker, typically a surface marker. When referring to a surface marker, the term refers to the absence of surface expression as detected by flow cytometry, for example, by staining with an antibody that specifically binds to the marker and detecting said antibody, wherein the staining is not detected by flow cytometry at a level substantially above the staining detected carrying out the same procedure with an isotype-matched control or fluorescence minus

one (FMO) gating control under otherwise identical conditions, and/or at a level substantially lower than that for cell known to be positive for the marker, and/or at a level substantially similar as compared to that for a cell known to be negative for the marker.

[0845] The term “vector,” as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as “expression vectors.”

V. EXEMPLARY EMBODIMENTS

[0846] Among the provided embodiments are:

1. A method of treating a subject having a systemic autoimmune disease, the method comprising administering a dose of CD19-directed genetically modified T cells from a composition comprising engineered T cells expressing a chimeric antigen receptor (CAR) to a subject having or suspected of having a severe systemic autoimmune disease, wherein the T cells of the dose are positive for expression of a CAR that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.
2. A method of treating a subject having systemic autoimmune disease, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having a moderate systemic autoimmune disease, wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.
3. The method of embodiment 1 or embodiment 2, wherein the systemic autoimmune disease is selected from the group consisting of systemic lupus erythematosus (SLE), Sjogren's' syndrome, progressive systemic sclerosis (i.e., scleroderma), idiopathic inflammatory myositis (IIM), including dermatomyositis, polymyositis and necrotizing myositis, mixed connective tissue disorder (MCTD), highly active relapsing-remitting multiple sclerosis, primary progressive MS, ANCA-associated vasculitis (AAV), Crohn's disease, myasthenia gravis, Behçet's, rheumatoid arthritis, IgA nephropathy, pemphigus vulgaris, myasthenia gravis, autoimmune hemolytic anemia, immune thrombocytopenia, IgG4-related diseases, membranous nephropathy, cutaneous lupus erythematosus, sarcoidosis, light chain amyloidosis,

acute respiratory distress syndrome, atopic eczema, hereditary angioedema, hidradenitis suppurative, inclusion-body myositis, inflammatory bowel disease, mastocytosis, multifocal motor neuropathy, necrotizing myopathy, neuromyelitis optica spectrum disorder, mixed connective tissue disorder, POEMS syndrome, primary biliary cholangitis, psoriasis, rhesus hemolytic disease, Still's disease, type 1 diabetes, urticaria, capillary leakage syndrome, cytokine release syndrome, erythema multiforme, pyoderma gangrenosum, x-linked agammaglobulinemia, antiphospholipid syndrome, and chronic inflammatory demyelinating polyneuropathy.

4. The method of any of embodiments 1-3, wherein the systemic autoimmune disease is rheumatoid arthritis.
5. The method of any of embodiments 1-3, wherein the systemic autoimmune disease is myositis.
6. The method of any of embodiments 1-3, wherein the systemic autoimmune disease is myasthenia gravis.
7. The method of any of embodiments 1-3, wherein the systemic autoimmune disease is bullous pemphigoid.
8. The method of any of embodiments 1-3, wherein the systemic autoimmune disease is immune thrombocytopenia.
9. The method of any of embodiments 1-3, wherein the systemic autoimmune disease is autoimmune hemolytic anemia.
10. The method of any of embodiments 1-3, wherein the systemic autoimmune disease is pemphigus vulgaris.
11. The method of any of embodiments 1-3, wherein the systemic autoimmune disease is demyelinating polyradiculoneuropathy.
12. The method of any of embodiments 1-3, wherein the systemic autoimmune disease is membranous nephropathy.
13. The method of any of embodiments 1-12, wherein the systemic autoimmune disease is a refractory disease.
14. The method of any of embodiments 1-13, wherein the subject is refractory to treatment with one or more prior therapies for the systemic autoimmune disease.
15. The method of any of embodiments 1-14, wherein the subject is refractory to treatment with two or more prior therapies for the systemic autoimmune disease.

16. The method of any of embodiments 1-15, wherein the systemic autoimmune disease is a severe disease.
17. A method of treating a subject having severe systemic lupus erythematosus (SLE), the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having severe systemic lupus erythematosus (SLE), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.
18. A method for reducing systemic lupus erythematosus (SLE) disease activity, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having severe systemic lupus erythematosus (SLE), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.
19. The method of embodiment 17 or embodiment 18 wherein the SLE in the subject has one or more of the following: renal, central nervous system, or hematologic involvement.
20. The method of any of embodiments 17-19, wherein the subject has at least one organ system categorized by the British Isles Lupus Assessment Group 2004 ("BILAG") as category A ("BILAG A") or at least two organ systems categorized as BILAG B.
21. The method of any of embodiments 17-20, wherein the subject fulfills the 2019 American College of Rheumatology (ACR)/ European League Against Rheumatism (EULAR) classification criteria of SLE and/or the subject has detectable anti-dsDNA, anti-histone, anti-chromatin or anti-Sm antibodies in their blood.
22. The method of any of embodiments 17-20, wherein the subject fulfills the 2019 American College of Rheumatology (ACR)/ European League Against Rheumatism (EULAR) classification criteria of SLE.
23. The method of any of embodiments 17-21, wherein the subject has detectable anti-dsDNA, anti-histone, anti-chromatin or anti-Sm antibodies in their blood.
24. The method of any of embodiments 17-23, wherein the subject has lupus nephritis.
25. A method of treating a subject having lupus nephritis, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having lupus nephritis, wherein the T cells of the dose are positive for expression of

a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

26. The method of any of embodiments 17-25, wherein the subject is refractory to treatment with one or more prior therapies for the lupus.

27. The method of any of embodiments 17-26, wherein the subject achieved an insufficient response to one or more prior therapies for the lupus.

28. The method of embodiment 26 or embodiment 27, wherein the two or more prior therapies for the lupus comprise a glucocorticoid, an antimalarial, an immunosuppressant, an anti-CD20 antibody, or an inhibitor of soluble B lymphocyte stimulator (BLyS).

29. The method of any of embodiments 26-28, wherein the two or more prior therapies are selected from any two or more of the following: mycophenolate mofetil (MFF), cyclophosphamide (cyc), belimumab, rituximab, anifrolumab, azathioprine, methotrexate cyclosporine (csp) or voclosporin.

30. The method of any of embodiments 17-29, wherein the subject does not have drug-induced SLE, clinically significant CNS pathology, related systemic autoimmune diseases, and/or SLE overlap syndromes.

31. The method of embodiment 30, wherein the subject does not have related systemic autoimmune diseases, including by not limited to multiple sclerosis, psoriasis, and inflammatory bowel disease.

32. The method of embodiment 30, wherein the subject does not have SLE overlap syndromes, including by not limited to rheumatoid arthritis, scleroderma, and mixed connective tissue disease.

33. The method of any of embodiments 17-32, wherein the subject is at high risk for organ failure.

34. The method of any of embodiments 1-17, wherein the method reduces the systemic autoimmune disease activity in the subject.

35. The method of any of embodiments 1-17, wherein reducing disease activity in the subject comprises a reduced inflammation in the subject.

36. The method of any of embodiments 17-33, wherein the method reduces SLE disease activity in the subject.

37. The method of any of embodiments 18-24 and 34-36, wherein reducing SLE disease activity in the subject comprises: a BILAG-Based Composite Lupus Assessment

(BICLA) response in the subject, reducing the subject's Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI) score compared to the subject's CLASI score pre-treatment, reducing the subject's tender and swollen joint count compared to the subject's tender and swollen joint count pre-treatment, the subject having a maximum of 1 BILAG-2004 B score following treatment, the subject having a BILAG-2004 score of C or better following treatment, the subject having an improvement in at least one patient reported outcome (PRO) compared to pre-treatment, and/or reducing the subject's SLE flare rate compared to the subject's flare rate pre-treatment.

38. The method of any of embodiments 18-24 and 34-36, wherein reducing SLE disease activity in a subject comprises: the subject achieves clinical remission as defined by The Definitions of Remission in Systemic Lupus Erythematosus (DORIS), and/or the subject achieves Lupus Low Disease Activity State (LLDAS).

39. The method of any of embodiments 2-33 and 37-38, wherein the subject achieves clinical remission of the lupus within 3 months or within 6 months of administering the dose of CD19-directed genetically modified T cells.

40. The method of embodiment 38 or embodiment 39, wherein the clinical remission is maintained for at least about 6 months, at least about 12 months, at least about 24 months, at least about 3 years, at least about 4 years, or at least about 5 years.

41. The method of any of embodiments 2-33 and 36-40, wherein the subject achieves prolonged remission of the lupus.

42. A method of treating a subject having idiopathic inflammatory myopathy (IIM), the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having idiopathic inflammatory myopathy (IIM), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

43. A method for reducing idiopathic inflammatory myopathy (IIM) disease activity, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having idiopathic inflammatory myopathy (IIM), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

44. The method of embodiment 42 or embodiment 43, wherein the subject is refractory to treatment with one or more prior therapies for the IIM.

45. The method of embodiment 42 or embodiment 43, wherein the subject achieved an insufficient response to one or more prior therapies for the IIM.

46. A method of treating a subject having systemic sclerosis (SSc), the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having systemic sclerosis (SSc), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

47. A method for reducing systemic sclerosis (SSc) disease activity, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having systemic sclerosis (SSc), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

48. The method of embodiment 46 or embodiment 47, wherein the subject is refractory to treatment with one or more prior therapies for the SSc.

49. The method of embodiment 46 or embodiment 47, wherein the subject achieved an insufficient response to one or more prior therapies for the SSc.

50. A method of treating a subject having multiple sclerosis (MS), the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having multiple sclerosis (MS), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

51. A method for reducing multiple sclerosis (MS) disease activity, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having multiple sclerosis (MS), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

52. The method of embodiment 50 or embodiment 51, wherein the subject is refractory to treatment with one or more prior therapies for the MS.

53. The method of embodiment 50 or embodiment 51, wherein the subject achieved an insufficient response to one or more prior therapies for the MS.

54. The method of any of embodiments 50-53, wherein the subject has or is suspected of having highly active relapse-remitting MS.

55. The method of any of embodiments 50-53, wherein the subject has or is suspected of having primary progressive MS.
56. The method of any of embodiments 37-40, wherein the subject has or is suspected of having active secondary progressive MS (aSPMS).
57. The method of any of embodiments 50-53, wherein the subject has or is suspected of having inactive secondary progress MS (iSPMS).
58. The method of any of embodiments 50-57, wherein the subject has an Expanded Disability Status Scale (EDSS) of ≥ 3.0 and ≤ 5.5 or of ≥ 3.0 and ≤ 6.0 .
59. The method of any of embodiments 50-58, wherein the subject can complete the 9-Hole Peg Test (9-HPT) for each hand in <240 seconds, and subjects can perform a Timed 25-Foot Walk Test (T25FWT) in < 150 seconds.
60. The method of any of embodiments 50-59, wherein the subject does not have MS lesions or symptoms that may place them at increased risk of neurotoxicity.
61. The method of any of embodiments 39-60, wherein the method reduces the autoimmune disease activity in the subject.
62. The method of any of embodiments 32-61, wherein reducing disease activity in the subject comprises a reduced inflammation in the subject.
63. The method of embodiment 61, wherein the reducing the autoimmune disease activity in the subject comprises reducing the subject's IMACS score after treatment compared to the subject's IMACS score before treatment, reducing the subject's skin lesions, muscle fatigue, and/or weakness compared to the subject's skin lesions, muscle fatigue, and/or weakness pre-treatment, or the subject having an improvement in at least one patient reported outcome (PRO) compared to pre-treatment.
64. The method of embodiment 62, wherein the reducing the autoimmune disease activity in the subject comprises reducing the subjects modified Rodnan skin score, European Scleroderma Study Group (EScSG) indices, minimum clinically important differences (MCID), patient reported short-form quality of life assessment (SF-36) Physical Component Summary (PCS) and/or Mental Component Summary (MCS) or a combination thereof or improving forced vital capacity.
65. The method of embodiment 63, wherein the reducing the autoimmune disease activity in the subject comprises improving the subjects score in any of the following tests; expanded disability status scale (EDSS), disease steps, multiple sclerosis functional composi

(MSFC), minimum clinically important differences (MCID), patient reported short-form quality of life assessment (SF-36) Physical Component Summary (PCS) and/or Mental Component Summary (MCS) or a combination thereof.

66. A method of treating a subject having autoimmune vasculitis (AAV), the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having autoimmune vasculitis (AAV), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

67. A method for reducing autoimmune vasculitis (AAV) disease activity, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having autoimmune vasculitis (AAV), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

68. A method of treating a subject having IgA nephropathy, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having IgA nephropathy, wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

69. A method for reducing IgA nephropathy disease activity, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having IgA nephropathy, wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

70. A method of treating a subject having pemphigus vulgaris, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having pemphigus vulgaris, wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

71. A method for reducing pemphigus vulgaris disease activity, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having pemphigus vulgaris, wherein the T cells of the dose are positive

for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

72. A method of treating a subject having myasthenia gravis, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having myasthenia gravis, wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

73. A method for reducing myasthenia gravis disease activity, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having myasthenia gravis, wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

74. The method of any of embodiments 1-73, wherein the dose is at or about 1×10^6 to 40×10^6 CAR-positive viable T cells.

75. The method of any of embodiments 1-73, wherein the dose is at or about 1×10^6 to 25×10^6 CAR-positive viable T cells.

76. The method of any of embodiments 1-73, wherein the dose is at or about 5×10^6 CAR-positive viable T cells.

77. The method of any of embodiments 1-73, wherein the dose is at or about 10×10^6 CAR-positive viable T cells.

78. The method of any of embodiments 1-73, wherein the dose is at or about 25×10^6 CAR-positive viable T cells.

79. The method of any of embodiments 1-73, wherein the dose is at or about 50×10^6 CAR-positive viable T cells.

80. The method of any of embodiments 1-79, wherein the T cells are autologous to the subject.

81. The method of any of embodiments 1-79, further comprising obtaining a leukapheresis sample from the subject for manufacturing the composition comprising engineered T cells.

82. The method of any of embodiments 1-81, wherein prior to the administration, the subject has been preconditioned with a lymphodepleting therapy.

83. The method of any of embodiments 1-82, wherein the method further comprises, immediately prior to the administration of the dose of CD19-directed genetically modified T cells, administering a lymphodepleting therapy to the subject, wherein the lymphodepleting therapy comprises the administration of fludarabine and/or cyclophosphamide.

84. The method of any of embodiments 1-83, wherein the administration of the dose of CD19-directed genetically modified T cells and/or the lymphodepleting therapy is carried out via outpatient delivery.

85. The method of any of embodiments 82-84, wherein the lymphodepleting therapy comprises the administration of fludarabine at 30 mg/m² body surface area of the subject, daily, and cyclophosphamide at 300 mg/m² body surface area of the subject, daily, each for 3 days.

86. The method of any of embodiments 82-84, wherein the dose of CD19-directed genetically modified T cells is administered between at or about 48 hours and at or about 9 days, inclusive, after completion of the lymphodepleting therapy.

87. The method of any of embodiments 1-86, wherein the dose of CD19-directed genetically modified T cells is administered to the subject by intravenous infusion.

88. The method of any of embodiments 1-87, wherein the CAR comprises an extracellular antigen-binding domain that binds CD19, a transmembrane domain, and an intracellular signaling domain.

89. The method of embodiment 88, wherein the CAR comprises a hinge spacer between the extracellular antigen-binding domain and the transmembrane domain, optionally wherein the hinge spacer is an immunoglobulin hinge or a CD8a hinge.

90. The method of embodiment 88 or embodiment 89, wherein the extracellular antigen-binding domain is an FMC63 monoclonal antibody-derived single chain variable fragment (scFv).

91. The method of any of embodiments 88-90, wherein the extracellular antigen-binding domain comprises a variable heavy chain set forth in SEQ ID NO:41 and a variable light chain set forth in SEQ ID NO:42.

92. The method of embodiment 90 or embodiment 91, wherein the scFv is set forth as SEQ ID NO: 43.

93. The method of embodiment 88 or embodiment 89, wherein the extracellular antigen-binding domain is a Hu19 single chain variable fragment (scFv).

94. The method of any one of embodiments 88, 89 and 93, wherein the extracellular antigen-binding domain comprises a variable heavy chain set forth in SEQ ID NO:114 and a variable light chain set forth in SEQ ID NO:112.

95. The method of embodiment 93 or 94, wherein the extracellular antigen-binding domain comprises in order a variable light chain set forth in SEQ ID NO: 112, a linker peptide set forth in SEQ ID NO: 113, and a variable heavy chain set forth in SEQ ID NO: 114.

96. The method of any one of embodiments 1-95, wherein the CAR is a monospecific CAR directed to CD19.

97. The method of any of embodiments 1-95, wherein the CAR is a tandem bispecific CAR directed against CD19 and at least one other antigen expressed on B cells.

98. The method of embodiment 97, wherein the other antigen expressed on B cells is selected from the group consisting of CD20, CD19, CD22, ROR1, BCMA, CD45, CD21, CD5, CD33, Igkappa, Iglambda, CD79a, CD79b or CD30.

99. The method of embodiment 97 or embodiment 98, wherein the other antigen expressed on B cells is CD20.

100. The method of embodiment 99, wherein the extracellular antigen-binding domain comprises a variable heavy chain and a variable light chain derived from a CD20 antibody selected from the group consisting of Leu16, C2B8, 11B8, 8G6-5, 2.1.2 and GA101.

101. The method of any of embodiments 88-100, wherein the transmembrane domain is a CD28 transmembrane domain.

102. The method of any of embodiments 88-101, wherein the transmembrane domain is a transmembrane domain from CD28, optionally a transmembrane domain that comprises the sequence of amino acids set forth in SEQ ID NO: 8 or a sequence of amino acids that exhibits at least or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:8.

103. The method of any of embodiments 99-102, wherein the intracellular signaling domain comprises a 4-1BB costimulatory domain and a CD3zeta activation domain.

104. The method of any of embodiments 1-103, wherein the CAR comprises, in order from N- to C-terminus, an FMC63 monoclonal antibody-derived single chain variable fragment (scFv), IgG4 hinge region, a CD28 transmembrane domain, a 4-1BB (CD137) costimulatory domain, and a CD3 zeta signaling domain.

105. The method of embodiment 103 or embodiment 104, wherein the 4-1BB costimulatory domain is or comprises the sequence set forth in SEQ ID NO: 12 or a variant thereof having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:12.

106. The method of any of embodiments 103-105, wherein the CD3zeta signaling domain is or comprises the sequence set forth in SEQ ID NO: 13, 14 or 15 or a sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto.

107. The method of any of embodiments 1-106, wherein the CAR contains in order from N-terminus to C-terminus: an extracellular antigen-binding domain that is the scFv set forth in SEQ ID NO: 43, the spacer set forth in SEQ ID NO:1, the transmembrane domain set forth in SEQ ID NO:8, the 4-1BB costimulatory signaling domain set forth in SEQ ID NO:12, and the signaling domain of a CD3-zeta (CD3 ζ) chain set forth in SEQ ID NO:13.

