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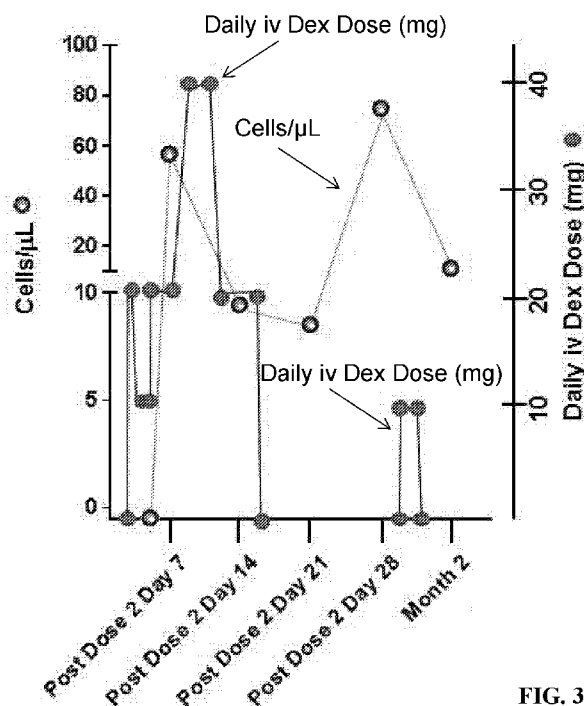


FIG. 31

(57) Abstract: Provided are methods of treatment, such as methods involving administering and/or determining dosing of, cell therapy, such as of cells engineered with a recombinant receptor, such as a T cell receptor (TCR) or chimeric antigen receptor (CAR). In some embodiments, the methods include determining a therapeutic range and/or window for dosing, for example, based on the estimated probabilities of risk of developing a toxicity and estimated probabilities of a treatment outcome or response, such as treatment, reduction nor amelioration of a sign or symptom thereof, or degree or durability thereof, following administration of the cell therapy or engineered cells. In some aspects, the methods involve administering an agent capable of modulating the engineered cells. Also provided are methods of ameliorating and/or treating a toxicity.



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METHODS FOR DOSING AND FOR MODULATION OF GENETICALLY ENGINEERED CELLS

Cross-Reference to Related Applications

[0001] This application claims priority from U.S. provisional application No. 62/593,878, filed December 1, 2017, entitled “METHODS FOR DOSING AND FOR MODULATION OF GENETICALLY ENGINEERED CELLS,” U.S. provisional application No. 62/596,773, filed December 8, 2017, entitled “METHODS FOR DOSING AND FOR MODULATION OF GENETICALLY ENGINEERED CELLS,” U.S. provisional application No. 62/633,599, filed February 21, 2018, entitled “METHODS FOR DOSING AND FOR MODULATION OF GENETICALLY ENGINEERED CELLS,” U.S. provisional application No. 62/679,763, filed June 1, 2018, entitled “METHODS FOR DOSING AND FOR MODULATION OF GENETICALLY ENGINEERED CELLS,” and U.S. provisional application No. 62/679,764, filed June 1, 2018, entitled “METHODS FOR DOSING AND FOR MODULATION OF GENETICALLY ENGINEERED CELLS,” the contents of which are incorporated by reference in their entirety.

Incorporation by Reference of Sequence Listing

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled 735042015240SeqList.txt, created November 30, 2018, which is 34 kilobytes in size. The information in the electronic format of the Sequence Listing is incorporated by reference in its entirety.

Field

[0003] The present disclosure relates in some aspect to methods of treatment, such as methods involving administering and/or determining dosing of, cell therapy, such as of cells engineered with a recombinant receptor, such as a T cell receptor (TCR) or chimeric antigen receptor (CAR). In some embodiments, the methods include determining a therapeutic range and/or window for dosing, for example, based on the estimated probabilities of risk of developing a toxicity and estimated probabilities of a treatment outcome or response, such as treatment, reduction nor amelioration of a sign or symptom thereof, or degree or durability thereof, following administration of the cell therapy or engineered cells. In some aspects, the methods involve administering an agent capable of modulating the engineered cells. Also

provided are methods of ameliorating and/or treating a toxicity.

Background

[0004] Various approaches are available for immunotherapy, for example, adoptive cell therapy methods involving administering T cells, such as those expressing genetically engineered antigen receptors, such as chimeric antigen receptors (CARs). In some aspects, available methods may not be entirely satisfactory. There is a need for additional strategies for immunotherapy and adoptive cell therapy, e.g., strategies to enhance persistence, activity and/or proliferation of administered cells and responses and strategies for modulating T cell phenotype, activity and/or expansion. Provided in some embodiments are methods, cells, compositions, articles of manufacture, and systems to address such needs.

Summary

[0005] Provided herein are methods of dosing or treating a subject, which in some aspects involve administering to the subject a dose of engineered cells, such as those engineered with a chimeric antigen receptor (CAR), and/or assessing and/or administering further agent(s) to subjects having been administered such engineered cells. In some of any such embodiments, the dose administered is within a therapeutic range and/or window and/or is sufficient to achieve an overall or peak amount or number of engineered cells, e.g., CAR+ cells, in a sample or tissue or bodily fluid of the subject, such as in the blood of the subject, within a specified range, such as within a specified or determined therapeutic range, optionally within or over a certain period of time following administration. In some aspects, the therapeutic range is determined based upon or relates to probabilities, such as estimated probabilities, e.g., probability of response and/or probability or risk of developing a sign or symptom of a toxicity, such as a severe and/or grade 3 or higher toxicity, such as neurotoxicity (NT), e.g., a grade 3 or higher toxicity.

[0006] In some of any such embodiments, the administering involves administration of a sub-optimal or reduced or low dose of cells which in some aspects is insufficient to be within or achieve or result within a therapeutic range and/or window and/or is insufficient to achieve an overall or peak amount or number of engineered cells, e.g., CAR+ cells, in a sample or tissue or bodily fluid of the subject, such as in the blood of the subject, within a specified range, such as within a specified or determined therapeutic range, optionally within or over a certain period of time following administration. In some aspects, such as in aspects of such embodiments, provided methods further include administering a compound to the subject other than or in

addition to the engineered cells. in some aspects, such agent may be an agent known or suspected of being capable of enhancing or increasing the likelihood, degree, rapidity, or level of expansion, persistence and/or exposure of the subject to the engineered cells, such as the CAR+ cells. In some aspects, the agent(s) increases or promotes expansion of the cells in vivo, and/or is capable of resulting in levels, degree or rapidity of expansion, peak levels, AUC, or other measure of the cells in the subject, such as CAR+ cells, expansion is within the therapeutic range and/or window. In some of any such embodiments, the therapeutic range in some aspects is determined based upon or relates to probabilities, such as estimated probabilities, e.g., probability of response and/or probability or risk of developing a sign or symptom of a toxicity, such as a severe and/or grade 3 or higher toxicity, such as neurotoxicity (NT), e.g., a grade 3 or higher toxicity.

[0007] In some of any such embodiments, the methods involve, e.g., subsequent to the administration, to the subject the cell therapy or engineered cells; monitoring levels of engineered or other cells in a sample of the subject such as a blood or blood-derived samples (such as peak CAR cells in the blood), optionally over time, for example, to assess whether the cells are within a therapeutic range and/or window. In some aspects, if the cells are not within a therapeutic range or window, the provided methods include an administration to the subject, such as administering a compound to enhance expansion or exposure to the engineered cells such as to enhance CAR+ cell expansion in vivo, e.g., such that the peak CAR+ expansion and/or levels and/or exposure and/or AUC is within the therapeutic or desired range.

[0008] In some of any such embodiments, the level of engineered, e.g., CAR+, cells in the sample is determined as the number of the cells, e.g., CAR+ cells, per microliter of the sample; In some of any such embodiments, the peak level is the highest such measurement following, optionally over a specified period of time following, administration of the cells or cell therapy to the subject.

[0009] In some of any such embodiments, the therapeutic range is a range in which the estimated probability of a toxicity or toxic outcome or sign or symptom thereof, such as a severe toxicity and/or a neurotoxicity (NT) or CRS, is less than 20%, less than 15%, less than 10% or less than 5%; in some aspects, the probability is based on a probability curve, e.g., based on outcomes of subjects treated with or administered the cell therapy and/or cells engineered to express the recombinant receptor. In some of any such embodiments, the estimated probability of achieving a treatment response, effect, amelioration or treatment is greater than 20%, 25%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more.

[0010] In some of any such embodiments, the toxicity is a neurotoxicity and/or is severe toxicity and/or is grade 3-5 neurotoxicity.

[0011] In some of any such embodiments, the response or indicator of response is a marrow response or an outcome measured in bone marrow of the subject. In some cases, the presence or absence of the marrow response is or is determined by flow cytometry and/or IgH sequencing and/or indicates or is a reduction or elimination of cells of the disease or condition in a sample of the subject, optionally an organ, tissue or fluid of the subject, such as a lymph node, bone marrow, tumor site, blood or other sample, of the subject.

[0012] In some of any such embodiments, the disease or condition is a cancer. In some aspects, the cancer is selected from the group consisting of sarcomas, carcinomas, lymphomas, non-Hodgkin lymphomas (NHLs), diffuse large B cell lymphoma (DLBCL), leukemia, CLL, ALL, AML and myeloma. In some cases, the cancer is a pancreatic cancer, bladder cancer, colorectal cancer, breast cancer, prostate cancer, renal cancer, hepatocellular cancer, lung cancer, ovarian cancer, cervical cancer, pancreatic cancer, rectal cancer, thyroid cancer, uterine cancer, gastric cancer, esophageal cancer, head and neck cancer, melanoma, neuroendocrine cancers, CNS cancers, brain tumors, bone cancer, or soft tissue sarcoma.

[0013] In some of any such embodiments, the chimeric antigen receptor (CAR) contains an extracellular antigen-recognition domain that specifically binds to the antigen and an intracellular signaling domain comprising an ITAM. In some aspects, the intracellular signaling domain contains an intracellular domain of a CD3-zeta (CD3 ζ) chain. In some of any such embodiments, the chimeric antigen receptor (CAR) further comprises a costimulatory signaling region. In some cases, the costimulatory signaling region comprises a signaling domain of CD28 or 4-1BB. In some instances, the costimulatory domain is a domain of CD28. In some instances, the costimulatory domain is a domain of 4-1BB.

[0014] In some of any such embodiments, the CAR specifically recognizes or binds an antigen selected from among antigens expressed by B cells, ROR1, B cell maturation antigen (BCMA), Her2, L1-CAM, CD19, CD20, CD22, mesothelin, CEA, and hepatitis B surface antigen, anti-folate receptor, CD23, CD24, CD30, CD33, CD38, CD44, EGFR, EGP-2, EGP-4, EPHA2, ErbB2, 3, or 4, erbB dimers, EGFR vIII, FBP, FCRL5, FCRH5, GPRC5D, fetal acetylcholine e receptor, GD2, GD3, HMW-MAA, IL-22R-alpha, IL-13R-alpha2, kdr, kappa light chain, Lewis Y, L1-cell adhesion molecule, (L1-CAM), Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, Preferentially expressed antigen of melanoma (PRAME), survivin, EGP2, EGP40, TAG72, B7-H6, IL-13 receptor a2 (IL-13Ra2), CA9, GD3, HMW-

MAA, CD171, G250/CAIX, HLA-AI MAGE A1, HLA-A2 NY-ESO-1, PSCA, folate receptor-a, CD44v6, CD44v7/8, avb6 integrin, 8H9, NCAM, VEGF receptors, 5T4, Foetal AchR, NKG2D ligands, CD44v6, dual antigen, and an antigen associated with a universal tag, a cancer-testes antigen, mesothelin, MUC1, MUC16, PSCA, NKG2D Ligands, NY-ESO-1, MART-1, gp100, oncofetal antigen, ROR1, TAG72, VEGF-R2, carcinoembryonic antigen (CEA), prostate specific antigen, PSMA, Her2/neu, estrogen receptor, progesterone receptor, ephrinB2, CD123, c-Met, GD-2, O-acetylated GD2 (OGD2), CE7, Wilms Tumor 1 (WT-1), a cyclin, cyclin A2, CCL-1, CD138, and a pathogen-specific antigen.