108. The method of any of embodiments 1-107, wherein the CAR comprises the amino acid sequence set forth in SEQ ID NO:59 or a sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto.

109. The method of any of embodiments 1-108, wherein the composition produced by a manufacturing process comprising:

(i) stimulating an input composition comprising primary T cells from the subject with an oligomeric stimulatory reagent, thereby generating a stimulated population, wherein the oligomeric stimulatory reagent comprises a plurality of cross-linked tetramers of a streptavidin or streptavidin mutein and wherein the streptavidin or streptavidin mutein are reversibly bound to a first agent comprising an anti-CD3 antibody or antigen binding fragment thereof and a second agent comprising an anti-CD28 antibody or antigen binding fragment thereof;

(ii) introducing into T cells of the stimulated population, a heterologous polynucleotide encoding the CAR that targets CD19, thereby generating a population of transformed cells;

(iii) incubating the population of transformed cells for up to 96 hours; and

(iv) harvesting T cells of the population of transformed cells, thereby producing a composition of CD19-directed genetically modified T cells wherein the harvesting is carried out at a time between 24 and 120 hours, inclusive, after the exposing to the stimulatory reagent is initiated.

110. The method of embodiment 109, wherein the anti-CD3 antibody or antigen binding fragment is a Fab and the anti-CD28 antibody or antigen binding fragment is a Fab.

111. The method of embodiment 109 or embodiment 110, wherein the first agent and the second agent each comprise a streptavidin-binding peptide that reversibly binds the first agent and the second agent to the oligomeric particle reagent, optionally wherein the streptavidin-binding peptide comprises the sequence of amino acids set forth in any of SEQ ID NOS:78-82.

112. The method of any of embodiments 109-111, wherein the streptavidin mutein molecule is a tetramer of a streptavidin mutein comprising amino acid residues Val44-Thr45-Ala46-Arg47 or Ile44-Gly45-Ala46-Arg47, optionally wherein the streptavidin mutein comprises the sequence set forth in any of SEQ ID NOS: 69, 84, 87, 88, 90, 85 or 59.

113. The method of any of embodiments 109-112, wherein the oligomeric particle reagent comprises between 1,000 and 5,000 streptavidin mutein tetramers, inclusive.

114. The method of any of embodiments 109-113, wherein the method further comprises, prior to harvesting the cells, adding biotin or a biotin analog after or during the incubation.

115. The method of any of embodiments 109-114, wherein the harvesting is carried out at a time between 48 and 120 hours, inclusive, after the exposing to the stimulatory reagent is initiated.

116. The method of any of embodiments 1-115, wherein the dose of autologous CD19-directed genetically modified T cells is cryopreserved prior to administration to the subject.

117. The method of embodiment 116, wherein the cryopreserved dose of autologous CD19-directed genetically modified T cells is thawed prior to administration to the subject.

118. The method of embodiment 117, wherein the dose of autologous CD19-directed genetically modified T cells is administered to the subject within about two hours of being thawed.

119. The method of any of embodiments 1-118, wherein the dose of autologous CD19-directed genetically modified T cells is provided in a formulation comprising a cryoprotectant.

120. The method of embodiment 119, wherein the formulation comprises dimethylsulfoxide (DMSO).

121. The method of embodiment 119 or embodiment 120, wherein the formulation comprises albumin, optionally human albumin.

122. The method of any of embodiments 1-121, wherein the dose of T cells comprises CD4⁺ T cells expressing the CAR and CD8⁺ T cells expressing the CAR at a ratio between about 1:5 and about 5:1.

123. The method of any of embodiments 1-122, wherein the dose of T cells comprises CD4⁺ T cells expressing the CAR and CD8⁺ T cells expressing the CAR at a ratio between about 1:3 and about 3:1.

124. The method of any of embodiments 1-123, wherein at least or at least about 90% of the cells in the composition are CD3⁺ cells.

125. The method of any of embodiments 1-124, wherein at least or at least about 91%, at least or at least about 92%, at least or at least about 93%, at least or at least about 94%, at least or at least about 95%, or at least or at least about 96% of the cells in the composition are CD3⁺ cells.

126. The method of any of embodiments 1-125, wherein at least 25% of the T cells in the composition are CAR⁺ T cells.

127. The method of any of embodiments 1-126, wherein at least 30%, at least 35%, at least 40%, at least 45% or at least 50% of the T cells in the composition are CAR⁺ T cells.

128. The method of any of embodiments 1-127, wherein between at or about 5% and at or about 30% of the CAR⁺ T cells in the composition express a marker of apoptosis, optionally between at or about 10% and at or about 15% of the CAR⁺ T cells in the composition, more optionally wherein the marker of apoptosis is Annexin V or active Caspase 3.

129. The method of any of embodiments 1-125, wherein less than 10% of the T cells, optionally the CAR⁺ T cells, in the composition express a marker of apoptosis, optionally wherein the marker of apoptosis is Annexin V or active Caspase 3.

130. The method of any of embodiments 1-125 and 129, wherein less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, or less than 4% of the T cells, optionally the CAR⁺ T cells, in the composition express a marker of apoptosis, optionally wherein the marker of apoptosis is Annexin V or active Caspase 3.

131. The method of any of embodiments 1-130, wherein at least 80% of the T cells in the composition are viable T cells, optionally wherein viability is determined by staining for acridine orange (AO) and propidium iodide (PI).

132. The method of any of embodiments 1-128, wherein at least or at least about 80% of the CAR⁺ T cells in the composition are of a naïve-like or central memory phenotype.

133. The method of embodiment 132, wherein the marker expressed on naïve-like or central memory T cell is selected from the group consisting of CD45RA, CD27, CD28, and CCR7.

134. The method of any of embodiments 1-133, wherein at least 85% of the CAR⁺ T cells in the composition are CCR7⁺.

135. The method of any of embodiments 1-134, wherein at least 85% of the CD8⁺CAR⁺ T cells in the composition are CCR7⁺ and at least 90% of the CD4⁺ CAR⁺ T cells in the composition are CCR7⁺.

136. The method of any of embodiments 1-135, wherein 85% to 98% of the CD8⁺ CAR⁺ T cells in the composition are CCR7⁺ and 94% to 99% of the CD4⁺ CAR⁺ T cells in the composition are CCR7⁺.

137. The method of any of embodiments 1-133, wherein the at least or at least about 80% of the CAR⁺ T cells in the composition that are of a naïve-like or central memory phenotype have a phenotype selected from one or more of phenotypes CCR7⁺CD45RA⁺, CCR7⁺CD45RA⁻, CD27⁺CCR7⁺, or CD62L⁻CCR7⁺.

138. The method of any of embodiments 1-137, wherein at least 40% of the CAR⁺ T cells in the composition are CD45RA⁺CCR7⁺.

139. The method of any of embodiments 1-138, wherein at least 50% of the CAR⁺ T cells in the composition are CD45RA⁺CCR7⁺.

140. The method of any of embodiments 1-139, wherein at least 60% of the CAR⁺ T cells in the composition are CD45RA⁺CCR7⁺.

141. The method of any of embodiments 1-140, wherein at least 70% of the CAR⁺ T cells in the composition are CD45RA⁺CCR7⁺.

142. The method of any of embodiments 1-141, wherein at least 80% of the CAR⁺ T cells in the composition are CD45RA⁺CCR7⁺.

143. The method of any of embodiments 1-142, wherein at least 20% of the CAR⁺ T cells in the composition are CD45RA⁻CCR7⁺.

144. The method of any of embodiments 1-143, wherein at least 30% of the CAR⁺ T cells in the composition are CD45RA⁻CCR7⁺.

145. The method of any of embodiments 1-144, wherein at least 40% of the CAR+ T cells in the composition are CD45RA-CCR7+.

146. The method of any of embodiments 1-145, wherein at least 50% of the CAR+ T cells in the composition are CD45RA-CCR7+.

147. The method of any of embodiments 1-146, wherein at least 60% of the CAR+ T cells in the composition are CD45RA-CCR7+.

148. The method of any of embodiments 1-147, wherein at least about 50% of CD4+CAR+ T cells in the composition are CCR7+CD45RA⁻.

149. The method of any of embodiments 1-147, wherein at least about 60% of CD4+CAR+ T cells in the composition are CCR7+CD45RA⁻.

150. The method of any of embodiments 1-147, wherein at least about 70% of CD4+CAR+ T cells in the composition are CCR7+CD45RA⁻.

151. The method of any of embodiments 1-150, wherein at least about 30% of CD8+CAR+ T cells in the composition are CCR7+CD45RA⁻.

152. The method of any of embodiments 1-150, wherein at least about 40% of CD8+CAR+ T cells in the composition are CCR7+CD45RA⁻.

153. The method of any of embodiments 1-150, wherein at least about 50% of CD8+CAR+ T cells in the composition are CCR7+CD45RA⁻.

154. The method of any of embodiment 1-153, wherein greater than or greater than about 50%, about 60%, about 70%, or about 80% of the subjects treated according to the method do not exhibit any grade of cytokine release syndrome (CRS).

155. The method of any of embodiment 1-154, wherein greater than or greater than about 40%, 50%, or about 60% of the subjects treated according to the method do not exhibit any grade of neurotoxicity.

156. The method of any of embodiments 1-155, wherein the subject is human.

157. The method of any of embodiments 1-156, wherein: (i) at least 60% of the T cells in the composition are viable, (ii) at least 25% of the T cells of the composition are CAR+ T cells; (iii) less than 10% of the cells of the composition are positive for an apoptotic marker, optionally wherein the marker of apoptosis is Annexin V or active Caspase 3; (iv) at least 85% of the CD8+CAR+ T cells in the composition are CCR7+; and/or (v) at least 90% of the CD4+CAR+ T cells in the composition are CCR7+.

158. The method of any of embodiments 1-157, wherein: (i) at least 80% of the T cells in the composition are viable, (ii) at least 45% of the T cells of the composition are CAR+, (iii) less than 4% of the cells of the composition are positive for an apoptotic marker, optionally wherein the marker of apoptosis is Annexin V or active Caspase 3; (iv) at least 85% of the CD8+CAR+ T cells in the composition are CCR7+; and/or (v) at least 90% of the CD4+ CAR+ T cells in the composition are CCR7+.

159. The method of any of embodiments 1-156, wherein: (i) at least 60% of the T cells in the composition are viable, (ii) at least 25% of the T cells of the composition are CAR+, (iii) less than 10% of the cells of the composition are positive for an apoptotic marker, optionally wherein the marker of apoptosis is Annexin V or active Caspase 3; (iv) and/or greater than at or about 40% of the CAR+ T cells in the composition are CCR7+CD45RA+.

160. The method of any of embodiments 1-156, wherein: (i) at least 80% of the T cells in the composition are viable; (ii) at least 45% of the T cells of the composition are CAR+; (iii) less than 4% of the cells of the composition are positive for an apoptotic marker, optionally wherein the marker of apoptosis is Annexin V or active Caspase 3; and/or (iv) at least 40% of the CAR+ T cells in the composition are CCR7+CD45RA+.

161. The method of any of embodiments 1-156, wherein: (i) at least 60% of the T cells in the composition are viable; (ii) at least 25% of the T cells of the composition are CAR+; (iii) less than 10% of the cells of the composition are positive for an apoptotic marker, optionally wherein the marker of apoptosis is Annexin V or active Caspase 3; and/or (iv) greater than 20% of the CAR+ T cells in the composition are CCR7+CD45RA-.

162. The method of any of embodiments 1-156, wherein: (i) at least 80% of the T cells in the composition are viable; (ii) at least 45% of the T cells of the composition are CAR+; (iii) less than 4% of the cells of the composition are positive for an apoptotic marker, optionally wherein the marker of apoptosis is Annexin V or active Caspase 3; and/or (iv) at least 20% of the CAR+ T cells in the composition are CCR7+CD45RA-.

VI. EXAMPLES

[0847] The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

Example 1 T cell compositions containing CAR+ T cells generated using non-expanded processes.

[0848] Engineered compositions of primary T cells containing T cells expressing an anti-CD19 chimeric antigen receptor (CAR) were produced by a process that utilized a stimulatory reagent composed of a soluble anti-CD3/anti-CD28 Fab conjugated oligomeric reagent in which anti-CD3/CD28 Fab fragments are linked to a recombinant streptavidin mutein (Streptactin) backbone (*e.g.*, PCT publication No. WO2018197949) to activate T cells prior to transduction with a viral vector but that did not involve a subsequent step for expansion of the transduced cells. The non-expanded process did not contain a cultivation step after transduction for the purpose of increasing (*e.g.*, expanding) the total number of viable cells at the end of the cultivation step compared to the beginning of the cultivation step. Although the incubating conditions were not carried out for purposes of expanding the cell population, the composition as harvested might have undergone expansion or exhibit an increase in cell number compared to the beginning of the incubation. For comparison, T cells from the same donor were engineered by a process in which cells were cultivated for purposes of expansion after transduction. The generated CD19-targeted CAR therapeutic T cell compositions were assessed for phenotype and activity.

[0849] All processes included engineering of T cells with the same CD19-targeted CAR. The CAR contained an anti-CD19 scFv (*e.g.*, SEQ ID NO:43), an immunoglobulin-derived spacer (*e.g.*, SEQ ID NO:1), a transmembrane domain derived from CD28 (*e.g.*, SEQ ID NO:8), a costimulatory region derived from 4-1BB (*e.g.*, SEQ ID NO:12), and a CD3-zeta intracellular signaling domain (*e.g.*, SEQ ID NO:13). A sequence of an exemplary anti-CD19 CAR used in this study is set forth in SEQ ID NO: 91. A lentiviral vector containing the anti-CD19 CAR transgene for transduction of T cells. The polynucleotide encoding the CAR contained in the viral vector further contained sequences encoding a truncated receptor, which served as a surrogate marker for CAR expression; separated from the CAR sequence by a T2A ribosome skip sequence.

A. Generation of CD19-targeted CAR T cell compositions.

[0850] In the non-expanded process, leukapheresis samples were collected from human donors, washed, and cryopreserved. The cryopreserved samples were thawed before separate compositions of CD4+ and CD8+ cells were selected from each sample by immunoaffinity-

based selection. The selected CD4+ and CD8+ T cell compositions were mixed at up to 900×10^6 total viable CD4+ and CD8+ T cells, and typically at an approximate 1:1 ratio, prior to stimulation incubation for 16-24 hours with anti-CD3/anti-CD28 Fab conjugated oligomeric streptavidin mutein reagents in a serum-free complete media containing recombinant IL-2 (100 IU/mL) recombinant IL-7 (600 IU/mL) and recombinant IL-15 (100 IU/mL). After stimulation, cells were transduced by spinoculation with a lentiviral vector encoding the anti-CD19 CAR. After the spinoculation, the cells resuspended in a basal serum-free media without the addition of recombinant cytokines and incubated at about 37.0 °C in an incubator. After about 48 hours after initiation of stimulation, D-biotin was then added and mixed with the cells to dissociate anti-CD3 and anti-CD28 Fab reagents from oligomeric streptavidin reagent. The cells were further incubated for another 48 hours (until about 4 days after initiation of stimulation), and then were formulated with a cryoprotectant.

[0851] For comparison to an expanded process, engineered CD4+ T cells and engineered CD8+ T cells each expressing the same anti-CD19 CAR were produced by a process involving subjecting enriched CD4+ and enriched CD8+ cell populations, separately, to process steps, including separate selection, cryopreservation, stimulation, transduction, expansion, and harvesting steps. Leukapheresis samples were collected from human donors, washed, and subjected to immunoaffinity-based selection for CD4+ and CD8+ T cell compositions. After selection, the separate CD4+ and CD8+ T cell compositions were activated with anti-CD3/anti-CD28 paramagnetic beads in serum-free complete media containing recombinant IL-2 and recombinant IL-15 (and additionally recombinant IL-7 for the CD4+ T cell composition), and then were separately subjected to lentiviral transduction with a vector encoding the anti-CD19 CAR. Paramagnetic beads were removed, and then transduced populations were separately incubated in the presence of recombinant IL-2 and recombinant IL-15 (and additionally recombinant IL-7 for the CD4+ T cell composition) and were cultivated in a rocking motion bioreactor for cell expansion until reaching a threshold of expansion, such as between 9 and 13 days from start of expansion. The cells were then separately harvested, formulated, and cryofrozen.

B. Memory T cell composition between CAR T cells produced with different manufacturing processes

[0852] *In vitro* studies were performed with manufactured anti-CD19 CAR+ T cells produced from the non-expanded process compared to donor matched anti-CD19 CAR+ T cells produced from the expanded process to determine the memory state of cells made from each process. CAR T cells were produced from 4 donors using both processes for all donors and the CAR T cells were stained for cell-surface expression of tyrosine phosphatase CD45RA and the homing receptor CCR7 on CAR+/CD4+ and CAR+/CD8+ T cells. Conventional T cell memory subtypes were categorized as follows: naïve-like cells were CD45RA+CCR7+, central memory cells were CD45RA-CCR7+, effector/effector memory cells were CD45RA-CCR7-, terminally differentiated effector memory re-expressing CD45RA (temra) cells were CD45RA+CCR7-. The CAR+ T cells from the non-expanded engineering process had high proportions of naïve-like and central memory with concomitant lower proportions of effector memory subtypes compared with CAR T cells produced using the expanded engineering process in both CAR+/CD4+ T cells (**FIG. 1A, *p<0.05**) and CAR+/CD8+ T cells (**FIG. 1B, *p<0.05**). These results support that the engineered cell compositions generated from the non-expanded process have a greater portion of cells with a naïve-like, less differentiated phenotype than cell compositions generated from the expanded process.

C. CAR T cell expansion potential between different manufacturing processes

[0853] CAR T cell population persistence in response to continued encounter with cognate antigen was shown to enable sustained generation of effector T cells and possible establishment of long-lived memory T cell populations. To determine the expansion potential of CAR T cell drug product from the non-expanded process compared to donor matched CAR T cell drug product produced from expanded process, absolute counts of live cells were measured every other day when cells were stimulated *in vitro* with microbeads coated with anti-idotype antibody for 10 days (**FIG. 2A-2C**). Fold expansion was calculated for each CAR T cell drug product by dividing the cell counts at each timepoint by the starting number of cells for each CAR T cell drug product (**FIG. 2A**). For each CAR T cell drug product, area under the curve was calculated from the fold expansion and donor matched CAR T cell drug products were plotted next to each other to compare which went through the largest fold expansion (**FIG. 2B, *p<0.05**) with statistical significance determined by a Mann-Whitney test. Fold expansion within each donor

comparing the drug products from the non-expanded process to the drug product of the expanded process was done by dividing the daily fold expansion for non-expanded drug product to the donor matched expansion process created drug product (**FIG. 2C**). Drug product produced without expansion process product exhibited robust expansion after exposure to CAR-specific stimulation, resulting in a 2- to 7-fold increase in expansion at the endpoint of the assay compared to donor-matched dual chain produced CAR+ T cells.

D. Cytokine production following stimulation

[0854] CAR T cell activated relies on antigen-specific stimulation for production and secretion of cytokines including IL-2, IFN γ , and TNF α . These cytokines are indicative of the level and multifunctional capacity of CAR+ T cell activity and provide complementary response that engage the endogenous innate and adaptive immune systems for antitumor immunity. Intracellular cytokine staining assays using flow cytometry were used to assess the frequency of CAR+ T cells producing IL-2, IFN γ , and TNF α after stimulation with microbeads coated with agonistic anti-idotype antibody.

[0855] Cytokine production was shown to be robust in both drug product made with the non-expanded engineering process and drug product made with the expanded engineering process and frequencies of cytokine positive cells were not significantly different between the two cell products across the four donors (**FIG. 3A-3D**).

E. Comparison of cytolytic activity of CAR T cells produced using different manufacturing processes

[0856] For functional assessment of cytolytic potential, anti-CD19 CAR T cell drug product from both the non-expanded engineering process and the expanded engineering process were interrogated for their ability to kill CD19-expressing fluorescent target cells (K562-CD19/NLR target cells) in an *in vitro* culture system at a ratio of 0.5:1 effector:target cell. Following manufacturing, all cells appeared highly potent in their ability to kill target cells with drug product from the non-expanded engineering process displaying a trend towards improved cytolytic potential with the % specific lysis displayed in **FIG. 4A**. This is reflected when the area under the curve for drug product from matched donors are plotted next to each other and for 3 of the donors, the drug product had a higher area under the curve when produced with the non-expanded engineering process (**FIG. 4B**) indicating that the drug product produced from the

non-expanded engineering process is either as cytolytic or slightly more cytolytic compared to drug product made from the expanded engineering process.

Example 2 Immune reset observed in patients treated with CAR T cells from non-expanded process

[0857] The anti-CD19 CAR T cell drug product from the non-expanded process described in Example 1 was used to treat human patients with relapsed or refractory (R/R) non-Hodgkins lymphoma (NHL). Following leukapheresis, patient T cells were purified and engineered followed by limited *ex vivo* expansion. Patients received a single infusion of either 10×10^6 or 25×10^6 cells of the anti-CD19 CAR T cell drug product after lymphodepleting chemotherapy, which constituted 3 days of fludarabine at 30 mg/m^2 and cyclophosphamide at 300 mg/m^2 . Transgene levels were measured in blood using droplet digital polymerase chain reaction and plotted using RStudio and is shown in **FIG. 5A**. The steady dotted line represents the limit of quantification which was 40 copies/ug. There was an increase in the transgene levels initially showing cellular expansion of the anti-CD19 CAR T cells in both doses of CAR T cells. **FIG. 5B** shows serum IgG and **FIG. 5C** shows serum IgA, with both showing a decrease in circulating immunoglobulin levels after administration of both doses CAR T cells. The steady dotted lines on **FIG. 5B** and **FIG. 5C** represent low normal immunoglobulin levels. Neutrophils, shown in **FIG. 5D**, lymphocytes, shown in **FIG. 5E**, and platelets, shown in **FIG. 5F**, all show a decrease in circulating cell levels with rebounds in cell levels by around 60 days post infusion with CAR T cells, demonstrating that the decrease in cell levels are transient and patients rapidly recover. This data demonstrates that hypogammaglobulinemia occurs indicating an immune reset (*i.e.*, the suppression of overactive B cells by the anti-CD19 CAR T cells with subsequent restoration of homeostatic immune system function) in subjects treated with the anti-CD19 CAR T cells. These results, including the observed hypogammaglobulinemia, support the use of the anti-CD19 CAR for treating systemic autoimmune diseases that have a B cell involvement, and clinical remission may be observed at similar doses in patients with autoimmune diseases such as SLE, IIM, SSc, or MS.

Example 3 Administration of Anti-CD19 CAR-Expressing Cells as a Monotherapy in severe, refractory Systemic lupus erythematosus (SLE) and lupus nephritis

[0858] Therapeutic CAR⁺ T cell compositions containing autologous T cells expressing a chimeric antigen-receptor (CAR) specific for CD19 are administered to subjects with severe, refractory systemic lupus erythematosus (SLE) and lupus nephritis.

[0859] Subjects with severe, refractory SLE (with or without lupus nephritis) are treated. Eligibility criteria include subjects with major organ disease ≥ 1 BILAG Grade A score or ≥ 2 with BILAG B moderate score. Subjects also have refractory SLE after an insufficient response to ≥ 2 treatments (*e.g.*, such as therapy with an anti-CD20 antibody (*e.g.*, rituximab), mycophenolate mofetil (MMF), cyclophosphamide (CYC), belimumab, rituximab, anifrolumab, azathioprine, methotrexate (mtx), cisplatin (CSP) and/or voclosporin).

[0860] The therapeutic CAR T cell compositions administered are generated by a process including immunoaffinity-based enrichment of CD4⁺ and CD8⁺ T cells from leukapheresis samples from the individual patients to be treated. The selected CD4⁺ and CD8⁺ T cell compositions are stimulated by incubation with anti-CD3/anti-CD28 Fab conjugated oligomeric streptavidin mutein reagent (see *e.g.*, WO2018/197949) in a serum-free complete media containing recombinant IL-2 (100 IU/mL) recombinant IL-7 (600 IU/mL) and recombinant IL-15 (100 IU/mL). After stimulation, cells are transduced with a viral vector encoding an anti-CD19 CAR (*e.g.*, SEQ ID NO:91). The CAR contains the FMC63 monoclonal antibody derived single chain variable fragment (scFv), IgG4 hinge region, CD28 transmembrane domain, 4-1BB (CD137) costimulatory domain, and CD3 zeta activation domain. The cells are incubated for 48 hours after the initiation of stimulation, and then D-biotin is added and mixed with the cells to dissociate anti-CD3 and anti-CD28 Fab reagents from oligomeric streptavidin reagents. The cells are further incubated for another approximately 48 hours, and then formulated with a cryoprotectant.

[0861] The CD4⁺ and CD8⁺ cryopreserved cell compositions are thawed prior to intravenous administration. Prior to CAR⁺ T cell infusion, subjects receive a lymphodepleting chemotherapy with fludarabine and cyclophosphamide for 3 days (*e.g.*, flu, 30 mg/m²/day; and Cy, 300mg/m²/day).

[0862] The subjects receive CAR-expressing T cells 2-7 days after lymphodepletion. Subjects are administered a single dose escalation of 10×10^6 CAR-expressing viable T cells, 25×10^6 CAR-expressing viable T cells, or 50×10^6 CAR-expressing viable T cells. The dose also

may be reduced so that subjects may also be administered a dose of 5×10^6 CAR-expressing viable T cells.

[0863] Primary endpoints include safety assessment, including development of cytokine release syndrome (CRS); neurotoxicity, including immune effector cell-associated neurotoxicity syndrome (ICANS); infections, including incidence of all grades as well as grade 3+ and severe infections; and blood and lymphatic events, including all grades, grade 3+ as well as incidences of neutropenia, anemia and thrombocytopenia. Secondary endpoints related to efficacy and pharmacokinetics (PK) are also assessed.

[0864] Study results demonstrate tolerable safety profiles including low grade CRS/ICANS, brief/reversible cytopenias, and low rate of severe infections. Subjects demonstrate high overall response rate (ORR), with deepening of responses to complete response rate (CRR) in majority of patients at 3-6 month follow-up. Pharmacodynamics data also are supportive of efficacy.

Example 4 Administration of Anti-CD19 CAR-Expressing Cells in severe, refractory Systemic Lupus Erythematosus (SLE)

[0865] Therapeutic CAR⁺ T cell compositions containing autologous T cells expressing a chimeric antigen-receptor (CAR) specific for CD19 are administered to subjects with severe, refractory systemic lupus erythematosus (SLE).

[0866] Subjects with severe, refractory SLE are treated. Eligibility criteria include subjects of all sexes being 18 years and older. Subjects are diagnosed with SLE, defined as followed: (1) fulfilling the 2019 American College of Rheumatology (ACR)/ European League Against Rheumatism (EULAR) classification criteria of SLE, and (2) having detectable anti-dsDNA, anti-histone, anti-chromatin or anti-Sm antibodies in their blood. Subjects also have active SLE disease at the time of screening, with major organ disease ≥ 1 BILAG Grade A score (excluding musculoskeletal, mucocutaneous and/or constitutional organ system diseases). Subjects also have SLE after an inadequate response to glucocorticoids, and an insufficient response to ≥ 2 treatments, used for at least 3 months each (*e.g.*, such as therapy with an anti-CD20 antibody (*e.g.*, rituximab), mycophenolic acid or its derivatives, cyclophosphamide (CYC), belimumab, rituximab, anifrolumab, azathioprine, methotrexate (mtx), cisplatin (CSP), obinutuzumab, cyclosporin, tacrolimus and/or voclosporin; methotrexate and azathioprine count as 1 for the purposes of the number of failed treatments). Insufficient response to treatments is

defined as a lack of response, insufficient response, or a lack of sustained response to appropriate doses. Intolerance is not considered insufficient response.

[0867] Exclusion criteria include subjects diagnosed with drug-induced SLE, as opposed to idiopathic SLE. Other systemic autoimmune diseases (eg, multiple sclerosis, psoriasis, inflammatory bowel disease, etc) are excluded. To note, however, subjects with type I autoimmune diabetes mellitus, thyroid autoimmune disease, Celiac disease, or secondary Sjögren's syndrome are not excluded from this study. Subjects also do not have SLE overlap syndromes, including, but not limited to, rheumatoid arthritis, scleroderma, and mixed connective tissue disease. Further, subjects have not recently, nor do they presently have clinically significant CNS pathology.

[0868] The therapeutic CAR T cell compositions administered are generated by a process including immunoaffinity-based enrichment of CD4⁺ and CD8⁺ T cells from leukapheresis samples from the individual patients to be treated, as described in Example 1. The selected CD4⁺ and CD8⁺ T cell compositions are stimulated by incubation with anti-CD3/anti-CD28 Fab conjugated oligomeric streptavidin mutein reagent (see *e.g.*, WO2018/197949) in a serum-free complete media containing recombinant IL-2 (100 IU/mL), recombinant IL-7 (600 IU/mL) and recombinant IL-15 (100 IU/mL). After stimulation, cells are transduced with a viral vector encoding an anti-CD19 CAR (*e.g.*, SEQ ID NO:91). The CAR contains the FMC63 monoclonal antibody derived single chain variable fragment (scFv), IgG4 hinge region, CD28 transmembrane domain, 4-1BB (CD137) costimulatory domain, and CD3 zeta activation domain. The cells are incubated for 48 hours after the initiation of stimulation, and then D-biotin is added and mixed with the cells to dissociate anti-CD3 and anti-CD28 Fab reagents from oligomeric streptavidin reagents. The cells are further incubated for another approximately 48 hours, and then formulated with a cryoprotectant.

[0869] The CD4⁺ and CD8⁺ cryopreserved cell compositions are thawed prior to intravenous administration. Subjects are administered a single dose of 10×10^6 CAR-expressing viable T cells, 25×10^6 CAR-expressing viable T cells, or 50×10^6 CAR-expressing viable T cells. The dose also may be reduced so that subjects may also be administered a dose of 5×10^6 CAR-expressing viable T cells. Prior to CAR⁺ T cell infusion, subjects receive a lymphodepleting chemotherapy with fludarabine and cyclophosphamide for 3 days (*e.g.*, flu, 30 mg/m²/day; and Cy, 300mg/m²/day). The subjects receive CAR-expressing T cells 2-7 days after lymphodepletion.

[0870] Primary endpoints include safety assessment, including a number of subjects developing treatment-emergent or serious adverse events (AEs), a number of subjects developing AEs of special interest, a number of subjects with laboratory abnormalities, a number of subjects with Dose Limiting Toxicities (DLT), and/or a recommendation for a Phase 2 dose.

[0871] Secondary endpoints related to efficacy and pharmacokinetics (PK) are also assessed, including, but not limited to, the list as follows: a proportion of subjects achieving remission, as defined by The Definitions of Remission in Systemic Lupus Erythematosus (DORIS), a proportion of subjects achieving Lupus Low Disease Activity State (LLDAS), a number of subjects experiencing a change in proteinuria measured by urine protein creatinine ratio (UPCR) or in the Health Assessment Questionnaire – Disability Index (HAQ-DI), a proportion of subjects achieving DORIS remission or LLDAS over time, a number of subjects experiencing a change in Systemic Lupus Erythematosus Disease Activity- 2000 (SLEDAI-2K) or in proteinuria measured by UPCR over time, the time to the first documentation of DORIS remission or LLDAS, the time from the CAR+ T cell infusion to the first disease flare, as monitored by the Safety of Estrogens in Lupus Erythematosus National Assessment-SLEDAI (SELENA-SLEDAI), the maximum observed blood concentration or time of the maximum observed blood concentration, and/or the area under the blood concentration-time curve curve from time zero to 28 days after dosing.

[0872] Study results are assessed to determine overall response rate (ORR) to the therapy. Safety profiles and pharmacodynamics data are also assessed.

Example 5 Administration of Anti-CD19 CAR-Expressing Cells in Multiple Sclerosis (MS)

[0873] Therapeutic CAR⁺ T cell compositions containing autologous T cells expressing a chimeric antigen-receptor (CAR) specific for CD19 are administered to subjects with Relapsing Forms of Multiple Sclerosis (RMS; cohort 1) or Progressive Forms of Multiple Sclerosis (PMS; cohort 2).

[0874] Subjects with RMS or PMS are treated. Eligibility criteria include subjects of all sexes being 18 years to 65 years old. In cohort 1 (RMS), subjects have an Expanded Disability Status Scale (EDSS) of ≥ 3.0 and ≤ 5.5 . Subjects also have a diagnosis of Multiple Sclerosis (MS) with relapsed/refractory MS or conversion to active secondary progressive multiple

sclerosis (aSPMS). Further, subjects have a worsening of disease within 12 months prior to screening and while on treatment with a high-efficacy disease modifying therapies (DMT) for at least 6 months. In cohort 2 (PMS), subjects have an Expanded Disability Status Scale (EDSS) of ≥ 3.0 and ≤ 6.0 . Subjects also have a diagnosis of primary progressive multiple sclerosis (PPMS) that is treatment-resistant or diagnosis of inactive secondary progressive multiple sclerosis (iSPMS).

[0875] Exclusion criteria include subjects that cannot complete the 9-Hole Peg Test (9-HPT) for each hand in <240 seconds, and subjects that cannot perform a Timed 25-Foot Walk Test (T25FWT) in < 150 seconds. Subjects also have not had MS lesions or symptoms that may place them at increased risk of neurotoxicity, including, but not limited to, tumefactive lesions (3 cm or greater within 5 years prior to Screening) or decreased level of consciousness, and/or presence of active, clinically significant concomitant central nervous system pathology other than MS that may confound the ability to interpret study results or complicate identification or evaluation of neurotoxicity.

[0876] The therapeutic CAR T cell compositions administered are generated by a process including immunoaffinity-based enrichment of CD4⁺ and CD8⁺ T cells from leukapheresis samples from the individual patients to be treated, as described in Example 1. The selected CD4⁺ and CD8⁺ T cell compositions stimulated by incubation with anti-CD3/anti-CD28 Fab conjugated oligomeric streptavidin mutein reagent (see *e.g.*, WO2018/197949) in a serum-free complete media containing recombinant IL-2 (100 IU/mL), recombinant IL-7 (600 IU/mL) and recombinant IL-15 (100 IU/mL). After stimulation, cells are transduced with a viral vector encoding an anti-CD19 CAR (*e.g.*, SEQ ID NO:91). The CAR contains the FMC63 monoclonal antibody derived single chain variable fragment (scFv), IgG4 hinge region, CD28 transmembrane domain, 4-1BB (CD137) costimulatory domain, and CD3 zeta activation domain. The cells are incubated for 48 hours after the initiation of stimulation, and then D-biotin is added and mixed with the cells to dissociate anti-CD3 and anti-CD28 Fab reagents from oligomeric streptavidin reagents. The cells are further incubated for another approximately 48 hours, and then formulated with a cryoprotectant.

[0877] The CD4⁺ and CD8⁺ cryopreserved cell compositions are thawed prior to intravenous administration. Subjects are administered a single dose of 10×10^6 CAR-expressing viable T cells, 25×10^6 CAR-expressing viable T cells, or 50×10^6 CAR-expressing viable T cells. The dose also may be reduced so that subjects may also be administered a dose of 5×10^6

CAR-expressing viable T cells. Prior to CAR+ T cell infusion, subjects receive a lymphodepleting chemotherapy with fludarabine and cyclophosphamide for 3 days (*e.g.*, flu, 30 mg/m²/day; and Cy, 300mg/m²/day). The subjects receive CAR-expressing T cells 2-7 days after lymphodepletion.

[0878] Primary endpoints include safety assessment, including a number of subjects developing treatment-emergent or serious adverse events (AEs), a number of subjects developing AEs of special interest, a number of subjects with laboratory and/or imaging abnormalities, a number of subjects with Dose Limiting Toxicities (DLT), and/or a recommendation for a Phase 2 dose.

[0879] Secondary endpoints related to efficacy and pharmacokinetics (PK) are also assessed, including, but not limited to, the list as follows: a number of subjects achieving no evidence of disease activity (NEDA), a number of subjects experiencing confirmed disability progression per the Expanded Disability Status Scale (EDSS), a number of subjects experiencing a change from baseline in magnetic resonance imaging (MRI) metrics, as assessed by 1) number of gadolinium-enhancing T1 lesions and 2) total number of new or enlarging hyperintense T2-weighted lesions, a number of subjects with disability improvement confirmed by EDSS, the maximum observed blood concentration or time of the maximum observed blood concentration, the area under the blood concentration-time curve from time zero to 28 days after dosing and/or the time to last measurable CAR-T concentrations (Tlast).

[0880] Study results are assessed to determine overall response rate (ORR) to the therapy. Safety profiles and pharmacodynamics data are also assessed.

Example 6 Methods of Manufacturing Engineered T Cells From Subjects With Systemic Lupus Erythematosus (SLE)

[0881] Leukapheresis samples were collected from healthy human donors or donors diagnosed with Systemic Lupus Erythematosus (SLE) (for engineering T cells in accord with methods described in Example 4), washed and cryopreserved. The cryopreserved samples were thawed and washed in a closed system centrifuge (*e.g.*, Sepax) and then selection reagents were added to the centrifuge as part of a closed system unit operation for selection of T cells by immunoaffinity-based selection. In this process, the selection reagents included equal volumes of anti-CD8 antibody conjugated to magnetic beads (CliniMACS® CD8 selection reagent;

Catalog Number 170-076-703, Miltenyi Biotec) and anti-CD4 antibody conjugated to magnetic beads (CliniMACS® CD4 selection reagent; Catalog No. 170-076-702, Miltenyi Biotec) for co-selection of CD4+ and CD8+ T cells in a simultaneous selection reaction. The anti-CD4 and anti-CD8 selection reagents were co-incubated with the T cells in the centrifuge at room temperature to produce CD8 bead/cell complexes and CD4 bead/cell complexes, washed to remove unbound material, and then the input material was transferred to a CliniMACS® System for magnetic positive selection of CD4+ and CD8+ T cells in three separate cycles in a closed-system unit operation, each cycle processing about 1/3 of the total input material. Selecting T cells in a single step, such as by co-selecting CD4+ and CD8+ T cells, improves manufacturing capacity, streamlines the process and reduces duration of the process, and reduces potential for deviations by eliminating several complex calculations and manipulation of cells prior to activation.