[0015] In some of any such embodiments, the cells are T cells. In some cases, the T cells are CD4+ or CD8+.

[0016] Also provided are articles of manufacture and compositions, such as those containing the cells and instructions for administration such as according to the methods and uses of any of the embodiments.

[0017] Provided here are methods of treatment including administering to a subject having a disease or condition, a dose of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR) for treating the disease or condition, after administering the dose of genetically engineered cells, monitoring CAR+ T cells in the blood of the subject to assess if the cells are within a therapeutic range, and if the genetically engineered cells are not within the therapeutic range, administering an agent to the subject capable of modulating, optionally increasing or decreasing, CAR+ T cell expansion or proliferation, in the subject, wherein the therapeutic range is: (i) based upon the range of peak CD3+ CAR+ T cells, or a CD8+CAR+ T cell subset thereof, in the blood among one or more subjects previously treated with the genetically engineered cells that is associated with an estimated probability of response of greater than or greater than about 65%, 70%, 75%, 80%, 85%, 90%, and an estimated probability of a toxicity of less than or about 30%; or (ii) peak CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 10 cells per microliter and 500 cells per microliter; or (iii) peak CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 2 cells per microliter and 200 cells per microliter.

[0018] Provided here are methods of treatment including monitoring, in the blood of a subject, the presence of genetically engineered cells containing T cells expressing a chimeric antigen receptor (CAR) to assess if the cells are within a therapeutic range, wherein the subject has been previously administered a dose of the genetically engineered cells for treating a disease

or condition; and if the genetically engineered cells are not within the therapeutic range, administering an agent to the subject capable of modulating, optionally increasing or decreasing, CAR+ T cell expansion or proliferation, in the subject, wherein the therapeutic range is: (i) based upon the range of peak CD3+ CAR+ T cells, or a CD8+CAR+ T cell subset thereof, in the blood among one or more subjects previously treated with the genetically engineered cells that is associated with an estimated probability of response of greater than or greater than about 65%, 70%, 75%, 80%, 85%, 90%, and an estimated probability of a toxicity of less than or about 30%, 25%, 20%, 15%, 10%, 5%; or (ii) peak CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 10 cells per microliter and 500 cells per microliter; or (iii) peak CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 2 cells per microliter and 200 cells per microliter. In some of any such embodiments, if the peak number of CAR+ T cells in the blood of the subject is less than the lowest number of peak CAR+ T cells in the therapeutic range, an agent is administered to the subject that is capable of increasing CAR+ T cell expansion or proliferation. In some cases, the agent is capable of CAR-specific expansion.

[0019] In some of any such embodiments, the agent is an anti-idiotypic antibody or antigen-binding fragment thereof specific to the CAR, an immune checkpoint inhibitor, a modulator of a metabolic pathway, an adenosine receptor antagonist, a kinase inhibitor, an anti-TGF β antibody or an anti-TGF β R antibody or a cytokine.

[0020] In some of any such embodiments, if the peak number of CAR+ T cells in the blood of the subject is greater than the highest number of peak CAR+ T cells in the therapeutic range, an agent is administered to the subject that is capable of decreasing CAR+ T cell expansion or proliferation. In some examples, the agent is a steroid. In some cases, the steroid is a corticosteroid. In some of any such embodiments, the steroid is dexamethasone or methylprednisolone.

[0021] In some of any such embodiments, the steroid is administered in an amount that is between or between about 1.0 mg and about 40 mg, between or between about 1.0 mg and about 20 mg, between or between about 2.0 mg and about 20 mg, between or between about 5.0 mg and about 25.0 mg, between or between about 10 mg and about 20 mg dexamethasone or equivalent thereof, each inclusive.

[0022] In some of any such embodiments, the steroid is administered in multiple doses over a period of at or more than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more or within a range

defined by any of the foregoing. In some of any such embodiments, the steroid is administered once per day, twice per day, or three times or more per day. In some of any such embodiments, the steroid is administered in an amount that is between or between about 1.0 mg and about 80 mg, between or between about 1.0 mg and about 60 mg, between or between about 1.0 mg and about 40 mg, between or between about 1.0 mg and about 20 mg, between or between about 1.0 mg and about 10 mg, between or between about 2.0 mg and about 80 mg, between or between about 2.0 mg and about 60 mg, between or between about 2.0 mg and about 40 mg, between or between about 2.0 mg and about 20 mg, between or between about 2.0 mg and about 10 mg, between or between about 5.0 mg and about 80 mg, between or between about 5.0 mg and about 60 mg, between or between about 5.0 mg and about 40 mg, between or between about 5.0 mg and about 20 mg, between or between about 5.0 mg and about 10 mg, between or between about 10 mg and about 80 mg, between or between about 10 mg and about 60 mg, between or between about 10 mg and about 40 mg, between or between about 10 mg and about 20 mg dexamethasone or equivalent thereof, each inclusive, per day or per 24 hours, or about 10 mg, 20 mg, 40 mg or 80 mg dexamethasone or equivalent thereof, per day or per 24 hours.

[0023] In some of any such embodiments, the subject is monitored for CAR+ T cells in the blood at a time that is at least 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days or 21 days after initiation of administration of the genetically engineered cells. In some of any such embodiments, the subject is monitored for CAR+ T cells in the blood at a time that is between or between about 11 to 22 days, 12 to 18 days or 14 to 16 days, each inclusive, after initiation of administration of the genetically engineered cells.

[0024] In some of any such embodiments, the agent is administered at a time that is greater than or greater than about 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days or 21 days after initiation of administration of the genetically engineered cells. In some of any such embodiments, the agent is administered at a time that is between or between about 11 to 22 days, 12 to 18 days or 14 to 16 days, each inclusive, after initiation of administration of the genetically engineered cells.

[0025] Provided are methods of modulating activity of engineered cells, the method including selecting a subject in which the level, amount or concentration of a volumetric measure of tumor burden or an inflammatory marker in a sample from the subject is at or above a threshold level, wherein the sample does not contain genetically engineered T cells expressing a chimeric antigen receptor (CAR) and/or is obtained from the subject prior to receiving

administration of genetically engineered T cells expressing a CAR; and administering to the selected subject an agent that is capable of decreasing expansion or proliferation of genetically engineered T cells expressing a CAR.

[0026] Provided are methods of modulating activity of engineered cells, the method including administering to a subject an agent that is capable of decreasing expansion or proliferation of genetically engineered T cells expressing a chimeric antigen receptor (CAR) in a subject, wherein the subject is one in which the level, amount or concentration of a volumetric measure of tumor burden or an inflammatory marker in a sample from the subject is at or above a threshold level.

[0027] In some of any such embodiments, the sample does not comprise genetically engineered T cells expressing a CAR and/or is obtained from the subject prior to receiving administration of genetically engineered T cells expressing a CAR.

[0028] In some of any such embodiments, the agent is administered prior to or concurrently with initiation of administration of a dose of genetically engineered cells including T cells expressing a chimeric antigen receptor. In some cases, the method further includes administering a dose of the genetically engineered cells.

[0029] In some of any such embodiments, the subject has a disease or condition and the genetically engineered cells are for treating the disease or condition.

[0030] In some of any such embodiments, prior to administering the agent, the selected subject is at risk of developing a toxicity following administration of the genetically engineered cells. In some of any such embodiments, the administration of the agent is sufficient to achieve peak CAR+ T cells in a therapeutic range in the subject, or in a majority of selected subjects so treated by the method or in greater than 75%, 80%, 85%, 90%, 95% of the selected subjects so treated by the method.

[0031] In some aspects, the therapeutic range is based upon the range of peak CD3+ CAR+ T cells, or a CD8+CAR+ T cell subset thereof, in the blood among one or more subjects previously treated with the genetically engineered cells that is associated with an estimated probability of response of greater than or greater than about 65%, 70%, 75%, 80%, 85%, 90%, and an estimated probability of a toxicity of less than or about 30%, 25%, 20%, 15%, 10%, 5%; or peak CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 10 cells per microliter and 500 cells per microliter; or peak CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 2 cells per microliter and 200 cells per microliter.

[0032] In some of any such embodiments, the therapeutic range is: (i) based upon the number or level of CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 10 cells per microliter and 500 cells per microliter; or (ii) based upon the number or level of CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 2 cells per microliter and 200 cells per microliter.

[0033] In some of any such embodiments, a volumetric measure of tumor burden is measured and the volumetric measure is a sum of the products of diameters (SPD), longest tumor diameters (LD), sum of longest tumor diameters (SLD), tumor volume, necrosis volume, necrosis-tumor ratio (NTR), peritumoral edema (PTE), and edema-tumor ratio (ETR). In some cases, the volumetric measure is a sum of the products of diameter (SPD). In some of any such embodiments, the volumetric measure is measured using computed tomography (CT), positron emission tomography (PET), and/or magnetic resonance imaging (MRI) of the subject.

[0034] In some of any such embodiments, an inflammatory marker in a sample from the subject is measured and the inflammatory marker is C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), albumin, ferritin, β 2 microglobulin (β 2-M), lactate dehydrogenase (LDH), a cytokine or a chemokine. In some cases, the inflammatory marker is LDH. In some examples, the inflammatory marker is a cytokine or a chemokine that is IL-7, IL15, MIP-1 α or TNF- α . In some of any such embodiments, the cytokine or chemokine is associated with macrophage or monocyte activation. In some of any such embodiments, the sample is or contains a blood sample, plasma sample, or serum sample. In some cases, the inflammatory marker is assessed using a colorimetric assay or an immunoassay. In some cases, the inflammatory marker is assessed using an immunoassay and the immunoassay is selected from enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), radioimmunoassay (RIA), surface plasmon resonance (SPR), Western Blot, Lateral flow assay, immunohistochemistry, protein array or immuno-PCR (iPCR).

[0035] In some of any such embodiments, the threshold value is a value that is within 25%, within 20%, within 15%, within 10%, or within 5% above the average value of the volumetric measure or inflammatory marker and/or is within a standard deviation above the average value of the volumetric measure or the inflammatory marker in a plurality of control subjects; is above the highest value of the volumetric measure or inflammatory marker, optionally within 50%, within 25%, within 20%, within 15%, within 10%, or within 5% above such highest fold change, measured in at least one subject from among a plurality of control subjects; and/or is

above the highest value of the volumetric measure or inflammatory marker as measured among more than 75%, 80%, 85%, 90%, 95%, or 98% of subjects from a plurality of control subjects.

[0036] In some of any such embodiments, the plurality of control subjects are a group of subjects prior to receiving a dose of the genetically engineered cells, wherein each of the control subjects of the group exhibited a peak CAR+ T cells in the blood greater than the highest peak CAR+ T cells in the therapeutic range; each of the control subjects of the group went on to develop at toxicity, optionally a neurotoxicity or cytokine release syndrome (CRS), a grade 2 or grade 3 or higher neurotoxicity or a grade 3 or higher CRS, after receiving a dose of the engineered cells for treating the same disease or condition; each of the control subjects of the group did not develop a response, optionally a complete response (CR) or partial response (PR), following administration of the dose of genetically engineered cells; and/or each of the control subjects of the group did not develop a durable response, optionally for at or about or greater than or about 3 months or at or about or greater than or about 6 months, following administration of the dose of genetically engineered cells.