[0882] A target number of selected material composed of enriched CD8+ and CD4+ T cells of up to 900×10^6 total viable CD4+ and CD8+ T cells (i.e. $\leq 900 \times 10^6$ cells) were transferred to the centrifuge (*e.g.*, Sepax) in a closed unit operation for T cell activation. T cell activation was carried out by incubation for 16-24 hours in the centrifuge with an anti-CD3/anti-CD28 Fab conjugated oligomeric streptavidin mutein reagent (*e.g.*, Expamer®), and in a serum-free complete media (SFM+) containing recombinant IL-2 (100 IU/mL) recombinant IL-7 (600 IU/mL) and recombinant IL-15 (100 IU/mL). The resuspended cells were transferred individually in separate runs to a sterile output bag by a closed unit operation via an operably connected heat-sealed sterile docking tube on the bag, in which the sterile bag was a highly permeable bag, in particular, a VueLife® HP Bag, Saint-Gobain. The serum-free complete media (SFM+) was composed of a basal media (*e.g.*, CTS™ OpTmizer basal media, Thermo Fisher) to which was added 2 mM of a dipeptide form of L-glutamine, L-alanyl-L-glutamine (*e.g.*, Glutamax™, Thermo Fisher) and the following supplements: a T cell supplement (*e.g.*, 2.% OpTmizer® T-cell Expansion Supplement, Thermo Fisher), an immune cell serum replacement (*e.g.*, 2.4% CTS™ Immune Cell Serum Replacement), 2 mM L-glutamine, and the recombinant cytokines (100 IU/mL IL-2, 600 IU/mL IL-7 and 100 IU/mL IL-15).

[0883] After stimulation, a target number of up to cells 250×10^6 total viable CD4+ and CD8+ T cells (i.e. $\leq 250 \times 10^6$ cells) suspended in the serum-free media described above with the recombinant cytokines were transduced by spinoculation using with a lentiviral vector

encoding the CAR (*e.g.*, anti-CD19 CAR). In this example, a lentiviral vector encoding the same anti-CD19 CAR as described in Example 1 was used to transduce T cells.

[0884] After the spinoculation, the cells were resuspended in SFM+ serum-free media formulation for the post-transduction incubation. The resuspended cells were transferred to a sterile output bag by a closed unit operation via an operably connected heat-sealed sterile docking tube on the bag, in which the sterile bag was a highly permeable bag, in particular, a VueLife® HP Bag, Saint-Gobain. The cells were incubated in the bag under static conditions (without rocking or perfusion) in an incubator at 37°C and 5% CO₂ until 96 hours post-stimulation. Specifically, for the incubation in the bag, the cells were cultured under static conditions until approximately 48 hours post-stimulation at which time D-Biotin was sterilely added to the cell culture bag to dissociate anti-CD3 and anti-CD28 Fab reagents from oligomeric streptavidin reagent. The cell culture bag with D-Biotin was further incubated for approximately 48 hours, in which the cell culture bag was gently mixed about 24 hours after the addition of D-Biotin during the 48 hour period.

[0885] The cells were harvested from culture on Day 5 of the process, which was approximately 72 hours post-transduction or approximately 96 hours post-stimulation, except that cells were allowed to be harvested on Day 6 (about 120 hours post-activation) if a threshold target cell number of 75×10^6 viable cells had not been met on Day 5. Generally, harvest of the cells occurred approximately 90-126 hours post-activation.

[0886] For harvest, the incubated cells were pulled into a conical enclosure portion of a continuous counterflow centrifuge system (CTS Rotea™ Counterflow Centrifugation System) and subjected to a centrifugal force of 1000 g and a flow rate of 10 mL/min until 50×10^6 cells were added to the conical closure to establish a fluidized cell bed. The engineered cells were then subjected to a centrifugal force of 1000 g and a flow rate of 28.5 mL/min (62.5 G/FR). Under these conditions, the media was then exchanged into an isotonic infusion solution containing PlasmaLyte pH 7.4 and 4.8% (v/v) % human serum albumin as a “washing” step, allowing elutriation of non-viable cells. Washed product (WP) was then subsequently re-suspended in a formulation medium containing 75% v/v CryoStor® CS-10 media and 25% (v/v) of the isotonic solution to produce a formulated drug product (FDP) containing 7.5% (v/v) DMSO.

[0887] The number of doublings was determined based on total nuclear count (TNC) of cells, by dual-fluorescence staining with acridine orange (AO) and either propidium iodide (PI)

or DAPI, using the following formula, where “starting TNC” is the target number of cells after stimulation going into transduction:

$$PDL = \frac{\ln(Harvested\ TNC) - \ln(Starting\ TNC)}{\ln 2}$$

[0888] Cumulative population doublings (cPDL) was then calculated as:

$$cPDL = PDL_0 + PDL_1 + PDL_2 \dots$$

[0889] Cells produced by the above process from three healthy donors and one donor with SLE was characterized. **Table E1** shows the number of cumulative doublings of T cells produced by the above processes for the four different donor cells. The results are also shown in **FIG. 6A**. cPDL of engineered cells produced by the process was consistently observed in all donors (at about 1.8-fold or higher).

Table E1	
	Process
Mean cPDL	2.03 (Δ +0.78)
Min	1.80 (Δ +0.87)
Max	2.41 (Δ +0.70)

[0890] As shown in **FIG. 6B**, the percent of viable TNC as determined by AO/PI staining demonstrated similar percent of viable T cells produced by the process.

[0891] Without wishing to be bound by theory, it was believed that a higher population doubling during production of engineered T cells as well as presence of T stimulatory cytokines (*e.g.*, IL-2, IL-15 and IL-7) are factors that can negatively correlate with enrichment of T cells with an early differentiated profile composed of naïve-like T cells and central memory T cells. To assess impact of the process on differentiation of T cells, engineered T cell compositions at harvest were stained with antibodies recognizing surface markers including CCR7 and CD45RA, and quantified by flow cytometry. The percentage of T cells that were indicative of effector memory CD45RA+ cells (CCR7-CD45RA+; EMRA), naïve-like T cells (CCR7+CD45RA+; NAIVE), effector memory (CCR7-CD45RA-; EM) or central memory T cells (CCR7+CD45RA-; CM) are shown in **Table E2** in cells of an output composition produced from the SLE donor. Following the engineering process, the results show that T cells

generated from the process have a high percentage of CD45RA+CCR7+ naïve-like cells and CD45RA-CCR7+ cells (central memory) compared to the percentage of more differentiated effector memory CCR7-CD45RA- and effector memory CD45RA+ (CCR7-CD45RA+) cells in the cell product. This result surprisingly supports that a process that retains some increased expansion potential is able to support an engineered T cell product with a desired early differentiation phenotype profile.

Table E2: Phenotype of Output Composition

Phenotype	CD4+ [%]	CD8+ [%]
CD45RA+CCR7+	47.7	79
CD45RA-CCR7+	50.1	20.3
CD45RA-CCR7-	2	0.1
CD45RA+CCR7-	0.2	0.5

Example 7 Administration of Anti-CD19 CAR-Expressing Cells in Treatment of Systemic Lupus Erythematosus (SLE)

[0892] Two subjects diagnosed with severe, refractory systemic lupus erythematosus (SLE) were treated with autologous T cells expressing a chimeric antigen receptor (CAR) specific for CD19, using the method substantially as described in Example 4. Both subjects were also diagnosed with lupus nephritis (LN). Both patients had previously undergone treatment with various medications, including hydroxychloroquine, azathioprine, prednisone, rituximab, mycophenolate mofetil, voclosporin, and belimumab. Leukapheresis samples were collected from the two subjects and CD19 CAR T cells were produced according to the methods described in Example 6. Prior to CAR+ T cell infusion, subjects received a lymphodepleting chemotherapy with fludarabine and cyclophosphamide for 3 days (*e.g.*, flu, 30 mg/m²/day; and Cy, 300mg/m²/day). The subjects received bridging therapy (5-40 mg injections of prednisone and/or methylprednisolone) while the CAR+ T cells were generated according to Example 6.

[0893] The subjects were then treated with the drug product at a dose of 10×10^6 viable CAR+ CD4+ and CD8+ T cells. The SLEDAI-2K scores of the participants, which measures SLE disease activity within the last 10 days, are shown in **Table E3**.

Table E3

Patient #	SLEDAI score before treatment	Most recent SLEDAI-2K score post-treatment	New lupus activity since CAR-T infusion	Immunomodulator or corticosteroid treatment after CAR-T infusion
1 (Female)	16	4	No	No
2 (Male)	12	0	No	No

[0894] The participants experienced adverse events related to the study treatments, including cytopenias, alopecia, fever, lymphopenia, and cytokine release syndrome (CRS). However, none of these adverse events were considered serious.

[0895] Treating patients with rheumatologic disease to low level disease activity state³ (SLEDAI-2K < or = 4) or no disease activity^{1,2} (SLEDAI-2K = 0) and/or the ability to wean and maintain patients off all disease-treatment correlate with improvements in long term outcomes. The reduction in SLEDAI-2K score, observation of no new disease activity, and successful cessation of all lupus-directed immunomodulator and corticosteroid treatments following CAR-T infusion demonstrate that the treatment was efficacious.

¹ Franklyn *et al.*, Asia-Pacific Lupus Collaboration. Definition and initial validation of a Lupus Low Disease Activity State (LLDAS). *Ann Rheum Dis.* 2016 Sep;75(9):1615-21. doi: 10.1136/annrheumdis-2015-207726. Epub 2015 Oct 12. PMID: 26458737.

² van Vollenhoven *et al.*, A framework for remission in SLE: consensus findings from a large international task force on definitions of remission in SLE (DORIS) *Annals of the Rheumatic Diseases* 2017;76:554-561.

³ van Vollenhoven *et al.*, 2021 DORIS definition of remission in SLE: final recommendations from an international task force. *Lupus Sci Med.* 2021 Nov;8(1):e000538. doi: 10.1136/lupus-2021-000538. Erratum in: *Lupus Sci Med.* 2022 Feb;9(1): PMID: 34819388; PMCID: PMC8614136.

[0896] The present invention is not intended to be limited in scope to the particular disclosed embodiments, which are provided, for example, to illustrate various aspects of the invention. Various modifications to the compositions and methods described will become apparent from

the description and teachings herein. Such variations may be practiced without departing from the true scope and spirit of the disclosure and are intended to fall within the scope of the present disclosure.

SEQUENCES

SEQ ID NO.	SEQUENCE	DESCRIPTION
1	ESKYGPPCPPCP	spacer (IgG4hinge) (aa) Homo sapiens
2	GAATCTAAGTACGGACCGCCCTGCCCCCTTGCCCT	spacer (IgG4hinge) (nt) homo sapiens
3	ESKYGPPCPPCPGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGSSFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK	Hinge-CH3 spacer Homo sapiens
4	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGSSFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK	Hinge-CH2-CH3 spacer Homo sapiens
5	RWPESPKAQASSVPTAQPQAEGSLAKATTAPATTRNTGRGGEEKKKEKEKEEQEERETKTPECPSHTQPLGVYLLTPAVQDLWLRDKATFTCFVVGSDLKDAHLTWEVAGKVPTGGVEEGLLERHSNGSQSHSRLTLPRSLWNAGTSVTCTLNHPSLPPQRLMALREPAAQAPVKLSLNLLASSDPPEAASWLLCEVSGFSPPNILLMWLEDQREVNTSGFAPARPPPQPGSTTFWAWSVLRVPAPPSPQPATYTCVVSHEDSRTL LNARSLEVS YVTDH	IgD-hinge-Fc Homo sapiens
6	LEGGGEGRGSLLTCGDVEENPGPR	T2A artificial
7	MLLLVTSLLLCELPHPAFLIPRKVCNGIGIGEFKDSLSINATNIKHFKNCTISISGLHILPVAFRGDSFTHPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGQFSLAVVSLNITSLGLRSLKEISDGDVHISGNKNLCYANTINWKKLFGTSGQKTKIISNRGENSKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLEGEPREFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVMGENNTLVWKYADAGHVCHLCHPNCTYGCTGPGLEGCP TNGPKIPSIATGMVGALLLLVVALGIGLFM	tEGFR artificial
8	FWVLVVVGGVLACYSLLVTVAFIIFWV	CD28 (amino acids 153-179 of Accession No. P10747) Homo sapiens
9	IEVMYPPPPYLDNEKSNGTIIHVKGKHLCPSPFLPGPSKPFWVLVVGGVLACYSLLVTVAFIIFWV	CD28 (amino acids 114-179 of Accession No. P10747) Homo sapiens
10	RSKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS	CD28 (amino acids 180-220 of P10747) Homo sapiens

11	RSKRSRGGHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS	CD28 (LL to GG) Homo sapiens
12	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL	4-1BB (amino acids 214-255 of Q07011.1) Homo sapiens
13	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHD GLYQGLSTATKDTYDALHMQALPPR	CD3 zeta Homo sapiens
14	RVKFSRSAEPPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHD GLYQGLSTATKDTYDALHMQALPPR	CD3 zeta Homo sapiens
15	RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPE MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHD GLYQGLSTATKDTYDALHMQALPPR	CD3 zeta Homo sapiens
16	RKVCNGIGIGEFKDSLSINATNIKHFNCTSSISGDLHILPVAFRGDS FTHTPPLDPQELDILKTVEITGFLLIQAWPENRTDLHAFENLEIIR GRTKQHGGFSLAVVSLNITSLGLRSLKEISDGDVVISGNKNLCYAN TINWKKLFGTSGQKTKIISNRGENSCKATGQVCHALCSPEGCWGP EPRDCVSCRNVSRGRECVDKCNLLEGEPRFVENSECIQCHPECLP QAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVMGENNTLVW KYADAGHVCHLCHPNCTYGCTGPGLEGCPNTPKIPSIATGMVG ALLLLLVALGIGLFM	tEGFR artificial
17	EGRGSLLTCGDVEENPGP	T2A artificial
18	GSGATNFSLLKQAGDVEENPGP	P2A
19	ATNFSLLKQAGDVEENPGP	P2A
20	QCTNYALLKLAGDVESNPGP	E2A
21	VKQTLNFDLLKLAGDVESNPGP	F2A
22	PGGG-(SGGGG)5-P- wherein P is proline, G is glycine and S is serine	linker
23	GSADDAKKDAKKDGKS	Linker
24	GSTSGSGKPGSGEGSTKG	Linker
25	gacatccagatgacccagaccacccagcctgagcgcagcctggcgaccgggtgaccatcagct gccggggccagccaggacatcagcaagtacctaactggtatcagcagaagcccgacggcaccgtcaa gtgtgtgatctaccacaccagccggctgcacagcggcgtgcccagccgggttagcggcagcggctcc ggcaccgactacagcctgaccatctccaacctggaacaggaagatatgccacctactttgccagcag ggcaacacactgccctacacctttggcgcggaacaaagctggaatcaccggcagcacctccggca gcggcaagcctggcagcggcgaggcagcaccaaggcgaggtgaagctgcaggaagcggccc tggcctggtggccccagccagagcctgagcgtgacctgcaccgtgagcggcgtgagcctgcccagc tacggcgtgagctggatccggcagccccaggaaggcctggaatggctggcgtgatctggggca gcgagaccactactacaacagcgcctgaagagcggctgacctcatcaaggacaacagcaagag ccaggtttctgaagatgaacagcctgcagaccgacgacaccgccatctactactgcgccaagcacta ctactacggcgagctacgccatggactactggggccaggcaccagcgtgacctgagcagc	Sequence encoding scFv
26	X ₁ PPX ₂ P X ₁ is glycine, cysteine or arginine X ₂ is cysteine or threonine	Hinge
27	Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro	Hinge
28	Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro	Hinge
29	ELKTPLGDTHTCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPE PKSCDTPPPCPRCP	Hinge
30	Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro	Hinge
31	Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro	Hinge

32	Tyr Gly Pro Pro Cys Pro Pro Cys Pro	Hinge
33	Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro	Hinge
34	Glu Val Val Val Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro	Hinge
35	RASQDISKYLN	CDR L1
36	SRLHSGV	CDR L2
37	GNTLPYTFG	CDR L3
38	DYGVS	CDR H1
39	VIWGSETTYNSALKS	CDR H2
40	YAMDYWG	CDR H3
41	EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGL EWLGVIWGSETTYNSALKSRLTIKDNSKSQVFLKMNSLQTDDT AIYYCAKHYYYGGSYAMDYWGQGSVTVSS	VH
42	DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVK LLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGN TLPYTFGGGKLEIT	VL
43	DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVK LLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGN TLPYTFGGGKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGGLVA PSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTY YNSALKSRLTIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGG SYAMDYWGQGSVTVSS	scFv
44	KASQNVGTNVA	CDR L1
45	SATYRNS	CDR L2
46	QQYNRYPYT	CDR L3
47	SYWMN	CDR H1
48	QIYPGDGDTNYNGKFKG	CDR H2
49	KTISSVVDYFDY	CDR H3
50	EVKLQQSGAELVRPGSSVKISCKASGYAFSSYWMNWVKQRPGQ GLEWIGQIYPGDGDTNYNGKFKGQATLTADKSSSTAYMQLSGLT SEDSAVYFCARKTISSVVDYFDYWGQGTITVTVSS	VH
51	DIELTQSPKFMSTSVGDRVSVTCKASQNVGTNVAWYQQKPGQSP KPLIYSATYRNSGVPDRFTGSGSGTDFLTITNVQSKDLADYFCQQ YNRYPYTSGGGTKLEIKR	VL
52	GGGGSGGGSGGGGS	Linker
53	EVKLQQSGAELVRPGSSVKISCKASGYAFSSYWMNWVKQRPGQ GLEWIGQIYPGDGDTNYNGKFKGQATLTADKSSSTAYMQLSGLT SEDSAVYFCARKTISSVVDYFDYWGQGTITVTVSSGGGGSGGGG SGGGSDIELTQSPKFMSTSVGDRVSVTCKASQNVGTNVAWYQQ KPGQSPKPLIYSATYRNSGVPDRFTGSGSGTDFLTITNVQSKDLA DYFCQQYNRYPYTSGGGTKLEIKR	scFv
54	HYYYGGSYAMDY	CDR H3
55	HTSRLHS	CDR L2
56	QQGNTLPYT	CDR L3
57	ACACGGCCTCGTGTATTACTGT	IGH primer
58	ACCTGAGGAGACGGTGACC	IGH Primer
59	MEAGITGTWYNQLGSTFIVTAGADGALTGTY IGARG NAESRYVL TGRYDSAPATDGSGTALGWTVAWKNNYRNAHSATTWSGQYVG GAEARINTQWLLTSGTTEANAWKSTLVGHDTFTKVKPSAAS	Mutein Streptavidin Ile ⁴⁴ - Gly ⁴⁵ -Ala ⁴⁶ -Arg ⁴⁷ Species: Streptomyces avidinii

60	Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser	Variable Heavy chain of anti-CD3 antibody OKT3
61	Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala His Phe Arg Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Gly Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Asn	Variable Light chain of anti-CD3 antibody OKT3
62	Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser Val Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Glu Tyr Ile Ile His Trp Ile Lys Leu Arg Ser Gly Gln Gly Leu Glu Trp Ile Gly Trp Phe Tyr Pro Gly Ser Asn Asp Ile Gln Tyr Asn Ala Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Val Tyr Met Glu Leu Thr Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg Arg Asp Asp Phe Ser Gly Tyr Asp Ala Leu Pro Tyr Trp Gly Gln Gly Thr Met Val Thr Val	Variable Heavy chain of anti-CD28 antibody CD28.3
63	Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Val Ser Val Gly Glu Thr Val Thr Ile Thr Cys Arg Thr Asn Glu Asn Ile Tyr Ser Asn Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Leu Ile Tyr Ala Ala Thr His Leu Val Glu Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Thr Ser Leu Gln Ser Glu Asp Phe Gly Asn Tyr Tyr Cys Gln His Phe Trp Gly Thr Pro Cys Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg	Variable Light chain of anti-CD28 antibody CD28.3
64	SAWSHPQFEKGGSGGGSGGSAWSHPQFEK	Twin-Strep-tag
65	MLLLVTSLLLCELPHPAFLIP	GMCSFR alpha chain signal sequence
66	atgcttctctggtgacaagccttctgctgtgtgagttaccacaccagcattctctctgatccca	GMCSFR alpha chain signal sequence
67	MALPVTALLLPLALLLHA	CD8 alpha signal peptide
68	DPSKDSKAQVSAAEAGITGTWYNQLGSTFIVTAGADGALTGTYESAVGNAESRYVLTGRYDSAPATDGSGTALGWTVAWKNNYRNAHSATTWSGQYVGGAEARINTQWLLTSGTTEANAWKSTLVGHDTFTKVKPSAASIDAACKAGVNNGNPLDAVQQ	Streptavidin Species: Streptomyces avidinii UniProt No. P22629
69	EAGITGTWYNQLGSTFIVTAGADGALTGTYESAVGNAESRYVLTGRYDSAPATDGSGTALGWTVAWKNNYRNAHSATTWSGQYVGGAEARINTQWLLTSGTTEANAWKSTLVGHDTFTKVKPSAAS	Minimal streptavidin Species: Streptomyces avidinii
70	His-Pro-Gln-Phe	Streptavidin-binding peptide

71	His-Pro-Xaa	Streptavidin Binding peptide Xaa is selected from Gln, Asp, and Met
72	Oaa-Xaa-His-Pro-Gln-Phe-Yaa-Zaa	Streptavidin- binding peptide Oaa is Trp, Lys or Arg; Xaa is any amino acid; Yaa is Gly or Glu Zaa is Gly, Lys or Arg
73	-Trp-Xaa-His-Pro-Gln-Phe-Yaa-Zaa-	Streptavidin- binding peptide Xaa is any amino acid; Yaa is Gly or Glu Zaa is Gly, Lys or Arg
74	Trp-Arg-His-Pro-Gln-Phe-Gly-Gly	Streptavidin binding peptide, Strep-tag®
75	WSHPQFEK	Strep-tag® II
76	Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(Xaa) _n -Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-	Sequential modules of streptavidin- binding peptide Xaa is any amino acid; n is either 8 or 12
77	Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer) _n -Trp-Ser-His-Pro-Gln-Phe-Glu-Lys	Sequential modules of streptavidin- binding peptide n is 2 or 3
78	WSHPQFEKGGGSGGGSGGGWSHPQFEK	Twin-Strep-tag
79	WSHPQFEKGGGSGGGSGGGWSHPQFEK	Twin-Strep-tag
80	WSHPQFEKGGGSGGGSGGSAWSHPQFEK	Twin-Strep-tag
81	SAWSHPQFEKGGGSGGGSGGGWSHPQFEK	Twin-Strep-tag
82	SAWSHPQFEKGGGSGGGSGGSAWSHPQFEK	Twin-Strep-tag
83	DPSKDSKAQVSAAEAGITGTWYNQLGSTFIVTAGADGALTGTYY T ARGNAESRYVLTGRYDSAPATDGS GTALGWTVAWKNNYRNA HSATTWSGQYVGGAEARINTQWLLTSGTTEANAWKSTLVGHDT FTKVKPSAASIDAACKAGVNNGNPLDAVQQ	Mutein Streptavidin Val44-Thr45- Ala46-Arg47

		Species: Streptomyces avidinii
84	EAGITGTWYNQLGSTFIVTAGADGALTGTYY VT ARGNAESRYVLT GRYDSAPATDGSGTALGWTVAWKNNYRNAHSATTWSGQYVGG AEARINTQWLLTSGTTEANAWKSTLVGHDTFTKVKPSAAS	Mutein Streptavidin Val44-Thr45- Ala46-Arg47 Species: Streptomyces avidinii
85	MEAGITGTWYNQLGSTFIVTAGADGALTGTYY VT ARGNAESRYVL TGRYDSAPATDGSGTALGWTVAWKNNYRNAHSATTWSGQYVG GAEARINTQWLLTSGTTEANAWKSTLVGHDTFTKVKPSAAS	Mutein Streptavidin Val44-Thr45- Ala46-Arg47 Species: Streptomyces avidinii
86	DPSKDSKAQVSAAEAGITGTWYNQLGSTFIVTAGADGALTGTYY IG ARG NAESRYVLTGRYDSAPATDGSGTALGWTVAWKNNYRNAH SATTWSGQYVGGAEARINTQWLLTSGTTEANAWKSTLVGHDTFT KVKPSAASIDAACKAGVNNGNPLDAVQQ	Mutein Streptavidin Ile44- Gly45-Ala-46- Arg47 Species: Streptomyces avidinii
87	EAGITGTWYNQLGSTFIVTAGADGALTGTYY IG ARGNAESRYVLT GRYDSAPATDGSGTALGWTVAWKNNYRNAHSATTWSGQYVGG AEARINTQWLLTSGTTEANAWKSTLVGHDTFTKVKPSAAS	Mutein Streptavidin Ile44- Gly45-Ala-46- Arg47 Species: Streptomyces avidinii
88	EAGITGTWYNQLGSTFIVTAGADGALTGTYY VT ARGNAESRYVLT GRYDSAPATDGSGTALGWTVAWKNNYRNAHSATTWSGQYVGG AEARINTQWLLTSGTTEENAGYSTLVGHDTFTKVKPSAAS	Mutein Streptavidin Val44-Thr45- Ala46-Arg47 and Glu117, Gly120, Try121 (mutein m1-9) Species: Streptomyces avidinii
89	DPSKDSKAQVSAAEAGITGTWYNQLGSTFIVTAGADGALTGTYY T ARGNAESRYVLTGRYDSAPATDGSGTALGWTVAWKNNYRNA HSATTWSGQYVGGAEARINTQWLLTSGTTEENAGYSTLVGHDTF TKVKPSAAS	Mutein Streptavidin Val44-Thr45- Ala46-Arg47 and Glu117, Gly120, Try121 (mutein m1-9)

		Species: Streptomyces avidinii
90	MEAGITGTWYNQLGSTFIVTAGADGALTGTYESAVGNAESRYVL TGRYDSAPATDGS GTALGWTVAWKNNYRNAHSATTWSGQYVG GAEARINTQWLLTSGTTEANAWKSTLVGHDTFTKVKPSAAS	Minimal streptavidin Species: Streptomyces avidinii
91	MLLLVTSLLLCELPHPAFLIPDIQMTQTSSLSASLGDRVTISCRASQ DISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTIS NLEQEDIATYFCQQGNTLPYTFGGGTKLEITGSTSGSGKPGSGEGSTKG EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLG VIWGSETTYNSALKSRLLTIKDNSKSQVFLKMNSLQDDTAIYYCAKH YYYGGSYAMDYWGQTSVTVSSESKYGPPCPPCFMFVWLVVVGGVLACY SLLVTVAFIIFWVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEE EGGCELRVKFSRSADAPAYQQGQNQLYNE LNLGRREEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQG LSTATKDTYDALHMQALPPR	CD19 CAR
92	atgctgctgctggtagcagcctgctgctgtgagctgccccaccccgctttctgctgaccccgaca tcagatgacccagaccacctccagcctgagcgcagcctggcgaccgggtgaccatcagctgccg ggccagccaggacatcagcaagtacctaactggtatcagcagaagcccgacggcaccgtcaagctg ctgatctaccacaccagccggctgcacagcggcgtgcccagccggttagcggcagcggctccggca ccgactacagcctgaccatctcaacctggaacaggaagatcgcacacacttttgccagcagggca acacactgccctacacctttggcgcggaacaaagctggaatcaccggcagcacctccggcagcgg caagcctggcagcggcgaggcgacgaccaagggcgaggtgaagctgcaggaaagcgccctggc ctggtggccccagccagagcctgagcgtgacctgcaccgtgagcggcgtgagcctgcccgactacg gcgtgagctggatccggcagccccccaggaagggcctggaatggctggcgctgactggggcagcg agaccactactacaacagcgccctgaagagccggctgaccatcatcaaggacaacagcaagacca ggtgttctgaagatgaacagcctgcagaccgacgacaccgccatctactactgcgccaagcactacta ctacggcggcagctacgccatggactactggggccaggggcaccagcgtgaccgtgagcagcgaatct aagtacggcagccctgcccccttgccctatgttctgggtgctggtggtggtggtggaggcgctgctggcct gtacagcctgctggtcaccgtggccttcacatcttttgggtgaaacggggcagaaagaaactcctgtat atatcaacaacacatttatgagaccagtacaaactactcaagaggaagatggctgtagctgccgatttc agaagaagaagaaggaggtgtgaactgcgggtgaagttcagcagaagcgccgacgcccctgccta ccagcagggccagaatcagctgtacaacgagctgaacctgggcagaaggggaagtagtacgacgtctg gataagcggagaggccgggaccctgagatggcgccgaagcctcgccggaagaacccccaggaag gcctgtataacgaactgcagaaagacaagatggccgagggcctacagcgagatcggcaggaaggcgga gcggagggcggggaaggccacgacggcctgtatcaggggcctgtccaccgccaccaaggataccta cgacgcctgcacatgcaggccctgcccccaagg	CD19 CAR
93	TTTTAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD	CD8α hinge domain
94	IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKP	CD28 hinge domain
95	AAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKP	CD28 hinge domain
96	IYIWAPLAGTCGVLLLSLVITLYC	CD8α transmembrane domain
97	MALPVTALLLPLALLLHAARPDQMTQTSSLSASLGDRVTISCRA SQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTD YSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITGGGGSGGGG	CD19 CAR

	SGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQ PPRKGLEWLGVIWGSETTYYNALSRLTIKDNSKSQVFLKMNS LQTDDTAIYYCAKHYYYGGSYAMDYWGQTSVTVSSTTTPAPRP PTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAG TCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSC RFEEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEY DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG MKGERRRRGKGHGGLYQGLSTATKDTYDALHMQALPPR	
98	MLLLVTSLLLCELPHPAFLLIPDIQMTQTSSLSASLGDRVTISCRA SQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTD YSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKEITGSTSGSGKPG SGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWI RQPPRKGLEWLGVIWGSETTYYNALSRLTIKDNSKSQVFLKM NSLQTDDTAIYYCAKHYYYGGSYAMDYWGQTSVTVSSAAIE VMYPYPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKPFVVLVVVG GYLACYSELLVTVAFIHFVRSKRSLHSDYMNMTPRRPGPTRKH YQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRR EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYS EIGMKGERRRRGKGHGGLYQGLSTATKDTYDALHMQALPPR	CD19 CAR
99	atgctccttctgctgacctcctgcttctctgcaactgccccatctgcttctgctg attcccgacattcagatgactcagaccactcctcctgctcgcctcctggcgaccgc gtgacctatcatccgcgccagccaggacatctcgaagtacctcaactggtaccagcag aagcccgacggaaccgtgaagctcctgatctaccacacctcccggctgcacagcggagtg cctgtctagattctcgggttcgggtcgggaactgactactccttactatttccaactg gagcaggagatattgccactactctgccaacaaggaaacacctgcccgtacactttt ggcgggggaaccaagctggaaatcactggcagcacatccggttccgggaagcccgctcc ggagaggcgagcaccagggggaagtaagctgcaggaatcaggacctggcctggtggcc ccgagccagtcactgtccgtgactgtactgtgtccggagtgtcgtcccggttaccgga gtgtcctggatcaggcagccacctcggaaaggattggaatgctcggagtcatctgggt tccgaaccacctattacaactcggcactgaaatcaggctcaccattatcaagataac tccaaagtcacaagtgttctgaagatgaatagcctgcagactgacgacacggcgatctac tattgcgccaagcactactactacggcgatcctacgctatggactactggggccaggggg accagctgaccgtgtcatccggaggcggcgagcggcgggggagggtccggagggggt ggttctgtggaggagatcgggagggcgtggcagcgaggtgcagttgcaacagtcagga gtgaactggtcaagccaggagccagcgtgaagatgagctgcaaggcctccggttacacc ttcacctctacaacatgcactgggtgaaacagaccccgggacaagggtcgaatggatt ggcgccatctacccgggaatggcgatacttcgtacaaccagaagttcaagggaaggcc acctgaccgccgacaagagctcctccaccgcgtatatgcagttgagctccctgacctcc gaggactcccgactactactgcgcacgggtccaactactatggaagctcgtactgttcc ttcgatgtctggggggccggcaccactgtgaccgtcagctccggggcgaggatccgggt ggagggcggaagcgggggtggaggatccgacattgtgctgactcagtcctccggcaatcctg tcggcctcaccggcgaaaaggtcacgatgactgttagagcgtcgtccagcgtgaactac atggattggtacaaaagaagcctggatcgtacccaagcctggatctacgctacatct aacctggcctccggcgtgccagcgcggttcagcgggtccggctcgggcacctcatactcg ctgacctctcccgcgtggaggtgaggacgccgcgacctactactgccagcagtggtcc ttcaacccgccgacttttgaggcggtactaagctggagatcaagcggccgcaactacc acctctgcccctggccgctgactccggcccaaccatcgaagccaacctctccttg cgccccgaagcttgccggcgccgggtggagcgtgcataccgggggctggacttt gctgcgatatctacatttggccccgctggccggcacttgcggcgtgctcctgctgtcg ctggtcatcacccttactgcaagaggggccggaagaagctgtttacatcttcaagcag ccgttcatcgcccgctgcagacgactcaggaagaggacggatgctcgtgcagattccct gaggagggaaggggggatgcgaactgcgcgtcaagtctcacggtccggcgacgcccc	Leader-CD19 VL- Whitlow linker CD19 VH (GGGGS)-5 CD20 VH (GGGGS)-3 CD20 VL CD8 hinge+TM-4- 1BB- CD3z (Construct CAR 1920)

	gcatatcaacagggccagaatcagctctacaacgagctgaacctgggaaggagagaggag tacgacgtgctggacaagcgacgcggacgcgacccggagatgggggggaaaccacggcgg aaaaaccctcaggaaggactgtacaacgaactccagaaagacaagatggcgggaagcctac tcagaaatcgggatgaaggagagcggaggaggggaaagggtcacgacgggctgtaccag ggactgagcaccgccactaaggatcctacgatgccttgcataatgaagcactcccacc cgg	
100	MLLLVTSLLLCELPHPAFLIPDIQMTQTTSSLSASLGDRVTISCRA SQDISKYLNWYQQ KPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIAT YFCQQGNTLPYTF GGGKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSL VTCTVSGVSLPDYG VSWIRQPPRKGLEWLGVIWGSETTYNSALKSRLTIHKDNSKSQVF LKMNSLQTDDAIY YCAKHYYYGGSYAMDYWGQTSVTVSSGGGSGGGGSGGGGS GGGSGGGGSEVQLQSG AELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGQGLEWIGAI YPGNGDTSYNQKFKGKA TLTADKSSSTAYMQLSSLTSEDSADYYCARSNYGSSYWFFDVW GAGTTVTVSSGGGSG GGSGGGGSDIVLTQSPAILSASPGEKVTMTCRASSSVNYMDWY QKKPGSSPKWIYATS NLASGVPARFSGSGSTSYSLTISRVEAEDAATYYCQQWSFNPTF GGGKLEIKAAATT TPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLS LVITLYCKRGRKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGG CELRVKFSRSADAP AYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP QEGLYNELQKDKMAEAY SEIGMKGERRRGKHDGLYQGLSTATKDTYDALHMQALPPR	Leader-CD19 VL- Whitlow linker CD19 VH (GGGS)-5 CD20 VH (GGGS)-3 CD20 VL CD8 hinge+TM-4- 1BB- CD3z (Construct CAR 1920)
101	atgctcctctcgtgacctccctgctctctcgaactgccccatcctgccttctcgtg attcccaggtgcagttgcaacagtcaggagctgaactggtcaagccaggagccagcgtg aagatgagctgcaaggcctccggttacacctcactctacaacatgactgggtgaaa cagaccccggaacaaggctcgatggattggcgcactctaccccggaatggcgatact tcgtacaaccagaagttaagggaaggccacctgaccgcccagaagactcctccacc gcgtatatgcagttgagctccctgacctccaggactccgccgactactactgcgcacgg tccaactactatggaagctcgtactggttcttcgatgtctggggggccggcaccactgtg accgtcagctccggggcgaggatccggtggaggcggaagcgggggtggagatccgac attgtgctgactcagtcgcccgcaatcctgtcggcctaccggcgaaaaggtcacgatg actgtagagcgtcgtccagcgtgaactacatggattgtacaaaagaagcctggatcg tcaccaagccttgatctacgtacatctaacctggcctccggcgtgccagcgcgggtc agcgggtccggctcgggacatcactcgtgacctctcccgtggaggctgaggac gccgcgactactactgccagcagtggtcctcaacccgccgacttttgaggcggtact aagctggagatcaaaaggagcgccggcagcggcgggggagggtccggagggggtggtct ggtggaggagatcgggagggcggtggcagcgacattcagatgactcagaccactcctc ctgtccgctccctggcgaccgctgacctctcatccgcgccagccaggacatctcg aagtacctcaactgtaccagcagaagcccgacggaaccgtgaagctcctgatctaccac acctccggctgcacagcggagtgccgtctagattctcgggttcgggtcgggaactgac tactcccttactattccaacctggagcaggagatattgccactactcttccaacaa ggaaacacctcgccgtacttttggcgggggaaccaagctggaatcactggcagcaca tcgggttcgggaagcccggtccggagaggcgagcaccagggggaagtcaggctgcag	Leader-CD20 VH (GGGS)3 - CD20 VL - (GGGS)5 -CD19 VL-Whitlow linker- CD19 VH CD8 hinge+TM-4-1BB- CD3z (Construct 2019)