[0037] In some of any such embodiments, the volumetric measure is SPD and the threshold value is or is about 30 cm², is or is about 40 cm², is or is about 50 cm², is or is about 60 cm², or is or is about 70 cm².

[0038] In some of any such embodiments, the inflammatory marker is LDH and the threshold value is or is about 300 units per liter, is or is about 400 units per liter, is or is about 500 units per liter or is or is about 600 units per liter.

[0039] In some of any such embodiments, the agent is a steroid. In some instances, the steroid is a corticosteroid. In some examples, the steroid is dexamethasone or methylprednisolone. In some of any such embodiments, the steroid is administered in an amount that is between or between about 1.0 mg and about 40 mg, between or between about 1.0 mg and about 20 mg, between or between about 2.0 mg and about 20 mg, between or between about 5.0 mg and about 25.0 mg, between or between about 10 mg and about 20 mg dexamethasone or equivalent thereof, each inclusive.

[0040] In some of any such embodiments, the steroid is administered in multiple doses over a period of at or more than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more or within a range defined by any of the foregoing. In some of any such embodiments, the steroid is administered once per day, twice per day, or three times or more per day. In some of any such embodiments, the steroid is administered in an amount that is between or between about 1.0 mg and about 80 mg, between or between about 1.0 mg and about 60 mg, between or between about 1.0 mg and

about 40 mg, between or between about 1.0 mg and about 20 mg, between or between about 1.0 mg and about 10 mg, between or between about 2.0 mg and about 80 mg, between or between about 2.0 mg and about 60 mg, between or between about 2.0 mg and about 40 mg, between or between about 2.0 mg and about 20 mg, between or between about 2.0 mg and about 10 mg, between or between about 5.0 mg and about 80 mg, between or between about 5.0 mg and about 60 mg, between or between about 5.0 mg and about 40 mg, between or between about 5.0 mg and about 20 mg, between or between about 5.0 mg and about 10 mg, between or between about 10 mg and about 80 mg, between or between about 10 mg and about 60 mg, between or between about 10 mg and about 40 mg, between or between about 10 mg and about 20 mg dexamethasone or equivalent thereof, each inclusive, per day or per 24 hours, or about 10 mg, 20 mg, 40 mg or 80 mg dexamethasone or equivalent thereof, per day or per 24 hours.

[0041] In some of any such embodiments, the volumetric measure or inflammatory marker is measured in the subject within 1 day, 2 days, 3 days, 4 days, 6 days, 8 days, 12 days, 16 days, 20 days, 24 days, 28 days or more prior to initiation of administration of the genetically engineered cells.

[0042] Provided are methods of dosing a subject, the method includes administering to a subject having a disease or condition, a dose of genetically engineered cells including T cells expressing a chimeric antigen receptor (CAR), wherein the dose contains a number of the genetically engineered cells that is sufficient to achieve peak CAR⁺ cells in the blood within a determined therapeutic range in the subject, or in a majority of subjects so treated by the method or in greater than 75%, 80%, 85%, 90%, 95% of the subjects so treated by the method, wherein the therapeutic range is: (i) based upon the range of peak CD3⁺ CAR⁺ T cells, or a CD8⁺CAR⁺ T cell subset thereof, in the blood among one or more subjects previously treated with the genetically engineered cells that is associated with an estimated probability of response of greater than or greater than about 65%, 70%, 75%, 80%, 85%, 90%, and an estimated probability of a toxicity of less than or about 30%, 25%, 20%, 15%, 10%, 5%; or (ii) peak CD3⁺CAR⁺ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 10 cells per microliter and 500 cells per microliter; or (iii) peak CD8⁺CAR⁺ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 2 cells per microliter and 200 cells per microliter.

[0043] In some of any such embodiments, the dose of genetically engineered cells contains from or from about 1×10^5 to 5×10^8 total CAR-expressing T cells, 1×10^6 to 2.5×10^8 total CAR-expressing T cells, 5×10^6 to 1×10^8 total CAR-expressing T cells, 1×10^7 to 2.5×10^8

total CAR-expressing T cells, 5×10^7 to 1×10^8 total CAR-expressing T cells, each inclusive. In some of any such embodiments, the dose of genetically engineered cells contains at least or at least about 1×10^5 CAR-expressing cells, at least or at least about 2.5×10^5 CAR-expressing cells, at least or at least about 5×10^5 CAR-expressing cells, at least or at least about 1×10^6 CAR-expressing cells, at least or at least about 2.5×10^6 CAR-expressing cells, at least or at least about 5×10^6 CAR-expressing cells, at least or at least about 1×10^7 CAR-expressing cells, at least or at least about 2.5×10^7 CAR-expressing cells, at least or at least about 5×10^7 CAR-expressing cells, at least or at least about 1×10^8 CAR-expressing cells, at least or at least about 2.5×10^8 CAR-expressing cells, or at least or at least about 5×10^8 CAR-expressing cells.

[0044] Provided are methods of dosing a subject, the method including administering to a subject having a disease or condition, a sub-optimal dose of genetically engineered cells including T cells engineered with a chimeric antigen receptor (CAR), wherein the dose contains a number of the genetically engineered cells that is insufficient to achieve peak CAR+ cells in the blood within a determined therapeutic range in the subject, or in a majority of subjects so treated by the method or in greater than 75%, 80%, 85%, 90%, 95% of the subjects so treated by the method; and subsequent to administering the genetically engineered cells, administering an agent to enhance CAR+ cell expansion or proliferation in the subject to achieve peak CAR+ T cells in the blood within the therapeutic range, wherein the therapeutic range is: (i) based upon the range of peak CD3+ CAR+ T cells, or a CD8+CAR+ T cell subset thereof, in the blood among one or more subjects previously treated with the genetically engineered cells that is associated with an estimated probability of response of greater than or greater than about 65%, 70%, 75%, 80%, 85%, 90% and an estimated probability of a toxicity of less than or about 30%, 25%, 20%, 15%, 10%, 5%; or (ii) peak CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 10 cells per microliter and 500 cells per microliter; or (iii) peak CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 2 cells per microliter and 200 cells per microliter.

[0045] In some of any such embodiments, after administering the dose of genetically engineered cells, the method includes monitoring the CAR+ T cells in the blood of the subject. In some of any such embodiments, following administration of the agent, the method achieves an increased frequency of peak CAR+ cells in the blood within a determined therapeutic range in the subject, compared to a method involving administration of the same dose of genetically engineered cells but without the agent; or peak CAR+ cells in the blood within a determined

therapeutic range in the subject, or in a majority of subjects so treated by the method or in greater than 75%, 80%, 85%, 90%, 95% of the subjects so treated by the method.

[0046] In some of any such embodiments, the dose of genetically engineered cells is less than or less than about 1×10^7 CAR-expressing cells, less than or less than about 5×10^6 CAR-expressing cells, less than or less than about 2.5×10^6 CAR-expressing cells, less than or less than about 1×10^6 CAR-expressing cells, less than or less than about 5×10^5 CAR-expressing cells, less than or less than about 2.5×10^5 CAR-expressing cells, less than or less than about 1×10^5 CAR-expressing cells.

[0047] In some of any such embodiments, the agent is capable of increasing expansion of the CAR+ T cells, optionally CAR-specific expansion. In some cases, the agent is an anti-idiotypic antibody or antigen-binding fragment thereof specific to the CAR, an immune checkpoint inhibitor, a modulator of a metabolic pathway, an adenosine receptor antagonist, a kinase inhibitor, an anti-TGF β antibody or an anti-TGF β R antibody or a cytokine.

[0048] In some of any such embodiments, among a plurality of subjects treated, the method achieves an increase in the percentage of subjects achieving a durable response, optionally a complete response (CR) or objective response (OR) or a partial response (PR), optionally that is durable for at or greater than 3 months or at or greater than 6 months, compared to a method that does not contain administering the agent. In some examples, the increase is greater than or greater than about 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold or more. In some of any such embodiments, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40% or at least 50% of subjects treated according to the method achieve a complete response (CR) that is durable for at or greater than 3 months or at or greater than 6 months; and/or at least 25%, at least 30%, at least 40%, at least 50%, at least 60% or at least 70% of the subjects treated according to the method achieve objective response (OR) that is durable for at or greater than 3 months or at or greater than 6 months.

[0049] In some of any such embodiments, greater than or greater than about 50%, greater than or greater than about 60%, greater than or greater than about 70%, or greater than or greater than about 80% of the subjects treated according to the method do not exhibit a grade 3 or greater cytokine release syndrome (CRS) and/or do not exhibit a grade 2 or greater or grade 3 or greater neurotoxicity; or greater than or greater than about 40%, greater than or greater than about 50% or greater than or greater than about 55% of the subjects treated according to the method do not exhibit any neurotoxicity or CRS.

[0050] In some of any such embodiments, peak CAR+ T cells is determined as the number

of CAR+ T cells per microliter in the blood of the subject. In some of any such embodiments, the therapeutic range is the range in which the estimated probability of toxicity is less than 20%, less than 15%, less than 10% or less than 5% and the estimated probability of achieving a response is greater than 65%, 70%, 75%, 80%, 85%, 90%, 95% or more.

[0051] In some of any such embodiments, the probability of toxicity is based on a toxicity selected from any neurotoxicity or cytokine release syndrome (CRS); severe toxicity or grade 3 or higher toxicity; severe CRS or a grade 3 or higher CRS; or severe neurotoxicity, grade 2 or higher neurotoxicity or grade 3 or higher neurotoxicity. In some of any such embodiments, the probability of a toxicity is based on the probability of a severe toxicity or a grade 3 or higher toxicity. In some cases, the severe toxicity is grade 3-5 neurotoxicity.

[0052] In some of any such embodiments, the probability of response is based on a response that is a complete response (CR), an objective response (OR) or a partial response (PR), optionally wherein the response is durable, optionally durable for at or at least 3 months or at or at least 6 months. In some of any such embodiments, the response is a marrow response as determined based on assessment of the presence of a malignant immunoglobulin heavy chain locus (IGH) ad/or an index clone in the bone marrow of the subject. In some cases, the malignant IGH and/or index clone is assessed by flow cytometry or IgH sequencing.

[0053] Provided is a method of assessing likelihood of a durable response, the method including detecting, in a biological sample from a subject, peak levels of one or more inflammatory marker and/or peak levels of genetically engineered cells including T cells expressing a chimeric antigen receptor (CAR), wherein the subject has been previously administered a dose of the genetically engineered cells for treating a disease or condition; and comparing, individually, the peak levels to a threshold value, thereby determining a likelihood that a subject will achieve a durable response to the administration of the genetically engineered cells.

[0054] In some of any such embodiments, the subject is likely to achieve a durable response if the peak levels of the one or more inflammatory marker is below a threshold value and the subject is not likely to achieve a durable response if the peak levels of the one or more inflammatory marker is above a threshold value; or the subject is likely to achieve a durable response if the peak level of the genetically engineered cells is within a therapeutic range between a lower threshold value and an upper threshold value and the subject is not likely to achieve a durable response if the peak level of the genetically engineered cells is below the lower threshold value or is above the upper threshold value.

[0055] In some of any such embodiments, if the subject is determined not likely to achieve a durable response, further including selecting a subject for treatment with a therapeutic agent or with an alternative therapeutic treatment other than the genetically engineered cells. In some aspects, if the subject is determined as not likely to achieve a durable response, further including administering a therapeutic agent or an alternative therapeutic treatment other than the genetically engineered cells.

[0056] Provided is a method of treatment including selecting a subject having received administration of genetically engineered cells including T cells expressing a chimeric antigen receptor (CAR) in which peak levels of one or more inflammatory markers in a sample from the subject is above a threshold value; and/or peak level of T cells including a chimeric antigen receptor (CAR) in a sample from the subject is below a lower threshold value or is above an upper threshold value; and administering to the subject a therapeutic agent or alternative therapeutic treatment other than the genetically engineered cells.

[0057] In some of any such embodiments, the response is a complete response (CR), objective response (OR) or partial response (PR). In some cases, the response is durable for at or greater than 3 months, 4 months, 5 months, or 6 months.

[0058] In some of any such embodiments, the peak levels are assessed and/or the sample is obtained from the subject at a time that is at least 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days or 21 days after initiation of administration of the genetically engineered cells. In some of any such embodiments, the peak levels are assessed and/or the sample is obtained from the subject at a time that is between or between about 11 to 22 days, 12 to 18 days or 14 to 16 days, each inclusive, after initiation of administration of the genetically engineered cells.

[0059] In some of any such embodiments, the peak level is a peak level of one or more inflammatory marker and the inflammatory marker is selected from C reactive protein (CRP), IL-2, IL-6, IL-10, IL-15, TNF-alpha, MIP-1alpha, MIP-1beta, MCP-1, CXCL10 or CCL13. In some of any such embodiments, the peak level of one or more inflammatory marker is assessed and the threshold value is within 25%, within 20%, within 15%, within 10% or within 5% and/or is within a standard deviation of the median or mean of the peak level of the inflammatory marker as determined among a group of control subjects having received administration of the genetically engineered cells, wherein each of the subjects of the group did not achieve a durable response, optionally a CR and/or PR, optionally at or greater than 3 months or 6 months following administration of the genetically engineered cells. In some instances, the control

subjects exhibited stable disease (SD) or progressive disease (PD) following administration of the genetically engineered cells, optionally at or greater than 3 months or 6 months following administration of the genetically engineered cells.

[0060] In some of any such embodiments, the peak level is a peak level of CAR+ T cells, or a CD8+ T cell subset thereof. In some of any such embodiments, the lower threshold value and upper threshold value is the lower and upper end, respectively, of a therapeutic range of peak CD3+ CAR+ T cells, or a CD8+CAR+ T cell subset thereof, in the blood among one or more subjects previously treated with the genetically engineered cells that is associated with an estimated probability of response of greater than or greater than about 65%, 70%, 75%, 80%, 85%, 90% and an estimated probability of a toxicity of less than or about 30%, 25%, 20%, 15%, 10%, 5%.

[0061] In some of any such embodiments, the therapeutic range is the range in which the estimated probability of toxicity is less than 20%, less than 15%, less than 10% or less than 5% and the estimated probability of achieving a response is greater than 65%, 70%, 75%, 80%, 85%, 90%, 95% or more. In some cases, the probability of toxicity is based on a toxicity selected from any neurotoxicity or cytokine release syndrome (CRS); severe toxicity or grade 3 or higher toxicity; severe CRS or a grade 3 or higher CRS; or severe neurotoxicity, grade 2 or higher neurotoxicity or grade 3 or higher neurotoxicity. In some of any such embodiments, the probability of response is based on a response that is a complete response (CR), an objective response (OR) or a partial response (PR), optionally wherein the response is durable, optionally durable for at or at least 3 months or at or at least 6 months.

[0062] In some of any such embodiments, peak CAR+ T cells is determined as the number of CAR+ T cells per microliter in the blood of the subject. In some of any such embodiments, the upper threshold value is between or between about 300 cells per microliter and 1000 cells per microliter or 400 cells per microliter and 600 cells per microliter, or is about 300 cells per microliter, 400 cells per microliter, 500 cells per microliter, 600 cells per microliter, 700 cells per microliter, 800 cells per microliter, 900 cells per microliter or 1000 cells per microliter; or the lower threshold value is less than or less than about 10 cells per microliter, 9 cells per microliter, 8 cells per microliter, 7 cells per microliter, 6 cells per microliter, 5 cells per microliter, 4 cells per microliter, 3 cells per microliter, 2 cells per microliter or 1 cell per microliter.

[0063] In some of any such embodiments, the sample is a blood sample or plasma sample. In some of any such embodiments, the method is carried out ex vivo.

[0064] In some of any such embodiments, the peak level of genetically engineered cells is above the upper threshold value and the therapeutic agent is an agent that is capable of decreasing CAR+ T cell expansion or proliferation. In some of any such embodiments, the peak level of CAR+ T cells is below a lower threshold value and the therapeutic agent is an agent that is capable of decreasing CAR+ T cell expansion or proliferation. In some cases, the agent is a steroid. In some cases, the steroid is a corticosteroid. In some examples, the steroid is dexamethasone or methylprednisolone. In some of any such embodiments, the steroid is administered in an amount that is between or between about 1.0 mg and about 40 mg, between or between about 1.0 mg and about 20 mg, between or between about 2.0 mg and about 20 mg, between or between about 5.0 mg and about 25.0 mg, between or between about 10 mg and about 20 mg dexamethasone or equivalent thereof, each inclusive.

[0065] In some of any such embodiments, the steroid is administered in multiple doses over a period of at or more than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more or within a range defined by any of the foregoing. In some of any such embodiments, the steroid is administered once per day, twice per day, or three times or more per day. In some of any such embodiments, the steroid is administered in an amount that is between or between about 1.0 mg and about 80 mg, between or between about 1.0 mg and about 60 mg, between or between about 1.0 mg and about 40 mg, between or between about 1.0 mg and about 20 mg, between or between about 1.0 mg and about 10 mg, between or between about 2.0 mg and about 80 mg, between or between about 2.0 mg and about 60 mg, between or between about 2.0 mg and about 40 mg, between or between about 2.0 mg and about 20 mg, between or between about 2.0 mg and about 10 mg, between or between about 5.0 mg and about 80 mg, between or between about 5.0 mg and about 60 mg, between or between about 5.0 mg and about 40 mg, between or between about 5.0 mg and about 20 mg, between or between about 5.0 mg and about 10 mg, between or between about 10 mg and about 80 mg, between or between about 10 mg and about 60 mg, between or between about 10 mg and about 40 mg, between or between about 10 mg and about 20 mg dexamethasone or equivalent thereof, each inclusive, per day or per 24 hours, or about 10 mg, 20 mg, 40 mg or 80 mg dexamethasone or equivalent thereof, per day or per 24 hours.

[0066] In some of any such embodiments, the peak level of CAR+ T cells is above the upper threshold value and the therapeutic agent is an agent that is capable of increasing expansion of the CAR+ T cells, optionally CAR-specific expansion.

[0067] In some of any such embodiments, the agent is an anti-idiotypic antibody or antigen-binding fragment thereof specific to the CAR, an immune checkpoint inhibitor, a modulator of a

metabolic pathway, an adenosine receptor antagonist, a kinase inhibitor, an anti-TGF β antibody or an anti-TGF β R antibody or a cytokine.

[0068] In some of any such embodiments, the disease or condition is a cancer. In some cases, the cancer is a B cell malignancy. In some examples, the cancer is selected from the group consisting of sarcomas, carcinomas, lymphomas, non-Hodgkin lymphomas (NHLs), diffuse large B cell lymphoma (DLBCL), leukemia, CLL, ALL, AML and myeloma. In some instances, the cancer is a pancreatic cancer, bladder cancer, colorectal cancer, breast cancer, prostate cancer, renal cancer, hepatocellular cancer, lung cancer, ovarian cancer, cervical cancer, pancreatic cancer, rectal cancer, thyroid cancer, uterine cancer, gastric cancer, esophageal cancer, head and neck cancer, melanoma, neuroendocrine cancers, CNS cancers, brain tumors, bone cancer, or soft tissue sarcoma.

[0069] In some of any such embodiments, the subject is a human.

[0070] In some of any such embodiments, the antigen is selected from among $\alpha\text{v}\beta 6$ integrin ($\alpha\text{v}\beta 6$ integrin), B cell maturation antigen (BCMA), B7-H3, B7-H6, carbonic anhydrase 9 (CA9, also known as CAIX or G250), a cancer-testis antigen, cancer/testis antigen 1B (CTAG, also known as NY-ESO-1 and LAGE-2), carcinoembryonic antigen (CEA), a cyclin, cyclin A2, C-C Motif Chemokine Ligand 1 (CCL-1), CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD138, CD171, epidermal growth factor protein (EGFR), type III epidermal growth factor receptor mutation (EGFR vIII), epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), ephrinB2, ephrine receptor A2 (EPHa2), estrogen receptor, Fc receptor like 5 (FCRL5; also known as Fc receptor homolog 5 or FCRH5), fetal acetylcholine receptor (fetal AchR), a folate binding protein (FBP), folate receptor alpha, ganglioside GD2, O-acetylated GD2 (OGD2), ganglioside GD3, glycoprotein 100 (gp100), G Protein Coupled Receptor 5D (GPRC5D), Her2/neu (receptor tyrosine kinase erb-B2), Her3 (erb-B3), Her4 (erb-B4), erbB dimers, Human high molecular weight-melanoma-associated antigen (HMW-MAA), hepatitis B surface antigen, Human leukocyte antigen A1 (HLA-A1), Human leukocyte antigen A2 (HLA-A2), IL-22 receptor alpha (IL-22Ra), IL-13 receptor alpha 2 (IL-13Ra2), kinase insert domain receptor (kdr), kappa light chain, L1 cell adhesion molecule (L1-CAM), CE7 epitope of L1-CAM, Leucine Rich Repeat Containing 8 Family Member A (LRRC8A), Lewis Y, Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, mesothelin, c-Met, murine cytomegalovirus (CMV), mucin 1 (MUC1), MUC16, natural killer group 2 member D (NKG2D) ligands, melan A (MART-1), neural cell adhesion molecule (NCAM), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME),

progesterone receptor, a prostate specific antigen, prostate stem cell antigen (PSCA), prostate specific membrane antigen (PSMA), Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1), survivin, Trophoblast glycoprotein (TPBG also known as 5T4), tumor-associated glycoprotein 72 (TAG72), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor receptor 2 (VEGFR2), Wilms Tumor 1 (WT-1), a pathogen-specific antigen, or an antigen associated with a universal tag, and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV or other pathogens.

[0071] In some of any such embodiments, the CAR specifically binds to an antigen associated with a disease or condition and/or expressed in cells associated with the disease or condition. In some examples, the antigen is selected from among 5T4, 8H9, avb6 integrin, B7-H6, B cell maturation antigen (BCMA), CA9, a cancer-testes antigen, carbonic anhydrase 9 (CAIX), CCL-1, CD19, CD20, CD22, CEA, hepatitis B surface antigen, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD138, CD171, carcinoembryonic antigen (CEA), CE7, a cyclin, cyclin A2, c-Met, dual antigen, EGFR, epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), EPHA2, ephrinB2, erb-B2, erb-B3, erb-B4, erbB dimers, EGFR vIII, estrogen receptor, Fetal AchR, folate receptor alpha, folate binding protein (FBP), FCRL5, FCRH5, fetal acetylcholine receptor, G250/CAIX, GD2, GD3, gp100, Her2/neu (receptor tyrosine kinase erbB2), HMW-MAA, IL-22R-alpha, IL-13 receptor alpha 2 (IL-13Ra2), kinase insert domain receptor (kdr), kappa light chain, Lewis Y, L1-cell adhesion molecule (L1-CAM), Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, MART-1, mesothelin, murine CMV, mucin 1 (MUC1), MUC16, NCAM, NKG2D, NKG2D ligands, NY-ESO-1, O-acetylated GD2 (OGD2), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME), PSCA, progesterone receptor, survivin, ROR1, TAG72, VEGF receptors, VEGF-R2, Wilms Tumor 1 (WT-1), a pathogen-specific antigen.