	gaatcaggacctggcctgggtgccccgagccagtcactgtccgtgactgtactgtgtcc ggagtgtcgtccccgattacggagtgctctggatcaggcagccacctcggaaaggattg gaatggctcggagtcacttgggttcggaaccacattacaactcggcactgaaatcc aggctcaccattatcaaggataactccaagtcacaagtggtcctgaagatgaatagcctg cagactgacgacacggcgatctactattgcgccaagcactactactacggcggatcctac gctatggactactggggccaggggaccagcgtgaccgtgcatccggcggcgaactacc acccctgccccctggcggcgactccggcccaaccatcgcaagccaaccctctccttg cgccccgaagcttgccggcgccgggtggagccgtgcataccgggggctggacttt gcctgcgatactacatttggccccgctggcggcacttgcggcgtgctcctgtctgc ctgtcatcacccttactcaagagggggccggaagaagctgtttacatctcaagcag ccgtcatcggccccgtgcagacgactcaggaagaggacggatgctcgtgcagattcct gaggagggaagagggggatgcgaactgcgcgtcaagtctcacggtccggcagcggccc gcatacaacaggggcagaatcagctctacaacgagctgaacctgggaaggagagaggag tacgacgtgctggacaagcgacgcggacgcgacccggagatgggggggaaccacggcgg aaaaacctcaggaaggactgtacaacgaactccagaagacaagatggcgggaagcctac tcagaaatcgggatgaaggagagcggaggggaaagggtcacgacgggctgtaccag ggactgagcaccgccactaaggatactacgatgccttgcatacgcaagcactcccacc cgg	
102	MLLVTSLLLCELPHPAFLLIPEVQLQQSGAELVKPGASVKMSCK ASGYTFTSYNMHWVK QTPGQGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQ LSSLTSEDSADYYCAR SNYYGSSYWFFDVWGAGTTVTVSSGGGSGGGGSGGGGSDIVLT QSPAILSASPGEKVTM TCRASSSVNYMDWYQKKPGSSPKWIYATSNLASGVPARFSGSG SGTSYSLTISRVEAED AATYYCQQWSFNPTFGGGTKLEIKGGGSGGGGSGGGGSGGGG SGGGSDIQMTQTTSS LSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLH SGVPSRFSGSGSGTD YSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITGSTSGSGKPG SGEGSTKGEVKLQ ESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLG IWGSETTYNSALKS RLTIKDNSKSKVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDY WGQTSVTVSSAAATT TPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLS LVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGG CELRVKFSRSADAP AYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP QEGLYNELQKDKMAEAY SEIGMKGERRRRGKHDGLYQGLSTATKDTYDALHMQALPPRPRT	Leader-CD20 VH (GGGGS)3 - CD20 VL - (GGGGS)5 -CD19 VL-Whitlow linker- CD19 VH CD8 hinge+TM-4-1BB- CD3z amino acid sequence (Construct CAR 2019)
103	EVQLQQSGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGQ GLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTS EDSADYYCARSNYYGSSYWFFDVWGAGTTVTVSS	Anti-CD20 Leu16 scFv heavy chain
104	DIVLTQSPAILSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKP WIYATSNLASGVPARFSGSGTSYSLTISRVEAEDAATYYCQQW SFNPTFGGGTKLEIK	Anti-CD20 Leu16 scFv light chain variable region
105	QSVVTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTA PKLLIYENTNRPSGV	Anti-CD19 scFv

	PDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLGWRVFGG GTKLTVLGSRRGGGS GGGSGGGGSLEMAEVQLVQSGAEVKKPGESLKISCKGSGYSFT NSWIGWVRQMPGKGLE WMGLIYPDDSDTRYSPSFQGGQVTISADSAINAYLQWSSLKASDT AMYYCARQSTYIYGG YYDTWGQGTLLTVSS	
106	QSVVTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTA PKLLIYENTNRPSGV PDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLGWRVFGG GTKLTVLG	Anti-CD19 variable light chain
107	MAEVQLVQSGAEVKKPGESLKISCKGSGYSFTNSWIGWVRQMPG KGLEWMGLIYPDDSDT RYSPSFQGGQVTISADSAINAYLQWSSLKASDTAMYYCARQSTYI YGGYYDTWGQGTLLTV VSS	Anti-CD19 variable heavy chain
108	QAVLTQPPSVSEAPRQRVTISCSGSSSNIGNNAVSWYQQLPGKAP KLLIYYDDLPSGV DRFSGSKSGTSASLAISGLQSEDEADYYCAAWDDSLNGWVFGGG TKVTVLGSRRGGGSGG GGSGGGGSLEEVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIG WVRQMPGKGLEWMI IYPGDSDDTRYSPSFQGGQVTISADKSISTAYLQWSSLKASDTAMYYC ARLSYSWSSWYWDF WGQGTLLTVSS	Anti-CD19 scFv
109	QAVLTQPPSVSEAPRQRVTISCSGSSSNIGNNAVSWYQQLPGKAP KLLIYYDDLPSGV DRFSGSKSGTSASLAISGLQSEDEADYYCAAWDDSLNGWVFGGG TKVTVLG	Anti-CD19 variable light chain
110	EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKG LEWMGHIYPGDSDDTRY SPSFQGGQVTISADKSISTAYLQWSSLKASDTAMYYCARLSYSWSS WYWDFWGQGTLLTVS S	Anti-CD19 variable heavy chain
111	FVPVFLPAKPTTTPAPRPPTPAPTASQ PLSLRPEACRPAAGGAVHTRGLDFACDI YIWAPLAGTCGVLLLSLVITLYCNHRN	CD8a hinge and transmembrane
112	EIVLTQSPGTLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYG SSRFTFGPGTKVDIK	Anti-CD19 variable light chain
113	GSTSGSGKPGSGEGSTKG	Linker
114	QVQLVQSGAEVKKPGSSVKVCKDSGGTFSSYAIWVRQAPGQG LEWMGGIPIFGTTNYAQGFQGRVTITADESTSTAYMELSSLRSED TAVYYCAREAVAADWLDWPWGQGTLLTVSS	Anti-CD19 variable heavy chain
115	MALPVTALLLPLALLLHAARP	CD8a signal sequence
116	MALPVTALLLPLALLLHAARPEIVLTQSPGTLSPGERATLSCRA SQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTD	Anti-CD19 CAR

	FTLTISRLEPEDFAVYYCQQYGSSRFTFGPGTKVDIKGSTSGSGKP GSGEGSTKGQVQLVQSGAEVKKPGSSVKVSCKDSGGTFSSYAIS WVRQAPQGQGLEWMGGIPIFGTTNYAQQFQGRVTITADESTSTAY MELSSLRSEDYAVYYCAREAVAADWLDWPWGQGT LVT VSSFPVFL LPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLD FACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRLHSDYM NMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQG QNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLY NELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD ALHMQALPPR	
117	MLLVTSLLLCELPHPAFLIPDIQMTQTSSLSASLGDRVTISCRA SQDISKYLNWYQQ KPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIAT YFCQQGNTLPYTF GGGKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSL VTCTVSGVSLPDYG VSWIRQPPRKGLEWLGVIWGSETTYNSALKSRLTIKDNSKSQV LKMNSLQTDDTAIY YCAKHYYGGSYAMDYWGQGSVTVSSAAAFVPVFLPAKPTTTP PAPRPPTPAPTIASQPL SLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLV ITLYCNHRNRSKRSRL LHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADA PAYQQGQNQLYNELNL GRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE AYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR	Anti-CD19 CAR
118	MALPVTALLLPLALLLHAARPEIVLTQSPGTLSPGERATLSCRA SQSVSSSYLAWYQQ KPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQYGSSRFTF GPGTKVDIKGSTSGSGKPGSGEGSTKGQVQLVQSGAEVKKPGSSV KVSCKDSGGTFSSYA ISWVRQAPQGQGLEWMGGIPIFGTTNYAQQFQGRVTITADESTSTA YMELSSLRSEDYAV YYCAREAVAADWLDWPWGQGT LVT VSSFPVFLPAKPTTTPAPRP PTPAPTIASQPLSLRP EACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYC NHRNRFSVVKRGRKK LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADA PAYQQGQNQLYNEL NLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM AEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMQALPPR	Anti-CD19 CAR
119	MALPVTALLLPLALLLHAARPEIVLTQSPGTLSPGERA TLSCRASQSVSSSYLAWYQQ KPGQAPRLLI YGASSRATGI PDRFSGSGSG TDFTLTISRLEPEDFAVYYC QQYGSSRFTF GPGTKVDIKG STSGSGKPGS GEGSTKGQVQLVQSGAEVKK PGSSVKVSCK DSGGTFSSYA ISWVRQAPGQ GLEWMGGIIP IFGTTNYAQQ FQGRVTITAD ESTSTAYMEL SSLRSEDYAV YYCAREAVAADWLDWPWGQGT LVT VSSFPV FLPKPTTTP APRPPTPAPT IASQPLSLRPEACRPAAGGA VHTRGLDFAC	Anti-CD19 CAR

	DIYIWAPLAG TCGVLLLSLV ITLYCNHRNRSKRSRLLHSD YMNMTPRRPG PTRKHYQPYAPPRDFAAYRSRVKFSRSADA PAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPPEMGGKPR RKNPQEGLYNELQKDKMAEA YSEIGMKGER RRGKGHDGLY QGLSTATKDT YDALHMQALPPR	
120	MALPVTALLL PLALLLHAAR PEIVLTQSPG TLSLSPGERA TLSCRASQSVSSSYLAWYQQ KPGQAPRLLI YGASSRATGI PDRFSGSGSG TDFTLTISRLEPEDFAVYYC QQYGSSRFTF GPGTKVDIKG STSGSGKPGS GEGSTKGQVQLVQSGAEVKK PGSSVKVSCK DSGGTFSSYA ISWVRQAPGQ GLEWMGGIIP IFGTTNYAQQ FQGRVTITAD ESTSTAYMEL SSLRSEDYAV YYCAREAVAADWLDPWGQGT LVTVSSFVPV FLPKPTTTP APRPPTPAPT IASQPLSLRPEACRPAAGGA VHTRGLDFAC DIYIWAPLAG TCGVLLLSLV ITLYCNHRNR FSVVKRGRKK LLYIFKQPFM RPVQTTQEED GCSCRFPEEE EGGCELRVKFSRSADAPAYQ QGQNQLYNELNLGRREEYDV LDKRRGRDPE MGGKPRRKNPQEGLYNELQK DKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDALHMQ ALPPR	Anti-CD19 CAR
121	MALPVTALLL PLALLLHAAR PEIVLTQSPG TLSLSPGERA TLSCRASQSVSSSYLAWYQQ KPGQAPRLLI YGASSRATGI PDRFSGSGSG TDFTLTISRLEPEDFAVYYC QQYGSSRFTF GPGTKVDIKG STSGSGKPGS GEGSTKGQVQLVQSGAEVKK PGSSVKVSCK DSGGTFSSYA ISWVRQAPGQ GLEWMGGIIP IFGTTNYAQQ FQGRVTITAD ESTSTAYMEL SSLRSEDYAV YYCAREAVAADWLDPWGQGT LVTVSSFVPV FLPKPTTTP APRPPTPAPT IASQPLSLRPEACRPAAGGA VHTRGLDFAC DIYIWAPLAG TCGVLLLSLV ITLYCNHRNQRRKYRSNKGE SPVEPAEPCR YSCPREEEGS TIPIQEDYRK PEPACSPRVK FSRSADAPAY QQGQNQLYNELNLGRREEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNELQ KDKMAEAYSE IGMKGERRRG KGHDGLYQGL STATKDTYDALHMQALPPR	Anti-CD19 CAR
122	MALPVTALLL PLALLLHAAR PEIVLTQSPG TLSLSPGERA TLSCRASQSVSSSYLAWYQQ KPGQAPRLLI YGASSRATGI PDRFSGSGSG TDFTLTISRLEPEDFAVYYC QQYGSSRFTF GPGTKVDIKG STSGSGKPGS GEGSTKGQVQLVQSGAEVKK PGSSVKVSCK DSGGTFSSYA ISWVRQAPGQ GLEWMGGIIPFGTTNYAQQ FQGRVTITADESTSTAYMELSSLRSEDYAVYYCAREAVAADWLD PWGQGT LVTVSSFVPV FLPKPTTTP APRPPTPAPT IASQPLSLRPEACRPAAGGA VHTRGLDFAC DIYIWAPLAG TCGVLLLSLV ITLYCNHRNRSKRSRLLHSD YMNMTPRRPG PTRKHYQPYA PPRDFAAYRS QRRKYRSNKGESPEVAEPC RYSCPREEEG STIPIQEDYR KPEPACSPRV KFSRSADAPA YQQGQNQLYNELNLGRREEYDVLDKRRGRDPPEMGGKPRRK NPQEGLYNELQKDKMAEAYS EIGMKGERRRRGKGHDGLYQG LSTATKDTYD ALHMQALPPR	Anti-CD19 CAR
123	MALPVTALLL PLALLLHAAR PEIVLTQSPG TLSLSPGERA TLSCRASQSV SSSYLAWYQQ KPGQAPRLLI YGASSRATGI PDRFSGSGSG TDFTLTISR	Anti-CD19 CAR

	EPEDFAVYYC QQYGSSRFTF GPGTKVDIKG STSGSGKPGS GEGSTKGQVQ LVQSGAEVKK PGSSVKVSCK DSGGTFSSYA ISWVRQAPGQ GLEWMGGIIP IFGTTNYAQQ FQGRVTITAD ESTSTAYMEL SSLRSEDYAV YYCAREAVAA DWLDPWGQGT LVTVSSFVPV FLPKPTTTP APRPPTPAPT IASQPLSLRP EACRPAAGGA VHTRGLDFAC DIYIWAPLAG TCGVLLLSLV ITLYCNHRNQ RRKYRSNKGE SPVEPAEPCR YSCPREEEGS TIPIQEDYRK PEPACSPRFS VVKRGRKKLL YIFKQPFMRP VQTTQEEDGC SCRFPREEEG GCELRVKFSR SADAPAYQQG QNQLYNELNL GRREEYDVLD KRRGRDPPEMG GKPRRKNPQE GLYNELQKDK MAEAYSEIGM KGERRRGKGH DGLYQGLSTA TKDTYDALHM QALPPR	
124	MLLLVTSLLL CELPHPAFL IPDIQMTQTT SLSASLGDR VTISCRASQD ISKYLNWYQQ KPDGTVKLLI YHTSRLHSGV PSRFSGSGSG TDYSLTISNL EQEDIATYFC QQGNTLPYTF GGGTKLEITG STSGSGKPGS GEGSTKGEVK LQESGPGVA PSQSLSVTCT VSGVSLPDYG VSWIRQPPRK GLEWLGVIWG SETTYNSAL KSRLTIKDN SKSQVFLKMN SLQTDDTAIY YCAKHYYGG SYAMDYWGQG TSVTVSSAAA FVPVFLPAKP TTPAPRPPT PAPTASQPL SLRPEACRPA AGGAVHTRGL DFACDIYIWA PLAGTCGVLL LSLVITLYCN HRNRSKRSRL LHSDYMNMTF RRPGPTRKHY QPYAPPRDFA AYRSRVKFSR SADAPAYQQG QNQLYNELNL GRREEYDVLD KRRGRDPPEMG GKPRRKNPQE GLYNELQKDK MAEAYSEIGM KGERRRGKGH DGLYQGLSTA TKDTYDALHM QALPPR	Anti-CD19 CAR
125	MLLLVTSLLL CELPHPAFL IPDIQMTQTT SLSASLGDR VTISCRASQD ISKYLNWYQQ KPDGTVKLLI YHTSRLHSGV PSRFSGSGSG TDYSLTISNL EQEDIATYFC QQGNTLPYTF GGGTKLEITG STSGSGKPGS GEGSTKGEVK LQESGPGVA PSQSLSVTCT VSGVSLPDYG VSWIRQPPRK GLEWLGVIWG SETTYNSAL KSRLTIKDN SKSQVFLKMN SLQTDDTAIY YCAKHYYGG	Anti-CD19 CAR

	SYAMDYWGQG TSVTVSSAAA FVPVFLPAKP TTTPAPRPPT PAPTIASQPL SLRPEACRPA AGGAVHTRGL DFACDIYIWA PLAGTCGVLL LSLVITLYCN HRNQRRKYRS NKGESPVEPA EPCRYSCPRE EEGSTIPIQE DYRKPEPACS PRFSVVKRGR KKLLYIFKQP FMRPVQTTQE EDGCSCRFPE EEEGGCELRV KFSRSADAPA YQQGQNQLYN ELNLGRREEY DVLDKRRGRD PEMGGKPRRK NPQEGLYNEL QKDKMAEAYS EIGMKGERRR GKGDGLYQG LSTATKDTYD ALHMQALPPR	
126	MLLVTSLLL CELHPAFLI IPDIQMTQTT SLSASLGDR VTISCRASQD ISKYLNWYQQ KPDGTVKLLI YHTSRLHSGV PSRFSGSGSG TDYSLTISNL EQEDIATYFC QQGNTLPYTF GGGTKLEITG STSGSGKPGS GEGSTKGEVK LQESGPGIVA PSQSLSVTCT VSGVSLPDYG VSWIRQPPRK GLEWLGVIWG SETTYYNAL KSRLTIKDN SKSQVFLKMN SLQTDDTAIY YCAKHYYGG SYAMDYWGQG TSVTVSSAAA FVPVFLPAKP TTTPAPRPPT PAPTIASQPL SLRPEACRPA AGGAVHTRGL DFACDIYIWA PLAGTCGVLL LSLVITLYCN HRNQRRKYRS NKGESPVEPA EPCRYSCPRE EEGSTIPIQE DYRKPEPACS PRVKFSRSAD APAYQQGQNQ LYNELNLGRR EEYDVLDKRR GRDPEMGGKP RRKNPQEGLY NELQKDKMAE AYSEIGMKGE RRRGKGHDGL YQGLSTATKD TYDALHMQAL PPR	Anti-CD19 CAR
127	MLLVTSLLL CELHPAFLI IPDIQMTQTT SLSASLGDR VTISCRASQD ISKYLNWYQQ KPDGTVKLLI YHTSRLHSGV PSRFSGSGSG TDYSLTISNL EQEDIATYFC QQGNTLPYTF GGGTKLEITG STSGSGKPGS GEGSTKGEVK LQESGPGIVA PSQSLSVTCT VSGVSLPDYG VSWIRQPPRK GLEWLGVIWG SETTYYNAL KSRLTIKDN SKSQVFLKMN SLQTDDTAIY YCAKHYYGG SYAMDYWGQG TSVTVSSAAA FVPVFLPAKP TTTPAPRPPT PAPTIASQPL SLRPEACRPA AGGAVHTRGL DFACDIYIWA PLAGTCGVLL LSLVITLYCN HRNRSKRSRL LHSDYMNMTF RRPGPTRKHY QPYAPPRDFA AYRSQRRKYR	Anti-CD19 CAR