[0072] In some of any such embodiments, the chimeric antigen receptor (CAR) contains an extracellular antigen-recognition domain that specifically binds to the antigen and an intracellular signaling domain containing an ITAM. In some cases, the intracellular signaling domain contains an intracellular domain of a CD3-zeta (CD3 ζ) chain. In some of any such embodiments, the chimeric antigen receptor (CAR) further contains a costimulatory signaling region. In some aspects, the costimulatory signaling region contains a signaling domain of CD28 or 4-1BB. In some of any such embodiments, the costimulatory domain is a domain of 4-1BB.

[0073] In some of any such embodiments, the cells are T cells. In some cases, the T cells

are CD4+ or CD8+. In some examples, the T cells are primary T cells obtained from a subject. In some of any such embodiments, the cells of the genetically engineered cells are autologous to the subject. In some of any such embodiments, the cells are allogeneic to the subject.

[0074] Also provided are kits containing a composition containing genetically engineered cells including T cells expressing a chimeric antigen receptor (CAR) and instructions for administering a dose of the cells to a subject following or based on the results of assessing if peak CAR+ T cells are within a therapeutic range, wherein the therapeutic range is: (i) based upon the range of peak CD3+ CAR+ T cells, or a CD8+CAR+ T cell subset thereof, in the blood among one or more subjects previously treated with the genetically engineered cells that is associated with an estimated probability of response of greater than or greater than about 65% and an estimated probability of a toxicity of less than or about 30%; or (ii) peak CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 10 cells per microliter and 500 cells per microliter; or (iii) peak CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 2 cells per microliter and 200 cells per microliter. In some of any such embodiments, the instructions specify that if the genetically engineered cells are not within the therapeutic range, administering an agent to the subject capable of modulating, optionally increasing or decreasing, CAR+ T cell expansion or proliferation, in the subject. In some of any such embodiments, the kit further contains the agent.

[0075] Provided are kits containing an agent capable of modulating, optionally increasing or decreasing, expansion or proliferation of genetically engineered cells including CAR+ T cells in a subject, and instructions for administering the agent to a subject, said subject having been administered the genetically engineered cells, based on results of assessing if peak CAR+ T cells are within a therapeutic range, wherein the therapeutic range is (i) based upon the range of peak CD3+ CAR+ T cells, or a CD8+CAR+ T cell subset thereof, in the blood among one or more subjects previously treated with the genetically engineered cells that is associated with an estimated probability of response of greater than or greater than about 65% and an estimated probability of a toxicity of less than or about 30%; or (ii) peak CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 10 cells per microliter and 500 cells per microliter; or (iii) peak CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 2 cells per microliter and 200 cells per microliter. In some of any such embodiments, the instructions specify that if the peak number of CAR+ T cells in the blood of the subject is less

than the lowest number of peak CAR+ T cells in the therapeutic range, an agent is administered to the subject that is capable of increasing CAR+ T cell expansion or proliferation. In some of any such embodiments, the agent is capable of CAR-specific expansion.

[0076] In some of any such embodiments, the agent is an anti-idiotypic antibody or antigen-binding fragment thereof specific to the CAR, an immune checkpoint inhibitor, a modulator of a metabolic pathway, an adenosine receptor antagonist, a kinase inhibitor, an anti-TGF β antibody or an anti-TGF β R antibody or a cytokine. In some of any such embodiments, if the peak number of CAR+ T cells in the blood of the subject is greater than the highest number of peak CAR+ T cells in the therapeutic range, an agent is administered to the subject that is capable of decreasing CAR+ T cell expansion or proliferation.

[0077] Provided are kits containing an agent capable of decreasing expansion or proliferation of genetically engineered cells including CAR+ T cells in a subject, and instructions for assessing a subject the level, amount or concentration of a volumetric measure of tumor burden or an inflammatory marker in a sample from the subject and administering to the subject the agent if the level, amount or concentration is at or above a threshold level, wherein the sample does not contain genetically engineered T cells expressing a chimeric antigen receptor (CAR) and/or is obtained from the subject prior to receiving administration of genetically engineered T cells expressing a CAR. In some of any such embodiments, the volumetric measure is a sum of the products of diameters (SPD), longest tumor diameters (LD), sum of longest tumor diameters (SLD), tumor volume, necrosis volume, necrosis-tumor ratio (NTR), peritumoral edema (PTE), and edema-tumor ratio (ETR). In some cases, the volumetric measure is a sum of the products of diameter (SPD).

[0078] In some of any such embodiments, the inflammatory marker is C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), albumin, ferritin, β 2 microglobulin (β 2-M), lactate dehydrogenase (LDH), a cytokine or a chemokine. In some examples, the inflammatory marker is LDH.

[0079] In some of any such embodiments, the agent is a steroid. In some cases, the steroid is a corticosteroid. In some examples, the steroid is dexamethasone or methylprednisolone. In some of any such embodiments, the steroid is formulated for administration in an amount that is between or between about 1.0 mg and about 40 mg, between or between about 1.0 mg and about 20 mg, between or between about 2.0 mg and about 20 mg, between or between about 5.0 mg and about 25.0 mg, between or between about 10 mg and about 20 mg dexamethasone or equivalent thereof, each inclusive.

[0080] In some of any such embodiments, the CAR specifically binds to an antigen associated with a disease or condition and/or expressed in cells associated with the disease or condition. In some of any such embodiments, the genetically engineered cells include T cells, optionally CD4+ or CD8+ T cells.

[0081] Also provided are articles of manufacture containing any of the kits provided herein.

[0082] Provided in some aspects are methods of ameliorating a toxicity, involving administering, to a subject exhibiting a sign or symptom of a toxicity, a treatment regimen for treating the toxicity, said subject having been administered a dose of genetically engineered cells containing T cells expressing a recombinant receptor, wherein the treatment regimen is selected from: (a) if, within 72, 96 or 120 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits a fever and/or one or more first physical signs or symptoms associated with a toxicity, optionally cytokine release syndrome (CRS), and/or one or more physical signs or symptoms associated with grade 1 CRS, administering (i) an agent capable of binding an interleukin-6 receptor (IL-6R), said agent administered no more than once every 24 hours, and (ii) one or more doses of a steroid, said steroid administered about every 12 to 24 hours; (b) if the subject exhibits one or more physical signs or symptoms associated with grade 2 CRS after receiving a dose of the genetically engineered cells, administering (i) an agent capable of binding an IL-6R, said agent administered no more than once every 24 hours, and (ii) one or more doses of a steroid, said steroid administered about every 12 to 24 hours; (c) if the subject exhibits one or more physical signs or symptoms associated with grade 3 CRS after receiving a dose of the genetically engineered cells, administering (i) an agent capable of binding an IL-6R, said agent administered no more than once every 24 hours, and (ii) one or more doses of a steroid, said steroid administered at least twice a day, optionally at least about every 12 hours; or (d) if the subject exhibits one or more physical signs or symptoms associated with grade 4 CRS after receiving a dose of the genetically engineered cells, administering (i) an agent capable of binding an IL-6R, said agent administered no more than once every 24 hours, and (ii) one or more doses of a steroid, said steroid administered at least twice a day, optionally at least about every 6 hours. In some of any such embodiments of the methods described herein, up to two doses of the agent is administered.

[0083] Also provided in some aspects are methods of ameliorating a toxicity, involving administering, to a subject exhibiting a sign or symptom of a toxicity, a treatment regimen for treating the toxicity, said subject having been administered a dose of genetically engineered cells comprising T cells expressing a recombinant receptor, wherein the treatment regimen comprises:

(a) if, within 72, 96 or 120 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits a fever and/or one or more first physical signs or symptoms associated with a toxicity, optionally cytokine release syndrome (CRS), and/or one or more physical signs or symptoms associated with grade 1 CRS, administering (i) an agent capable of binding an interleukin-6 receptor (IL-6R) , said agent administered no more than once every 24 hours, and (ii) one or more doses of a steroid, said steroid administered about every 12 to 24 hours; (b) if the subject exhibits one or more physical signs or symptoms associated with grade 2 CRS after receiving a dose of the genetically engineered cells, administering (i) an agent capable of binding an IL-6R , said agent administered no more than once every 24 hours, and (ii) one or more doses of a steroid, said steroid administered about every 12 to 24 hours; (c) if the subject exhibits one or more physical signs or symptoms associated with grade 3 CRS after receiving a dose of the genetically engineered cells, administering (i) an agent capable of binding an IL-6R , said agent administered no more than once every 24 hours, and (ii) one or more doses of a steroid, said steroid administered at least twice a day, optionally at least about every 12 hours; and (d) if the subject exhibits one or more physical signs or symptoms associated with grade 3 CRS after receiving a dose of the genetically engineered cells, administering (i) an agent capable of binding an IL-6R , said agent administered no more than once every 24 hours, and (ii) one or more doses of a steroid, said steroid administered at least twice a day, optionally at least about every 6 hours.

[0084] Provided in other aspects are methods of ameliorating a toxicity, involving administering, to a subject exhibiting a sign or symptom of a toxicity, a treatment regimen for treating the toxicity, said subject having been administered a dose of genetically engineered cells containing T cells expressing a recombinant receptor, wherein the treatment regimen is, if, within 72, 96 or 120 hours of administration of the dose of genetically engineered, the subject exhibits a fever and/or one or more first physical signs or symptoms associated with a toxicity, optionally cytokine release syndrome (CRS), and/or one or more physical signs or symptoms associated with grade 1 CRS, administering (i) an agent capable of binding an interleukin-6 receptor (IL-6R) and (ii) one or more doses of a steroid. In some of any such embodiments, the agent capable of binding IL-6R is administered in one or more doses.

[0085] Also provided herein are methods of ameliorating a toxicity, comprising administering, to a subject exhibiting one or more physical signs or symptom of a toxicity, one or more agent capable of reducing and/or ameliorating the one or more physical signs or symptoms associated with the toxicity, said subject having been administered a dose of

genetically engineered cells comprising T cells expressing a recombinant receptor, wherein the one or more agent is administered in a treatment regimen comprising: (a) administering one or more agent if : (i) at or greater than 72 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits a fever, and exhibits one or more physical signs or symptoms associated with the toxicity, optionally cytokine release syndrome (CRS), and/or exhibits a rapid progression of the physical signs or symptoms associated with the toxicity; or (ii) within 48 or 72 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits a fever and/or one or more physical signs or symptoms associated with grade 2 or higher CRS; (b) administering one or more agent if, within 24, 48 or 72 hours after administration of the one or more agent in (a), the subject does not exhibit an improvement of the fever and/or the one or more physical signs or symptoms associated with the toxicity and/or exhibits a rapid progression of the physical signs or symptoms associated with the toxicity, which one or more agent optionally are different from the one or more agent administered in (a) and/or is administered at the same or higher dose and/or frequency as the one or more agent administered in (a); (c) administering one or more agent if, within 24, 48 or 72 hours after administration of the one or more agent in (b), the subject does not exhibit an improvement of the fever and/or the one or more physical signs or symptoms associated with the toxicity and/or exhibits a rapid progression of the physical signs or symptoms associated with the toxicity, which one or more agent optionally are different from the one or more agent administered in (a) or (b) and/or is administered at the same or higher dose and/or frequency as the one or more agent administered in (a) or (b); and (d) administering one or more agent if, after administration of the one or more agent in (c), the subject does not exhibit an improvement of the fever and/or the one or more physical signs or symptoms associated with the toxicity, which one or more agent optionally are different from the one or more agent administered in (a), (b) or (c) and/or is administered at the same or higher dose and/or frequency as the one or more agent administered in (a), (b) or (c).