	SNKGESPVEP AEPCRYSCPR EEGSTIPIQ EDYRKPEPAC SPRVKFSRSA DAPAYQQGQN QLYNELNLGR REEYDVLDKR RGRDPEMGGK PRRKNPQEG YNELQKDKMA EAYSEIGMKG ERRRGKGHDG LYQGLSTATK DTYDALHMQA LPPR	
128	MALPVTALLL PLALLLHAAR PEIVLTQSPG TLSLSPGERA TLSCRASQSV SSSYLAWYQQ KPGQAPRLLI YGASSRATGI PDRFSGSGSG TDFTLTISRL EPEDFAVYYC QQYGSSRFTF GPGTKVDIKG STSGSGKPGS GEGSTKGQVQ LVQSGAEVKK PGSSVKVSCK DSGGTFSYA ISWVRQAPGQ GLEWMGGIIP IFGTTNYAQQ FQGRVTITAD ESTSTAYMEL SSLRSEDYAV YYCAREAVAA DWLDPWGQGT LVTVSSFVPV FLPKPTTTP APRPPTPAPT IASQPLSLRP EACRPAAGGA VHTRGLDFAC DIYIWAPLAG TCGVLLSLV ITLYCNHRNR SKRSRLHSD YMNMTPRRPG PTRKHYPYA PPRDFAAYRS QRRKYRSNKG ESPVEPAEPC RYSCPREEEG STIPIQEDYR KPEPACSPQV RKAATSYEK SDGVYTGLST RNQETYETLK HEKPPQ	Anti-CD19 CAR
129	MLLVTSLLL CELHPAFLI IPDIQMTQTT SSLSASLGDR VTISCRASQD ISKYLNWYQQ KPDGTVKLLI YHTSRLHSGV PSRFSGSGSG TDYSLTISNL EQEDIATYFC QQGNTLPYTF GGGTKLEITG STSGSGKPGS GEGSTKGQVQ LQESGPGIVA PSQSLSVTCT VSGVSLPDYG VSWIRQPPRK GLEWLGVIWG SETTYNSAL KSRLTIKDN SKSQVFLKMN SLQTDDBAIY YCAKHYYYGG SYAMDYWGQG TSVTVSSAAA FVPVFLPAKP TTPAPRPPT PAPTASQPL SLRPEACRPA AGGAVHTRGL DFACDIYIWA PLAGTCGVLL LSLVITLYCN HRNRSKRSRL LHSDYMNMTM RRPGRPTRKHYPYAPPRDFA AYRSQRRKYR SNKGESPVEP AEPCRYSCPR EEGSTIPIQ EDYRKPEPAC SPQVRKAAIT SYEKSDGVYT GLSTRNQETY ETLKHEKPPQ	Anti-CD19 CAR
130	MALPVTALLL PLALLLHAAR PEIVLTQSPG TLSLSPGERA TLSCRASQSV SSSYLAWYQQ KPGQAPRLLI YGASSRATGI PDRFSGSGSG TDFTLTISRL EPEDFAVYYC QQYGSSRFTF GPGTKVDIKG STSGSGKPGS GEGSTKGQVQ	Anti-CD19 CAR

	LVQSGAEVKK PGSSVKVSK DSGGTFSSYA ISWVRQAPGQ GLEWMGGIIP IFGTTNYAQQ FQGRVTITAD ESTSTAYMEL SSLRSEDNAV YYCAREAVAA DWLDPWGQGT LVTVSSFVPV FLPKPTTTP APRPPTPAPT IASQPLSLRP EACRPAAGGA VHTRGLDFAC DIYIWAPLAG TCGVLLLSLV ITLYCNHRNR SKRSRLHSD YMNMTPRRPG PTRKHYPYA PPRDFAAYRS QVRKAAITSY EKSDGVYTGL STRNQETYET LKHEKPPQ	
131	MLLVTSLLL CELHPAFL IPDIQMTQTT SSLASLGDR VTISCRASQD ISKYLNWYQQ KPDGTVKLLI YHTSRLHSGV PSRFSGSGSG TDYSLTISNL EQEDIATYFC QQGNTLPYTF GGGTKLEITG STSGSGKPGS GEGSTKGEVK LQESGPGGLVA PSQSLSVTCT VSGVSLPDYG VSWIRQPPRK GLEWLGVWIG SETTYNSAL KSRLTIKDN SKSQVFLKMN SLQTDDTAIY YCAKHYYGG SYAMDYWGQG TSVTVSSAAA FVPVFLPAKP TTPAPRPPT PAPTIASQPL SLRPEACRPA AGGAVHTRGL DFACDIYIWA PLAGTCGVLL LSLVITLYCN HRNRSKRSRL LHSDYMNMT PRRPGPTRKHYPY APPRDFAYRS QVRKAA ITSYEKSDGV YTGLSTRNQE TYETLKHEKP PQ	Anti-CD19 CAR
132	EVQLVESGGG LVQGRSLRL SCAASGFTFN DYAMHWVRQA PGKGLEWVST ISWNSGSIGY ADSVKGRFTI SRD NAKKSLY LQMNSLRAED TALYYCAKDI QYGNYYYGMD VWGQGTTVTV SS	Anti-CD20 heavy chain
133	EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD ASNRATGIPA RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ RSNWPITFGQ GTRLEIK	Anti-CD20 light chain
134	MALPVTALLLPLALLLHAARPEIVLTQSPATLSLSPGERATLSCRA SQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTD FTLTISSLEPEDFAVYYCQQRSNWPITFGQGTRLEIKGSTSGGGSG GSGGGGSSEVQLVESGGGLVQGRSLRLSCAASGFTFNDYAMH WVRQAPGKGLEWVSTISWNSGSIGYADSVKGRFTISRDNKKSL YLQMNSLRAEDTALYYCAKDIQYGNYYYGMDVWGQGTTVTVS SGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQ PPRKGLEWLGVWIGSETTYNSALKSRLTIKDN SKSQVFLKMNS LQTDDTAIYYCAKHYYGGSYAMDYWGQTSVTVSSGSTSGSG KPGSGEGSTKGIQMTQTTSSLASLGDRVTISCRASQDISKYLNW YQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQE DIATYFCQQGNTLPYTFGGGTKLEITESKYGPCPPCPMFWVLV VGGVLACYSLLVTVAFIIFWVKRGRKKLLYIFKQPFMRPVQTTQE EDGCSCRFEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNL	Anti- CD19/CD20 CAR

	GRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	
135	METDTLLLWV LLLWVPGSTG DIVLTQSPAI LSASPGKVT MTCRASSSVN YMDWYQKKPG SSPKPWIYAT SNLASGVPAR FSGSGSGTSY SLTISRVEAE DAATYYCQQW SFNPPTFGGG TKLEIKGSTS GGGSGGGSGG GGSSEVQLQQ SGAELVKPGA SVKMSCKASG YTFTSYNMHW VKQTPGQGLE WIGAIYPGNG DTSYNQKFKG KATLTADKSS STAYMQLSSL TSEDSADYYC ARSNYYGSSY WFFDVWGAGT TVTVSSGGGG SEVKLQESGP GLVAPSQSL VTCTVSGVSL PDYGVSWIRQ PPRKGLEWLG VIWGSETTTY NSALKSRLTI IKDNSKSQVF LKMNSLQTDD TAIYYCAKHY YYGGSYAMDY WGQGTSVTVS SGSTSGSGKP GSGEGSTKGD IQMTQTTSSL SASLGDRVTI SCRASQDISK YLNWYQQKPD GTVKLLIYHT SRLHSGVPSR FSGSGSGTDY SLTISNLEQE DIATYFCQQG NTPYTFGGG TKLEITESKY GPPCPPCPMF WVLVVVGGVL ACYSLLVTV FIIFWVKRGR KKLLYIFKQP FMRPVQTTQE EDGCSCRFPE EEEGGCELRV KFSRSADAPA YQQGQNQLYN ELNLGRREEY DVLDKRRGRD PEMGGKPRRK NPQEGLYNEL QKDKMAEAYS EIGMKGERRR KGHDGLYQG LSTATKDTYD ALHMQALPPR	Anti- CD19/CD20 CAR
136	QVQLQSGGG LVQPGGSLKL SCAASGIDFS RYWMSWVRR PGKGLEWIGE INPDSSTINY APSLKDKFII SRDNAKNTLY LQMSKVRSED TALYYCASLY YDYGDAWDYWG GQGTSVTVSS	Anti-BCMA heavy chain variable region
137	DIVMTQSQRF MTTSGDRVS VTCKASQSV SNVAWYQQKP RQSPKALIFS ASLRFSGVPA RFTGSGSGTD FTLTISNLQS EDLAEYFCQQ YNNYPLTFGA GTKLELK	Anti-BCMA light chain variable region

CLAIMS

WHAT IS CLAIMED:

1. A method of treating a subject having a systemic autoimmune disease, the method comprising administering a dose of CD19-directed genetically modified T cells from a composition comprising engineered T cells expressing a chimeric antigen receptor (CAR) to a subject having or suspected of having a severe systemic autoimmune disease, wherein the T cells of the dose are positive for expression of a CAR that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

2. A method of treating a subject having systemic autoimmune disease, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having a moderate systemic autoimmune disease, wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

3. The method of claim 1 or claim 2, wherein the systemic autoimmune disease is selected from the group consisting of systemic lupus erythematosus (SLE), Sjogren's syndrome, progressive systemic sclerosis (i.e., scleroderma), idiopathic inflammatory myositis (IIM), including dermatomyositis, polymyositis and necrotizing myositis, mixed connective tissue disorder (MCTD), highly active relapsing-remitting multiple sclerosis, primary progressive MS, ANCA-associated vasculitis (AAV), Crohn's disease, myasthenia gravis, Behçet's, rheumatoid arthritis, IgA nephropathy, pemphigus vulgaris, myasthenia gravis, autoimmune hemolytic anemia, immune thrombocytopenia, IgG4-related diseases, membranous nephropathy, cutaneous lupus erythematosus, sarcoidosis, light chain amyloidosis, acute respiratory distress syndrome, atopic eczema, hereditary angioedema, hidradenitis suppurative, inclusion-body myositis, inflammatory bowel disease, mastocytosis, multifocal motor neuropathy, necrotizing myopathy, neuromyelitis optica spectrum disorder, mixed connective tissue disorder, POEMS syndrome, primary biliary cholangitis, psoriasis, rhesus hemolytic disease, Still's disease, type 1 diabetes, urticaria, capillary leakage syndrome, cytokine release syndrome, erythema multiforme, pyoderma gangrenosum, x-linked agammaglobulinemia, antiphospholipid syndrome, and chronic inflammatory demyelinating polyneuropathy.

4. The method of any of claims 1-3, wherein the systemic autoimmune disease is rheumatoid arthritis.
5. The method of any of claims 1-3, wherein the systemic autoimmune disease is myositis.
6. The method of any of claims 1-3, wherein the systemic autoimmune disease is myasthenia gravis.
7. The method of any of claims 1-3, wherein the systemic autoimmune disease is bullous pemphigoid.
8. The method of any of claims 1-3, wherein the systemic autoimmune disease is immune thrombocytopenia.
9. The method of any of claims 1-3, wherein the systemic autoimmune disease is autoimmune hemolytic anemia.
10. The method of any of claims 1-3, wherein the systemic autoimmune disease is pemphigus vulgaris.
11. The method of any of claims 1-3, wherein the systemic autoimmune disease is demyelinating polyradiculoneuropathy.
12. The method of any of claims 1-3, wherein the systemic autoimmune disease is membranous nephropathy.
13. The method of any of claims 1-12, wherein the systemic autoimmune disease is a refractory disease.

14. The method of any of claims 1-13, wherein the subject is refractory to treatment with one or more prior therapies for the systemic autoimmune disease.

15. The method of any of claims 1-14, wherein the subject is refractory to treatment with two or more prior therapies for the systemic autoimmune disease.

16. The method of any of claims 1-15, wherein the systemic autoimmune disease is a severe disease.

17. A method of treating a subject having severe systemic lupus erythematosus (SLE), the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having severe systemic lupus erythematosus (SLE), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

18. A method for reducing systemic lupus erythematosus (SLE) disease activity, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having severe systemic lupus erythematosus (SLE), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

19. The method of claim 17 or claim 18 wherein the SLE in the subject has one or more of the following: renal, central nervous system, or hematologic involvement.

20. The method of any of claims 17-19, wherein the subject has at least one organ system categorized by the British Isles Lupus Assessment Group 2004 ("BILAG") as category A ("BILAG A") or at least two organ systems categorized as BILAG B.

21. The method of any of claims 17-20, wherein the subject fulfills the 2019 American College of Rheumatology (ACR)/ European League Against Rheumatism (EULAR) classification criteria of SLE and/or the subject has detectable anti-dsDNA, anti-histone, anti-chromatin or anti-Sm antibodies in their blood.

22. The method of any of claims 17-20, wherein the subject fulfills the 2019 American College of Rheumatology (ACR)/ European League Against Rheumatism (EULAR) classification criteria of SLE.

23. The method of any of claims 17-21, wherein the subject has detectable anti-dsDNA, anti-histone, anti-chromatin or anti-Sm antibodies in their blood.

24. The method of any of claims 17-23, wherein the subject has lupus nephritis.

25. A method of treating a subject having lupus nephritis, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having lupus nephritis, wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

26. The method of any of claims 17-25, wherein the subject is refractory to treatment with one or more prior therapies for the lupus.

27. The method of any of claims 17-26, wherein the subject achieved an insufficient response to one or more prior therapies for the lupus.

28. The method of claim 26 or claim 27, wherein the two or more prior therapies for the lupus comprise a glucocorticoid, an antimalarial, an immunosuppressant, an anti-CD20 antibody, or an inhibitor of soluble B lymphocyte stimulator (BLyS).

29. The method of any of claims 26-28, wherein the two or more prior therapies are selected from any two or more of the following: mycophenolate mofetil (MFF), cyclophosphamide (cyc), belimumab, rituximab, anifrolumab, azathioprine, methotrexate cyclosporine (csp) or voclosporin.

30. The method of any of claims 17-29, wherein the subject does not have drug-induced SLE, clinically significant CNS pathology, related systemic autoimmune diseases, and/or SLE overlap syndromes.

31. The method of claim 30, wherein the subject does not have related systemic autoimmune diseases, including by not limited to multiple sclerosis, psoriasis, and inflammatory bowel disease.

32. The method of claim 30, wherein the subject does not have SLE overlap syndromes, including by not limited to rheumatoid arthritis, scleroderma, and mixed connective tissue disease.

33. The method of any of claims 17-32, wherein the subject is at high risk for organ failure.

34. The method of any of claims 1-17, wherein the method reduces the systemic autoimmune disease activity in the subject.

35. The method of claim 34, wherein reducing disease activity in the subject comprises a reduced inflammation in the subject.

36. The method of any of claims 17-33, wherein the method reduces SLE disease activity in the subject.

37. The method of any of claims 18-24 and 34-36, wherein reducing SLE disease activity in the subject comprises: a BILAG-Based Composite Lupus Assessment (BICLA) response in the subject, reducing the subject's Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI) score compared to the subject's CLASI score pre-treatment, reducing the subject's tender and swollen joint count compared to the subject's tender and swollen joint count pre-treatment, the subject having a maximum of 1 BILAG-2004 B score following treatment, the subject having a BILAG-2004 score of C or better following treatment, the subject having an improvement in at least one patient reported outcome (PRO) compared to

pre-treatment, and/or reducing the subject's SLE flare rate compared to the subject's flare rate pre-treatment.

38. The method of any of claims 18-24 and 34-36, wherein reducing SLE disease activity in a subject comprises: the subject achieves clinical remission as defined by The Definitions of Remission in Systemic Lupus Erythematosus (DORIS), and/or the subject achieves Lupus Low Disease Activity State (LLDAS).

39. The method of any of claims 2-33 and 37-38, wherein the subject achieves clinical remission of the lupus within 3 months or within 6 months of administering the dose of CD19-directed genetically modified T cells.

40. The method of claim 38 or claim 39, wherein the clinical remission is maintained for at least about 6 months, at least about 12 months, at least about 24 months, at least about 3 years, at least about 4 years, or at least about 5 years.

41. The method of any of claims 2-33 and 36-40, wherein the subject achieves prolonged remission of the lupus.

42. A method of treating a subject having idiopathic inflammatory myopathy (IIM), the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having idiopathic inflammatory myopathy (IIM), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

43. A method for reducing idiopathic inflammatory myopathy (IIM) disease activity, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having idiopathic inflammatory myopathy (IIM), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

44. The method of claim 42 or claim 43, wherein the subject is refractory to treatment with one or more prior therapies for the IIM.
45. The method of claim 42 or claim 43, wherein the subject achieved an insufficient response to one or more prior therapies for the IIM.
46. A method of treating a subject having systemic sclerosis (SSc), the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having systemic sclerosis (SSc), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.
47. A method for reducing systemic sclerosis (SSc) disease activity, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having systemic sclerosis (SSc), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.
48. The method of claim 46 or claim 47, wherein the subject is refractory to treatment with one or more prior therapies for the SSc.
49. The method of claim 46 or claim 47, wherein the subject achieved an insufficient response to one or more prior therapies for the SSc.
50. A method of treating a subject having multiple sclerosis (MS), the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having multiple sclerosis (MS), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.
51. A method for reducing multiple sclerosis (MS) disease activity, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject

having or suspected of having multiple sclerosis (MS), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

52. The method of claim 50 or claim 51, wherein the subject is refractory to treatment with one or more prior therapies for the MS.

53. The method of claim 50 or claim 51, wherein the subject achieved an insufficient response to one or more prior therapies for the MS.

54. The method of any of claims 50-53, wherein the subject has or is suspected of having a relapsing form of MS.

55. The method of any of claims 50-53, wherein the subject has or is suspected of having a progressive form of MS.

56. The method of any of claims 50-54, wherein the subject has or is suspected of having highly active relapse-remitting MS.

57. The method of any of claims 50-53, wherein the subject has or is suspected of having primary progressive MS.

58. The method of any of claims 50-53, wherein the subject has or is suspected of having active secondary progressive MS (aSPMS).

59. The method of any of claims 50-53, wherein the subject has or is suspected of having inactive secondary progress MS (iSPMS).

60. The method of any of claims 50-59, wherein the subject has an Expanded Disability Status Scale (EDSS) of ≥ 3.0 and ≤ 5.5 or of ≥ 3.0 and ≤ 6.0 .

61. The method of any of claims 50-60, wherein the subject can complete the 9-Hole Peg Test (9-HPT) for each hand in <240 seconds, and subjects can perform a Timed 25-Foot Walk Test (T25FWT) in < 150 seconds.

62. The method of any of claims 50-61, wherein the subject does not have MS lesions or symptoms that may place them at increased risk of neurotoxicity.

63. The method of any of claims 39-62, wherein the method reduces the autoimmune disease activity in the subject.

64. The method of any of claims 32-63, wherein reducing disease activity in the subject comprises a reduced inflammation in the subject.