[0086] In some of any such embodiments, the one or more agent is selected from an agent capable of binding an interleukin-6 receptor (IL-6R) or one or more steroid, optionally one or more doses of the one or more steroid.

[0087] Also provided herein are methods of ameliorating a toxicity, comprising administering, to a subject exhibiting one or more physical signs or symptom of a toxicity, one or more agent capable of reducing and/or ameliorating the one or more physical signs or symptoms associated with the toxicity, said subject having been administered a dose of

genetically engineered cells comprising T cells expressing a recombinant receptor, wherein the one or more agent is administered in a treatment regimen comprising: (a) administering one or more agent if: (i) at or greater than 72 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits one or more physical signs or symptoms associated with the toxicity, optionally neurotoxicity (NT); or (ii) within 48 or 72 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits one or more physical signs or symptoms associated with the toxicity; (b) administering one or more agent if, within 24, 48 or 72 hours after administration of the one or more agent in (a), the subject does not exhibit an improvement of the one or more physical signs or symptoms associated with the toxicity and/or exhibits a progression of the physical signs or symptoms associated with the toxicity, which one or more agent optionally are different from the one or more agent administered in (a) and/or is administered at the same or higher dose and/or frequency as the one or more agent administered in (a); and (c) administering one or more agent if, within 24, 48 or 72 hours after administration of the one or more agent in (b), the subject does not exhibit an improvement of the one or more physical signs or symptoms associated with the toxicity and/or exhibits a rapid progression of the physical signs or symptoms associated with the toxicity, which one or more agent optionally are different from the one or more agent administered in (a) or (b) and/or is administered at the same or higher dose and/or frequency as the one or more agent administered in (a) or (b). In some of any such embodiments, the one or more agent is one or more steroid, optionally one or more doses of the one or more steroid.

[0088] In some of any such embodiments, up to two doses of the agent is administered. In some of any such embodiments, the dose of the agent capable of binding IL-6R and a dose of steroid is administered simultaneously, or a dose of the steroid is administered within about 1, 2, 3 or 4 hours of the dose of the agent capable of binding IL-6R. In some of any such embodiments, the agent capable of binding IL-6R is administered no more than once every 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or more hours. In some of any such embodiments, up to two doses of the agent is administered.

[0089] In some of any such embodiments, the steroid is administered every 6, 9, 12, 15, 18, 21, 24, 36 or 48 hours, or a range defined by any two of the foregoing values. In some of any such embodiments, the steroid is or contains a corticosteroid, which optionally is a glucocorticoid. In some of any such embodiments, the steroid is selected from among cortisones, dexamethasones, hydrocortisones, methylprednisolones, prednisolones and prednisones. In some of any such embodiments, the steroid is or contains dexamethasone, prednisone or

methylprednisolone. In certain embodiments, the steroid is dexamethasone or methylprednisolone.

[0090] In some of any such embodiments, the steroid is for administration at an equivalent dosage amount of from or from about 1.0 mg to at or about 40 mg, from or from about 1.0 mg to at or about 20 mg, from or from about 2.0 mg to at or about 20 mg, from or from about 5.0 mg to at or about 25.0 mg, or from or from about 10 mg to at or about 20 mg dexamethasone or equivalent thereof, each inclusive. In some of any such embodiments, the steroid is administered at an equivalent dosage amount of between or between about 0.5 mg/kg and about 5 mg/kg, or about 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg or 5 mg/kg methylprednisolone or equivalent thereof, each inclusive. In some of any such embodiments, multiple doses of the steroid is administered. In some of any such embodiments, the steroid is administered for 2, 3, 4, 5 or more days. In other embodiments, the steroid is administered at an equivalent dosage amount of from or from about 10 mg to about 80 mg dexamethasone or equivalent thereof, per day or per 24 hours, or about 10 mg, 20 mg, 40 mg or 80 mg dexamethasone or equivalent thereof, per day or per 24 hours.

[0091] In some of any such embodiments, the steroid is administered in multiple doses over a period of at or more than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or within a range defined by any of the foregoing. In some of any such embodiments, the steroid is administered for 2, 3, 4, 5 or more days. In some of any such embodiments, the steroid is administered once per day, twice per day, or three times or more per day.

[0092] In some of any such embodiments, the steroid is administered at an equivalent dosage amount of between or between about 1.0 mg and about 80 mg, between or between about 1.0 mg and about 60 mg, between or between about 1.0 mg and about 40 mg, between or between about 1.0 mg and about 20 mg, between or between about 1.0 mg and about 10 mg, between or between about 2.0 mg and about 80 mg, between or between about 2.0 mg and about 60 mg, between or between about 2.0 mg and about 40 mg, between or between about 2.0 mg and about 20 mg, between or between about 2.0 mg and about 10 mg, between or between about 5.0 mg and about 80 mg, between or between about 5.0 mg and about 60 mg, between or between about 5.0 mg and about 40 mg, between or between about 5.0 mg and about 20 mg, between or between about 5.0 mg and about 10 mg, between or between about 10 mg and about 80 mg, between or between about 10 mg and about 60 mg, between or between about 10 mg and about 40 mg, between or between about 10 mg and about 20 mg dexamethasone or equivalent thereof, each inclusive, per day or per 24 hours, or from or from about 10 mg to about 80 mg

dexamethasone or equivalent thereof, per day or per 24 hours, or about 10 mg, 20 mg, 40 mg or 80 mg dexamethasone or equivalent thereof, per day or per 24 hours

[0093] In some of any such embodiments, the multiple doses contain an initial dose of steroids of between about 1 and about 3 mg/kg, such as 2 mg/kg methylprednisolone or equivalent thereof, followed by subsequent doses of between about 1 and about 5 mg/kg, or about 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg or 5 mg/kg methylprednisolone or equivalent thereof, divided between 1, 2, 3, 4 or 5 times over a day or over 24 hours. In some of any such embodiments, the high dose of steroid is dexamethasone at dosage amount of at or about 10 mg, 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, 60 mg, 65 mg, 70 mg, 75 mg or 80 mg dexamethasone or equivalent thereof, or a range defined by any of the foregoing, each inclusive.

[0094] In some of any such embodiments, the steroid is formulated for intravenous or oral administration.

[0095] In some of any such embodiments, the agent capable of binding IL-6R is a recombinant anti-IL-6 receptor antibody or an antigen-binding fragment thereof is or contains an agent selected from among tocilizumab or sarilumab or an antigen-binding fragment thereof. In some of any such embodiments, the recombinant anti-IL-6R antibody is or contains tocilizumab or an antigen-binding fragment thereof. In some of any such embodiments, the anti-IL-6R antibody is for administration in a dosage amount of from or from about 1 mg/kg to 20 mg/kg, 2 mg/kg to 19 mg/kg, 4 mg/kg to 16 mg/kg, 6 mg/kg to 14 mg/kg or 8 mg/kg to 12 mg/kg, each inclusive, or the anti-IL-6R antibody is administered in a dosage amount of at least or at least about or about 1 mg/kg, 2 mg/kg, 4 mg/kg, 6 mg/kg, 8 mg/kg, 10 mg/kg, 12 mg/kg, 14 mg/kg, 16 mg/kg, 18 mg/kg, 20 mg/kg. In some of any such embodiments, the anti-IL-6R antibody is formulated for single dosage administration of an amount from or from about 30 mg to about 5000 mg, from about 50 mg to about 1000 mg, from about 50 mg to about 500 mg, from about 50 mg to about 200 mg, from about 50 mg to about 100 mg, from about 100 mg to about 1000 mg, from about 100 mg to about 500 mg, from about 100 mg to about 200 mg, from about 200 mg to about 1000 mg, from about 200 mg to about 500 mg, or from about 500 mg to about 1000 mg. In some of any such embodiments, the anti-IL-6R antibody is formulated for intravenous administration.

[0096] In some of any such embodiments, the method further involves, if the subject exhibits one or more first physical signs or symptoms associated with the toxicity, optionally CRS, within 72 hours of administration of the dose of genetically engineered cells, if the

physical signs or symptoms associated with the toxicity, optionally CRS, does not improve, if the physical signs or symptoms associated with the toxicity is severe or aggressive and/or if the grade of toxicity, optionally CRS, becomes more severe, administering an additional dose of steroids, optionally at a high dose. In some of any such embodiments, the high dose of steroid is methylprednisolone at about 1 to about 4 mg/kg initial dose followed by about 1 to about 4 mg mg/kg/day divided 2, 3, 4, 5 or 6 times per day, or equivalents thereof.

[0097] In some of any such embodiments, the method further involves administering to the subject a dose of genetically engineered cells containing T cells expressing a recombinant receptor for treating a disease or condition prior to administering the treatment regimen. In some of any such embodiments, the recombinant receptor is or contains a chimeric receptor and/or a recombinant antigen receptor. In some of any such embodiments, the recombinant receptor is capable of binding to a target antigen that is associated with, specific to, and/or expressed on a cell or tissue of a disease, disorder or condition. In some of any such embodiments, the disease, disorder or condition is an infectious disease or disorder, an autoimmune disease, an inflammatory disease, or a tumor or a cancer. In some of any such embodiments, the target antigen is a tumor antigen. In certain embodiments, the target antigen is selected from among $\alpha\beta6$ integrin (avb6 integrin), B cell maturation antigen (BCMA), B7-H3, B7-H6, carbonic anhydrase 9 (CA9, also known as CAIX or G250), a cancer-testis antigen, cancer/testis antigen 1B (CTAG, also known as NY-ESO-1 and LAGE-2), carcinoembryonic antigen (CEA), a cyclin, cyclin A2, C-C Motif Chemokine Ligand 1 (CCL-1), CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD138, CD171, epidermal growth factor protein (EGFR), type III epidermal growth factor receptor mutation (EGFR vIII), epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), ephrinB2, ephrine receptor A2 (EPHa2), estrogen receptor, Fc receptor like 5 (FCRL5; also known as Fc receptor homolog 5 or FCRH5), fetal acetylcholine receptor (fetal AchR), a folate binding protein (FBP), folate receptor alpha, ganglioside GD2, O-acetylated GD2 (OGD2), ganglioside GD3, glycoprotein 100 (gp100), G Protein Coupled Receptor 5D (GPRC5D), Her2/neu (receptor tyrosine kinase erb-B2), Her3 (erb-B3), Her4 (erb-B4), erbB dimers, Human high molecular weight-melanoma-associated antigen (HMW-MAA), hepatitis B surface antigen, Human leukocyte antigen A1 (HLA-A1), Human leukocyte antigen A2 (HLA-A2), IL-22 receptor alpha(IL-22Ra), IL-13 receptor alpha 2 (IL-13Ra2), kinase insert domain receptor (kdr), kappa light chain, L1 cell adhesion molecule (L1-CAM), CE7 epitope of L1-CAM, Leucine Rich Repeat Containing 8 Family Member A (LRRC8A), Lewis Y, Melanoma-associated antigen

(MAGE)-A1, MAGE-A3, MAGE-A6, mesothelin, c-Met, murine cytomegalovirus (CMV), mucin 1 (MUC1), MUC16, natural killer group 2 member D (NKG2D) ligands, melan A (MART-1), neural cell adhesion molecule (NCAM), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME), progesterone receptor, a prostate specific antigen, prostate stem cell antigen (PSCA), prostate specific membrane antigen (PSMA), Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1), survivin, Trophoblast glycoprotein (TPBG also known as 5T4), tumor-associated glycoprotein 72 (TAG72), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor receptor 2 (VEGFR2), Wilms Tumor 1 (WT-1), a pathogen-specific antigen, or an antigen associated with a universal tag, and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV or other pathogens.

[0098] In some of any such embodiments, the recombinant receptor is or contains a functional non-TCR antigen receptor or a TCR or antigen-binding fragment thereof. In some of any such embodiments, the recombinant receptor is a chimeric antigen receptor (CAR). In some of any such embodiments, the recombinant receptor contains an extracellular domain containing an antigen-binding domain. In some of any such embodiments, the antigen-binding domain is or contains an antibody or an antibody fragment thereof, which optionally is a single chain fragment. In some of any such embodiments, the fragment contains antibody variable regions joined by a flexible linker. In some of any such embodiments, the fragment contains an scFv.

[0099] In some of any such embodiments, the recombinant receptor contains an intracellular signaling region. In some of any such embodiments, the intracellular signaling region contains an intracellular signaling domain. In some of any such embodiments, the intracellular signaling domain is or contains a primary signaling domain, a signaling domain that is capable of inducing a primary activation signal in a T cell, a signaling domain of a T cell receptor (TCR) component, and/or a signaling domain containing an immunoreceptor tyrosine-based activation motif (ITAM). In some of any such embodiments, the intracellular signaling domain is or contains an intracellular signaling domain of a CD3 chain, optionally a CD3-zeta (CD3 ζ) chain, or a signaling portion thereof.

[0100] In some of any such embodiments, the recombinant receptor further contains a transmembrane domain disposed between the extracellular domain and the intracellular signaling region. In some of any such embodiments, the intracellular signaling region further contains a costimulatory signaling domain. In some of any such embodiments, the costimulatory signaling domain contains an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof. In some of any such embodiments, the costimulatory signaling

domain contains an intracellular signaling domain of a CD28, a 4-1BB or an ICOS or a signaling portion thereof. In some of any such embodiments, the costimulatory signaling domain is between the transmembrane domain and the intracellular signaling domain.

[0101] In some of any such embodiments, the cells are T cells. In some of any such embodiments, the T cells are CD4+ or CD8+. In some of any such embodiments, the T cells are primary T cells obtained from a subject. In some of any such embodiments, the cells of the genetically engineered cells are autologous to the subject. In some of any such embodiments, the cells are allogeneic to the subject.

[0102] Also provided are methods of treatment, the methods comprising: (a) administering, to a subject having a disease or condition, a dose of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR) for treating the disease or condition; (b) after administering the dose of genetically engineered cells, monitoring CAR+ T cells in the blood of the subject to assess if the cells are within a therapeutic range, and (c) if the genetically engineered cells are not within the therapeutic range, administering to the subject an agent capable of modulating, optionally increasing or decreasing, CAR+ T cell expansion or proliferation, in the subject, wherein the therapeutic range is: (i) peak CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 10 cells per microliter and 500 cells per microliter; or (ii) peak CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 2 cells per microliter and 200 cells per microliter.

[0103] Also provided are method of treatment, the methods comprising: (a) monitoring, in the blood of a subject, the presence of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR) to assess if the cells are within a therapeutic range, wherein the subject has been previously administered a dose of the genetically engineered cells for treating a disease or condition; and (b) if the genetically engineered cells are not within the therapeutic range, administering to the subject an agent capable of modulating, optionally increasing or decreasing, CAR+ T cell expansion or proliferation, in the subject, wherein the therapeutic range is: (i) peak CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 10 cells per microliter and 500 cells per microliter; or (ii) peak CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 2 cells per microliter and 200 cells per microliter.

[0104] In some of any such embodiments, if the peak number of CAR+ T cells in the blood

of the subject is less than the lowest number of peak CAR+ T cells in the therapeutic range, an agent that is capable of increasing CAR+ T cell expansion or proliferation is administered to the subject. In some of any such embodiments, the agent is capable of increasing the CAR-specific expansion.

[0105] In some of any such embodiments, the agent is an anti-idiotypic antibody or antigen-binding fragment thereof specific to the CAR, an immune checkpoint inhibitor, a modulator of a metabolic pathway, an adenosine receptor antagonist, a kinase inhibitor, an anti-TGF β antibody or an anti-TGF β R antibody or a cytokine.

[0106] In some of any such embodiments, if the peak number of CAR+ T cells in the blood of the subject is greater than the highest number of peak CAR+ T cells in the therapeutic range, an agent that is capable of decreasing CAR+ T cell expansion or proliferation is administered to the subject.

[0107] Provided herein are methods of treatment comprising: (a) administering, to a subject having a disease or condition, a dose of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR) for treating the disease or condition; (b) after administering the dose of genetically engineered cells, monitoring CAR+ T cells in the blood of the subject, and (c) administering to the subject an agent capable of decreasing, CAR+ T cell expansion or proliferation, in the subject if: (i) the amount of CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, is greater than at or about 500 cells per microliter; or (ii) the amount of CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, is greater than at or about 200 cells per microliter.

[0108] Provided also herein are methods of treatment, the method comprising: (a) monitoring, in the blood of a subject, the presence of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR), wherein the subject has been previously administered a dose of the genetically engineered cells for treating a disease or condition; and (b) administering to the subject an agent capable of decreasing, CAR+ T cell expansion or proliferation, in the subject if: (i) the amount of CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, is greater than at or about 500 cells per microliter; or (ii) the amount of CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, is greater than at or about 200 cells per microliter.

[0109] In some of any such embodiments, the agent is one or more steroid. In some of any such embodiments, the steroid is dexamethasone or methylprednisolone.

[0110] In some of any such embodiments, the steroid is administered in an amount that is

between or between about 1.0 mg and at or about 40 mg, between or between about 1.0 mg and at or about 20 mg, between or between about 2.0 mg and at or about 20 mg, between or between about 5.0 mg and at or about 25.0 mg, between or between about 10 mg and at or about 20 mg dexamethasone or equivalent thereof, each inclusive. In some of any such embodiments, the steroid is administered in multiple doses over a period of at or more than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more or within a range defined by any of the foregoing. In some of any such embodiments, the steroid is administered once per day, twice per day, or three times or more per day.

[0111] In some of any such embodiments, the steroid is administered in an amount that is between or between about 1.0 mg and at or about 80 mg, between or between about 1.0 mg and at or about 60 mg, between or between about 1.0 mg and at or about 40 mg, between or between about 1.0 mg and at or about 20 mg, between or between about 1.0 mg and at or about 10 mg, between or between about 2.0 mg and at or about 80 mg, between or between about 2.0 mg and at or about 60 mg, between or between about 2.0 mg and at or about 40 mg, between or between about 2.0 mg and at or about 20 mg, between or between about 2.0 mg and at or about 10 mg, between or between about 5.0 mg and at or about 80 mg, between or between about 5.0 mg and at or about 60 mg, between or between about 5.0 mg and at or about 40 mg, between or between about 5.0 mg and at or about 20 mg, between or between about 5.0 mg and at or about 10 mg, between or between about 10 mg and at or about 80 mg, between or between about 10 mg and at or about 60 mg, between or between about 10 mg and at or about 40 mg, between or between about 10 mg and at or about 20 mg dexamethasone or equivalent thereof, each inclusive, per day or per 24 hours, or about 10 mg, 20 mg, 40 mg or 80 mg dexamethasone or equivalent thereof, per day or per 24 hours.

[0112] In some of any such embodiments, the subject is monitored for CAR+ T cells in the blood at a time that is at least 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days or 21 days after initiation of administration of the genetically engineered cells; or at a time that is between or between about 11 to 22 days, 12 to 18 days or 14 to 16 days, each inclusive, after initiation of administration of the genetically engineered cells.

[0113] In some of any such embodiments, the agent is administered at a time that is greater than or greater than about 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days or 21 days after initiation of administration of the genetically engineered cells; or at a time that is between or between at or about 11 to at or about

22 days, 12 to 18 days or 14 to 16 days, each inclusive, after initiation of administration of the genetically engineered cells.

Brief Description of the Drawings

[0114] **FIG. 1** shows an estimated probability curve of response and an estimated probability of developing Grade 3-5 neurotoxicity constructed based on the number of CD4+/truncated receptor+ or CD8+/truncated receptor+ CAR-T cells in the blood.

[0115] **FIG. 2A** shows the number of CD3⁺/CAR⁺ T cells in peripheral blood measured at certain time points post-infusion for subjects grouped by best overall response.

[0116] **FIGS. 2B-2D** show CD3⁺/CAR⁺ T cell, CD4⁺/CAR⁺ T cell, and CD8⁺/CAR⁺ T cell levels in peripheral blood measured at certain time points post-infusion for subjects who achieved a response, grouped by continued response at 3 months.

[0117] **FIG. 3** shows the percentage of subjects who experienced laboratory abnormalities and treatment-emergent adverse events (TEAEs) that occurred in $\geq 20\%$ of subjects. *: One Grade 5 AE of multi-organ failure unrelated to study treatment and due to progression of lymphoma; †: One Grade 5 AE of diffuse alveolar damage, investigator assessed as related to fludarabine, cyclophosphamide, and CAR T cell therapy, occurred on day 23 in a subject who refused mechanical ventilation for progressive respiratory failure while neutropenic on growth factors and broad spectrum antibiotics and antifungals.

[0118] **FIG. 4** is a Kaplan Meier curve depicting observed time to onset of CRS and neurotoxicity.

[0119] **FIG. 5A** and **FIG. 5B** depict the three month overall response rates (M3 ORR) at among subgroups of treated subjects in the full (**FIG. 5A**) and core (**FIG. 5B**) cohort of subjects.

[0120] **FIG. 6A** and **FIG. 6B** show the duration of response (CR/PR, CR or PR) and overall survival in the full (**FIG. 6A**) and core (**FIG. 6B**) cohort of subjects.

[0121] **FIG. 7A** shows the pharmacokinetics of the CAR⁺ T cells in peripheral blood at various time points post-treatment at different dose levels. **FIG. 7B** shows the pharmacokinetics of the CAR⁺ T cells in peripheral blood at various time points post-treatment between responders (CR + PR) and nonresponders (PD) at month 3. **FIG. 7C** shows the pharmacokinetics of the CAR⁺ T cells in peripheral blood at various time points post-treatment in subjects that did or did not develop any neurotoxicity.

[0122] **FIG. 8** shows levels of analytes measured in the serum of subjects prior to administration of the CAR⁺ T cells and correlation to the development of neurotoxicity.

[0123] **FIG. 9** shows a graph plotting progression-free time (months) and indicating best overall response and response durability, and individual clinical outcomes observed over time in individual subjects within a Full cohort and a Core cohort of NHL subjects treated with an anti-CD19 cell therapy containing CAR-T-expressing CD4+ and CD8+ T cells. ^a: Patients achieved BOR at month 1 except where otherwise noted; ^b: Complete resolution of CNS involvement by lymphoma observed in 2 patients; ^c: One patient re-expanded after biopsy upon disease progression.