65. The method of claim 63, wherein the reducing the autoimmune disease activity in the subject comprises reducing the subject's IMACS score after treatment compared to the subject's IMACS score before treatment, reducing the subject's skin lesions, muscle fatigue, and/or weakness compared to the subject's skin lesions, muscle fatigue, and/or weakness pre-treatment, or the subject having an improvement in at least one patient reported outcome (PRO) compared to pre-treatment.

66. The method of claim 64, wherein the reducing the autoimmune disease activity in the subject comprises reducing the subjects modified Rodnan skin score, European Scleroderma Study Group (EScSG) indices, minimum clinically important differences (MCID), patient reported short-form quality of life assessment (SF-36) Physical Component Summary (PCS) and/or Mental Component Summary (MCS) or a combination thereof or improving forced vital capacity.

67. The method of claim 65, wherein the reducing the autoimmune disease activity in the subject comprises improving the subjects score in any of the following tests; expanded disability status scale (EDSS), disease steps, multiple sclerosis functional composi (MSFC), minimum clinically important differences (MCID), patient reported short-form quality of life

assessment (SF-36) Physical Component Summary (PCS) and/or Mental Component Summary (MCS) or a combination thereof.

68. A method of treating a subject having autoimmune vasculitis (AAV), the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having autoimmune vasculitis (AAV), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

69. A method for reducing autoimmune vasculitis (AAV) disease activity, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having autoimmune vasculitis (AAV), wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

70. A method of treating a subject having IgA nephropathy, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having IgA nephropathy, wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

71. A method for reducing IgA nephropathy disease activity, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having IgA nephropathy, wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

72. A method of treating a subject having pemphigus vulgaris, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having pemphigus vulgaris, wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

73. A method for reducing pemphigus vulgaris disease activity, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having pemphigus vulgaris, wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

74. A method of treating a subject having myasthenia gravis, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having myasthenia gravis, wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

75. A method for reducing myasthenia gravis disease activity, the method comprising administering a dose of CD19-directed genetically modified T cells to a subject having or suspected of having myasthenia gravis, wherein the T cells of the dose are positive for expression of a chimeric antigen receptor (CAR) that binds CD19 and the dose is from 1×10^6 to 50×10^6 CAR-positive viable T cells.

76. The method of any of claims 1-75, wherein the dose is at or about 1×10^6 to 40×10^6 CAR-positive viable T cells.

77. The method of any of claims 1-75, wherein the dose is at or about 1×10^6 to 25×10^6 CAR-positive viable T cells.

78. The method of any of claims 1-75, wherein the dose is at or about 5×10^6 CAR-positive viable T cells.

79. The method of any of claims 1-75, wherein the dose is at or about 10×10^6 CAR-positive viable T cells.

80. The method of any of claims 1-75, wherein the dose is at or about 25×10^6 CAR-positive viable T cells.

81. The method of any of claims 1-75, wherein the dose is at or about 50×10^6 CAR-positive viable T cells.

82. The method of any of claims 1-81, wherein the T cells are autologous to the subject.

83. The method of any of claims 1-81, further comprising obtaining a leukapheresis sample from the subject for manufacturing the composition comprising engineered T cells.

84. The method of any of claims 1-83, wherein prior to the administration, the subject has been preconditioned with a lymphodepleting therapy.

85. The method of any of claims 1-84, wherein the method further comprises, immediately prior to the administration of the dose of CD19-directed genetically modified T cells, administering a lymphodepleting therapy to the subject, wherein the lymphodepleting therapy comprises the administration of fludarabine and/or cyclophosphamide.

86. The method of any of claims 1-85, wherein the administration of the dose of CD19-directed genetically modified T cells and/or the lymphodepleting therapy is carried out via outpatient delivery.

87. The method of any of claims 84-86, wherein the lymphodepleting therapy comprises the administration of fludarabine at 30 mg/m^2 body surface area of the subject, daily, and cyclophosphamide at 300 mg/m^2 body surface area of the subject, daily, each for 3 days.

88. The method of any of claims 84-86, wherein the dose of CD19-directed genetically modified T cells is administered between at or about 48 hours and at or about 9 days, inclusive, after completion of the lymphodepleting therapy.

89. The method of any of claims 1-88, wherein the dose of CD19-directed genetically modified T cells is administered to the subject by intravenous infusion.

90. The method of any of claims 1-89, wherein the CAR comprises an extracellular antigen-binding domain that binds CD19, a transmembrane domain, and an intracellular signaling domain.

91. The method of claim 90, wherein the CAR comprises a hinge spacer between the extracellular antigen-binding domain and the transmembrane domain, optionally wherein the hinge spacer is an immunoglobulin hinge or a CD8a hinge.

92. The method of claim 90 or claim 91, wherein the extracellular antigen-binding domain is an FMC63 monoclonal antibody-derived single chain variable fragment (scFv).

93. The method of any of claims 90-92, wherein the extracellular antigen-binding domain comprises a variable heavy chain set forth in SEQ ID NO:41 and a variable light chain set forth in SEQ ID NO:42.

94. The method of claim 92 or claim 93, wherein the scFv is set forth as SEQ ID NO: 43.

95. The method of claim 90 or claim 91, wherein the extracellular antigen-binding domain is a Hu19 single chain variable fragment (scFv).

96. The method of any one of claims 90, 91, and 95, wherein the extracellular antigen-binding domain comprises a variable heavy chain set forth in SEQ ID NO:114 and a variable light chain set forth in SEQ ID NO:112.

97. The method of claim 95 or 96, wherein the extracellular antigen-binding domain comprises in order a variable light chain set forth in SEQ ID NO: 112, a linker peptide set forth in SEQ ID NO: 113, and a variable heavy chain set forth in SEQ ID NO: 114.

98. The method of any one of claims 1-97, wherein the CAR is a monospecific CAR directed to CD19.

99. The method of any of claims 1-97, wherein the CAR is a tandem bispecific CAR directed against CD19 and at least one other antigen expressed on B cells.

100. The method of claim 99, wherein the other antigen expressed on B cells is selected from the group consisting of CD20, CD19, CD22, ROR1, BCMA, CD45, CD21, CD5, CD33, Iglambda, CD79a, CD79b or CD30.

101. The method of claim 99 or claim 100, wherein the other antigen expressed on B cells is CD20.

102. The method of claim 101, wherein the extracellular antigen-binding domain comprises a variable heavy chain and a variable light chain derived from a CD20 antibody selected from the group consisting of Leu16, C2B8, 11B8, 8G6-5, 2.1.2 and GA101.

103. The method of any of claims 90-102, wherein the transmembrane domain is a CD28 transmembrane domain.

104. The method of any of claims 90-103, wherein the transmembrane domain is a transmembrane domain from CD28, optionally a transmembrane domain that comprises the sequence of amino acids set forth in SEQ ID NO: 8 or a sequence of amino acids that exhibits at least or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:8.

105. The method of any of claims 101-104, wherein the intracellular signaling domain comprises a 4-1BB costimulatory domain and a CD3zeta activation domain.

106. The method of any of claims 1-105, wherein the CAR comprises, in order from N- to C-terminus, an FMC63 monoclonal antibody-derived single chain variable fragment

(scFv), IgG4 hinge region, a CD28 transmembrane domain, a 4-1BB (CD137) costimulatory domain, and a CD3 zeta signaling domain.

107. The method of claim 105 or claim 106, wherein the 4-1BB costimulatory domain is or comprises the sequence set forth in SEQ ID NO: 12 or a variant thereof having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:12.

108. The method of any of claims 105-107, wherein the CD3zeta signaling domain is or comprises the sequence set forth in SEQ ID NO: 13, 14 or 15 or a sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto.

109. The method of any of claims 1-108, wherein the CAR contains in order from N-terminus to C-terminus: an extracellular antigen-binding domain that is the scFv set forth in SEQ ID NO: 43, the spacer set forth in SEQ ID NO:1, the transmembrane domain set forth in SEQ ID NO:8, the 4-1BB costimulatory signaling domain set forth in SEQ ID NO:12, and the signaling domain of a CD3-zeta (CD3 ζ) chain set forth in SEQ ID NO:13.

110. The method of any of claims 1-109, wherein the CAR comprises the amino acid sequence set forth in SEQ ID NO:59 or a sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto.

111. The method of any of claims 1-110, wherein the composition produced by a manufacturing process comprising:

(i) stimulating an input composition comprising primary T cells from the subject with an oligomeric stimulatory reagent, thereby generating a stimulated population, wherein the oligomeric stimulatory reagent comprises a plurality of cross-linked tetramers of a streptavidin or streptavidin mutein and wherein the streptavidin or streptavidin mutein are reversibly bound to a first agent comprising an anti-CD3 antibody or antigen binding fragment thereof and a second agent comprising an anti-CD28 antibody or antigen binding fragment thereof;

(ii) introducing into T cells of the stimulated population, a heterologous polynucleotide encoding the CAR that targets CD19, thereby generating a population of transformed cells;

(iii) incubating the population of transformed cells for up to 96 hours; and

(iv) harvesting T cells of the population of transformed cells, thereby producing a composition of CD19-directed genetically modified T cells wherein the harvesting is carried out at a time between 24 and 120 hours, inclusive, after the exposing to the stimulatory reagent is initiated.

112. The method of claim 111, wherein the anti-CD3 antibody or antigen binding fragment is a Fab and the anti-CD28 antibody or antigen binding fragment is a Fab.

113. The method of claim 111 or claim 112, wherein the first agent and the second agent each comprise a streptavidin-binding peptide that reversibly binds the first agent and the second agent to the oligomeric particle reagent, optionally wherein the streptavidin-binding peptide comprises the sequence of amino acids set forth in any of SEQ ID NOS:78-82.

114. The method of any of claims 111-113, wherein the streptavidin mutein molecule is a tetramer of a streptavidin mutein comprising amino acid residues Val44-Thr45-Ala46-Arg47 or Ile44-Gly45-Ala46-Arg47, optionally wherein the streptavidin mutein comprises the sequence set forth in any of SEQ ID NOS: 69, 84, 87, 88, 90, 85 or 59.

115. The method of any of claims 111-114, wherein the oligomeric particle reagent comprises between 1,000 and 5,000 streptavidin mutein tetramers, inclusive.

116. The method of any of claims 111-115, wherein the method further comprises, prior to harvesting the cells, adding biotin or a biotin analog after or during the incubation.

117. The method of any of claims 111-116, wherein the harvesting is carried out at a time between 48 and 120 hours, inclusive, after the exposing to the stimulatory reagent is initiated.

118. The method of any of claims 1-117, wherein the dose of autologous CD19-directed genetically modified T cells is cryopreserved prior to administration to the subject.

119. The method of claim 118, wherein the cryopreserved dose of autologous CD19-directed genetically modified T cells is thawed prior to administration to the subject.

120. The method of claim 119, wherein the dose of autologous CD19-directed genetically modified T cells is administered to the subject within about two hours of being thawed.

121. The method of any of claims 1-120, wherein the dose of autologous CD19-directed genetically modified T cells is provided in a formulation comprising a cryoprotectant.

122. The method of claim 121, wherein the formulation comprises dimethylsulfoxide (DMSO).

123. The method of claim 121 or claim 122, wherein the formulation comprises albumin, optionally human albumin.

124. The method of any of claims 1-123, wherein the dose of T cells comprises CD4⁺ T cells expressing the CAR and CD8⁺ T cells expressing the CAR.

125. The method of any of claims 1-124, wherein the dose of T cells comprises CD4⁺ T cells expressing the CAR and CD8⁺ T cells expressing the CAR at a ratio between about 1:5 and about 5:1, optionally at a ratio between about 1:3 and about 3:1.

126. The method of any of claims 1-125, wherein at least or at least about 90% of the cells in the composition are CD3⁺ cells.

127. The method of any of claims 1-126, wherein at least or at least about 91%, at least or at least about 92%, at least or at least about 93%, at least or at least about 94%, at least

or at least about 95%, or at least or at least about 96% of the cells in the composition are CD3⁺ cells.

128. The method of any of claims 1-127, wherein at least 25% of the T cells in the composition are CAR⁺ T cells.

129. The method of any of claims 1-128, wherein at least 30%, at least 35%, at least 40%, at least 45% or at least 50% of the T cells in the composition are CAR⁺ T cells.

130. The method of any of claims 1-129, wherein between at or about 5% and at or about 30% of the CAR⁺ T cells in the composition express a marker of apoptosis, optionally between at or about 10% and at or about 15% of the CAR⁺ T cells in the composition, more optionally wherein the marker of apoptosis is Annexin V or active Caspase 3.

131. The method of any of claims 1-127, wherein less than 10% of the T cells, optionally the CAR⁺ T cells, in the composition express a marker of apoptosis, optionally wherein the marker of apoptosis is Annexin V or active Caspase 3.

132. The method of any of claims 1-127 and 131, wherein less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, or less than 4% of the T cells, optionally the CAR⁺ T cells, in the composition express a marker of apoptosis, optionally wherein the marker of apoptosis is Annexin V or active Caspase 3.

133. The method of any of claims 1-132, wherein at least 80% of the T cells in the composition are viable T cells, optionally wherein viability is determined by staining for acridine orange (AO) and propidium iodide (PI).

134. The method of any of claims 1-130, wherein at least or at least about 80% of the CAR⁺ T cells in the composition are of a naïve-like or central memory phenotype.

135. The method of claim 134, wherein the marker expressed on naïve-like or central memory T cell is selected from the group consisting of CD45RA, CD27, CD28, and CCR7.

136. The method of any of claims 1-135, wherein at least 85% of the CAR⁺ T cells in the composition are CCR7⁺.

137. The method of any of claims 1-136, wherein at least 85% of the CD8⁺CAR⁺ T cells in the composition are CCR7⁺ and at least 90% of the CD4⁺ CAR⁺ T cells in the composition are CCR7⁺.

138. The method of any of claims 1-137, wherein 85% to 98% of the CD8⁺ CAR⁺ T cells in the composition are CCR7⁺ and 94% to 99% of the CD4⁺ CAR⁺ T cells in the composition are CCR7⁺.

139. The method of any of claims 1-135, wherein the at least or at least about 80% of the CAR⁺ T cells in the composition that are of a naïve-like or central memory phenotype have a phenotype selected from one or more of phenotypes CCR7⁺CD45RA⁺, CCR7⁺CD45RA⁻, CD27⁺CCR7⁺, or CD62L⁺CCR7⁺.

140. The method of any of claims 1-139, wherein at least 40% of the CAR⁺ T cells are CD45RA⁺CCR7⁺.

141. The method of any of claims 1-140, wherein at least 50% of the CAR⁺ T cells are CD45RA⁺CCR7⁺.

142. The method of any of claims 1-141, wherein at least 60% of the CAR⁺ T cells are CD45RA⁺CCR7⁺.

143. The method of any of claims 1-142, wherein at least 70% of the CAR⁺ T cells are CD45RA⁺CCR7⁺.

144. The method of any of claims 1-143, wherein at least 80% of the CAR⁺ T cells are CD45RA⁺CCR7⁺.

145. The method of any of claims 1-144, wherein at least 20% of the CAR+ T cells are CD45RA-CCR7+.

146. The method of any of claims 1-145, wherein at least 30% of the CAR+ T cells are CD45RA-CCR7+.

147. The method of any of claims 1-146, wherein at least 40% of the CAR+ T cells are CD45RA-CCR7+.

148. The method of any of claims 1-147, wherein at least 50% of the CAR+ T cells are CD45RA-CCR7+.

149. The method of any of claims 1-148, wherein at least 60% of the CAR+ T cells are CD45RA-CCR7+.

150. The method of any of claims 1-149, wherein at least about 50% of CD4+CAR+ T cells in the composition are CCR7+CD45RA⁻.

151. The method of any of claims 1-149, wherein at least about 60% of CD4+CAR+ T cells in the composition are CCR7+CD45RA⁻.

152. The method of any of claims 1-149, wherein at least about 70% of CD4+CAR+ T cells in the composition are CCR7+CD45RA⁻.

153. The method of any of claims 1-152, wherein at least about 30% of CD8+CAR+ T cells in the composition are CCR7+CD45RA⁻.

154. The method of any of claims 1-152, wherein at least about 40% of CD8+CAR+ T cells in the composition are CCR7+CD45RA⁻.

155. The method of any of claims 1-152, wherein at least about 50% of CD8+CAR+ T cells in the composition are CCR7+CD45RA⁻.

156. The method of any of claims 1-155, wherein:

- (i) at least 60% of the T cells in the composition are viable;
- (ii) at least 25% of the T cells of the composition are CAR+ T cells;
- (iii) at least 85% of the CD8+CAR+ T cells in the composition are CCR7+; and
- (iv) at least 90% of the CD4+ CAR+ T cells in the composition are CCR7+.

157. The method of any of claims 1-155, wherein:

- (i) at least 80% of the T cells in the composition are viable;
- (ii) at least 45% of the T cells of the composition are CAR+;
- (iii) at least 85% of the CD8+CAR+ T cells in the composition are CCR7+; and
- (iv) at least 90% of the CD4+ CAR+ T cells in the composition are CCR7+.

158. The method of any of claims 1-155, wherein:

- (i) at least 60% of the T cells in the composition are viable,
- (ii) at least 25% of the T cells of the composition are CAR+; and
- (iii) greater than at or about 40% of the CAR+ T cells in the composition are CCR7+CD45RA+.

159. The method of any of claims 1-155, wherein:

- (i) at least 80% of the T cells in the composition are viable;
- (ii) at least 45% of the T cells of the composition are CAR+; and
- (iii) at least 40% of the CAR+ T cells in the composition are CCR7+CD45RA+.

160. The method of any of claims 1-155, wherein:

- (i) at least 60% of the T cells in the composition are viable;
- (ii) at least 25% of the T cells of the composition are CAR+; and
- (iii) greater than 20% of the CAR+ T cells in the composition are CCR7+CD45RA-.

161. The method of any of claims 1-155, wherein:

- (i) at least 80% of the T cells in the composition are viable;
- (ii) at least 45% of the T cells of the composition are CAR+; and

(iii) at least 20% of the CAR+ T cells in the composition are CCR7+CD45RA-.

162. The method of any of claims 1-161, wherein less than 10% of the cells of the composition are positive for an apoptotic marker, optionally wherein the marker of apoptosis is Annexin V or active Caspase 3.

163. The method of any of claims 1-161, wherein less than 4% of the cells of the composition are positive for an apoptotic marker, optionally wherein the marker of apoptosis is Annexin V or active Caspase 3.

164. The method of any of claim 1-163, wherein greater than or greater than about 50%, about 60%, about 70%, or about 80% of the subjects treated according to the method do not exhibit any grade of cytokine release syndrome (CRS).

165. The method of any of claim 1-164, wherein greater than or greater than about 40%, 50%, or about 60% of the subjects treated according to the method do not exhibit any grade of neurotoxicity.

166. The method of any of claims 1-165, wherein the subject does not receive administration of immunosuppressant for treating the disease after administering the dose of CD19-directed genetically modified T cells.

167. The method of any of claims 1-166, wherein the subject is human.