[0124] **FIG. 10A** depicts the median (\pm quartiles) number of CAR-expressing CD3+ cells/ μ L blood, assessed by flow cytometry using an antibody specific for a truncated receptor (CD3, circle; N=87); or median (\pm quartiles) number of copies integrated CAR transgene/ μ g genomic DNA, assessed by quantitative polymerase chain reaction (qPCR) using primers specific for a woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) present in the vector encoding the CAR (qPCR, square; N=85) in blood samples from 87 subjects that have been administered anti-CD19 CAR-expressing cells. The cutoff for CAR+ cell detection in flow cytometry was set at ≥ 25 events in the CAR+ gate, and limit of detection for qPCR was ≥ 12.5 copies of CAR transgene per μ g of genomic DNA. **FIG. 10B** depicts the relative numbers of CD4+ and CD8+ CAR-expressing cells/ μ L in blood and bone marrow samples from 67 subjects that have been administered anti-CD19 CAR-expressing cells, on day 11 ± 3 days. The line represents the line of unity and is not a regression line.

[0125] **FIGS. 11A and 11B** depict the median (\pm quartiles) area under the curve between days 0 and 28 (AUC_{0-28} ; **FIG. 11A**) and maximum serum concentration (C_{max} ; CAR+ cells/ μ L blood; **FIG. 11B**) of CD4+ and CD8+ CAR+ cells in subject subgroups with diffuse large B-cell lymphoma de novo or transformed from indolent lymphoma (DLBCL, NOS; N=27), transformed follicular lymphoma (tFL; N=10), DLBCL transformed from marginal zone lymphoma or chronic lymphocytic leukemia (tMZL/tCLL; N=4), or mantle cell lymphoma (MCL; N=5), who have received CAR-expressing T cells at DL1.

[0126] **FIGS. 12A and 12B** depict the median (\pm quartiles) area under the curve between days 0 and 28 (AUC_{0-28} ; **FIG. 12A**) and maximum serum concentration (C_{max} ; CAR+ cells/ μ L blood; **FIG. 12B**) of CD3+, CD4+ and CD8+ CAR+ cells in subjects who have received CAR+ cells at DL1 or DL2.

[0127] **FIGS. 13A-13D** depict the median (\pm quartiles) number of CAR-expressing CD4+ and CD8+ CAR+ cells/ μ L blood over time, in subjects that developed cytokine release syndrome (any CRS) compared to subjects that have not developed CRS (no CRS) (CD4+: **FIG. 13A**;

CD8+: **FIG. 13B**) or in subjects that developed neurotoxicity (any NT) compared to subjects that have not developed NT (no NT) (CD4+: **FIG. 13C**; CD8+: **FIG. 13D**).

[0128] **FIGS. 14A** and **14B** depict the number of peak CD3⁺ CAR⁺ cells/ μ L (CD3+ C_{max}) in subjects grouped by subjects who had the best overall response (BOR) of CR, PR or PD, or a 3-month (M3) durable response of CR, PR or PD.

[0129] **FIG. 15A** depicts pre-lymphodepletion blood analyte levels in serum samples from subjects that exhibited high CAR+ cell expansion (CD3+ C_{max} > 500) and subjects that exhibited low CAR+ cell expansion (CD3+ C_{max} < 500). **FIG. 15B** depicts the peak blood analyte levels in serum samples from subjects that exhibited high CAR+ cell expansion (CD3+ C_{max} > 500) and subjects that exhibited low CAR+ cell expansion (CD3+ C_{max} < 500).

[0130] **FIG. 16** depicts a plot depicting pre-lymphodepletion sum of product dimensions (SPD; cm²) against AUC₀₋₂₈ (cells*day/ μ L) of CD3+ CAR+ cells, for individual subjects administered DL1 or DL2 of CAR+ cells.

[0131] **FIGS. 17A** and **17B** depict pre-lymphodepletion blood analyte levels in serum samples from subjects that developed cytokine release syndrome (CRS grade 1-4) compared to subjects that have not developed CRS (CRS grade 0) (**FIG. 17A**) or in subjects that developed neurotoxicity (NT grade 0) compared to subjects that have not developed NT (NT grade 1-4) (**FIG. 17B**). The units were: Ferritin and D-dimer (μ g/L); CRP (mg/L) and cytokines (pg/mL).

[0132] **FIG. 18** depicts the assessment of pre-lymphodepletion patient parameter sum of product dimensions (SPD; cm²), indicative of tumor burden, and lactate dehydrogenase (LDH; U/L) level, in subjects that developed cytokine release syndrome (any CRS) compared to subjects that have not developed CRS (no CRS) or in subjects that developed neurotoxicity (any NT) compared to subjects that have not developed NT (no NT).

[0133] **FIG. 19A** is a plot depicting pre-lymphodepletion SPD (cm²) against pre-lymphodepletion LDH (U/L) levels, in individuals that have developed neurotoxicity (Grade 1-4 NT) or subjects that have not developed NT (Grade 0 NT) (left panel), and in individuals that have developed CRS (Grade 1-4 CRS) or subjects that have not developed CRS (Grade 0 CRS) (right panel). Dotted lines represent levels of SPD (50 cm² or higher) or LDH (500 U/L or higher) that is associated with higher rates of CRS or NT. **FIG. 19B** depicts the odds ratio estimates for developing CRS or NT based on the levels of SPD (50 cm² or higher) or LDH (500 U/L or higher), with 95% confidence intervals (CI). **FIG. 19C** depicts the odds ratio estimates for developing CRS or NT based on the levels of SPD or LDH, including the odds ratio estimates for values lower than the threshold, with 95% confidence intervals (CI).

[0134] **FIG. 20** depicts pre-lymphodepletion tumor burden parameter (SPD) and blood analyte levels in for subjects that had a durable response at 3 months versus for subjects that did not have a response at 3 months. The units were: Ferritin and D-dimer ($\mu\text{g/L}$); CRP and SAA-1 (mg/L) and cytokines (pg/mL).

[0135] **FIGS. 21A** and **21B** depict peak blood analyte levels in serum samples from subjects that developed cytokine release syndrome (any CRS) compared to subjects that have not developed CRS (no CRS) (**FIG. 21A**) or in subjects that developed neurotoxicity (any NT) compared to subjects that have not developed NT (no NT) (**FIG. 21B**). The units were: CRP (mg/L), SAA-1 (mg/L) and cytokines (pg/mL).

[0136] **FIG. 22A** depicts peak blood analyte levels in serum samples from subjects that had a best overall response (BOR) of complete response (CR) or partial response (PR) ($N=57$) compared to levels in subjects that had stable disease (SD) or progressive disease (PD) ($N=17$). **FIG. 22B** depicts peak blood analyte levels in serum samples from subjects that had a 3-month response of SD/PD ($N=31$), compared to subjects who had a 3-month response CR/PR ($N=35$). The units were: CRP (mg/L), SAA-1 (mg/L) and cytokines (pg/mL).

[0137] **FIGS. 23A-23C** depict estimated probability curves for response, toxicity and durable response outcomes, based on the maximum serum concentration of CD3+ (**FIG. 23A**), CD4+ (**FIG. 23B**) or CD8+ (**FIG. 23C**) CAR-expressing cells (C_{max} ; cells/ μL blood). The estimated probability curves for overall response rate (ORR; including subjects with complete response (CR) and partial response (PR)), 3-month response (M3 response; including CR and PR at month 3 after administration), any NT, any CRS, Grade 3-4 NT, Grade 3-5 NT or Grade 2-5 CRS.

[0138] **FIG. 24** depicts month 3 objective response rates (ORR) among subgroups of treated subjects, with the 95% confidence interval.

[0139] **FIGS. 25A** and **25B** depict the duration of response (DOR) for the full cohort (**FIG. 25A**) and the core cohort (**FIG. 25B**), and **FIGS. 25C** and **25D** depict the overall survival for the full cohort (**FIG. 25C**) and the core cohort (**FIG. 25D**), , for subjects who achieved CR, PR, all subjects that showed a response, non-responders, and all treated subjects. Median F/U was 6.3 months for duration of response.

[0140] **FIG. 26** shows the percentage of subjects who experienced treatment-emergent adverse events (TEAEs) in the FULL DLBCL cohort occurring in $\geq 20\%$ of patients. Data for 5 patients with MCL treated with conforming product at DL1 with at least 28 days of follow-up were not included. ^b : One grade 5 AE of septic shock unrelated to CAR+ T cell administration. ^c

: One grade 5 AE of diffuse alveolar damage, investigator assessed as related to fludarabine, cyclophosphamide, and CAR⁺ T cells, occurred on day 23 in a patient who refused mechanical ventilation for progressive respiratory failure while neutropenic on growth factors and broad-spectrum antibiotics and antifungals. ^d : Laboratory anomalies.

[0141] **FIG. 27** shows the percentage of subjects who developed CRS or neurotoxicity over time, in the full cohort.

[0142] **FIG. 28** shows the percentage of subjects who experienced treatment-emergent adverse events (TEAEs) in the FULL DLBCL cohort occurring in $\geq 20\%$ of the subject at a study time point described in Example 6. Data for 6 subjects with MCL treated with conforming product at DL1 with at least 28 days of follow-up were not included. ^b : One grade 5 AE of septic shock unrelated to CAR⁺ T cell administration, occurred in the setting of disease progression. ^c : One grade 5 AE of diffuse alveolar damage, investigator assessed as related to fludarabine, cyclophosphamide, and CAR⁺ T cells, occurred on day 23 in a patient who refused mechanical ventilation for progressive respiratory failure while neutropenic on growth factors and broad-spectrum antibiotics and antifungals. ^d : Laboratory anomalies.

[0143] **FIG. 29** depict the six (6) month objective response rates (ORR) among subgroups of treated subjects, with the 95% confidence interval. ^a Includes all DLBCL subjects treated at all dose levels in the CORE cohort.

[0144] **FIGS. 30A** and **30B** depict the duration of response (DOR) for the full cohort (**FIG. 30A**) and the core cohort (**FIG. 30B**), and **FIGS. 30C** and **30D** depict the overall survival for the full cohort (**FIG. 30C**) and the core cohort (**FIG. 30D**), for subjects who achieved CR, PR, all subjects that showed a response, non-responders, and all treated subjects. NE, not estimable.

[0145] **FIG. 31** shows the CAR⁺ T cell concentration (cells/ μ L; left axis) and the daily dose of dexamethasone administration (daily IV dose, mg; right axis) in a subject who has been administered two doses of autologous engineered CAR⁺ T cells, at various time points after the administration of the second dose.

Detailed Description

I. METHOD FOR DETERMINING THERAPEUTIC DOSAGE RANGE

[0146] Among the embodiments provided herein are methods, uses, compositions and articles of manufacture involving and related to the administration of cell therapies such as those including engineered cells to subjects having or suspected of having a disease or condition, such as those specifically recognized by the cells of the therapy and/or those expressing antigens that

are specifically bound or recognized by the cells. The provided embodiments in some aspects relate to dosing a subject, e.g., administering a particular dose of the cell therapy to the subject, such as administering a dose that is or is suspected of being within a therapeutic dosage range and/or window, which generally is a range and/or window that achieves or is likely to achieve a desired level of the engineered cells in a sample, fluid, tissue, organ or location of the subject. Also provided are methods for ameliorating and/or treating a toxicity. Also provided are methods for modulating activity of engineered cells used for cell therapy. In some aspects, also provided are methods of assessing likelihood of a response, such as a durable response. In some aspects, also provided are related uses, and kits and articles of manufacture related to the provided methods.

[0147] Adoptive cell therapies (including those involving the administration of cells expressing recombinant and/or chimeric receptors specific for a disease or disorder of interest, such as chimeric antigen receptors (CARs) and/or other recombinant antigen receptors, as well as other adoptive immune cell and adoptive T cell therapies) can be effective in the treatment of cancer and other diseases and disorders. In certain contexts, available approaches to adoptive cell therapy may not always be entirely satisfactory. In some contexts, optimal response to therapy can depend on the ability of the administered cells to recognize and bind to a target, *e.g.*, target antigen, to traffic, localize to and successfully enter appropriate sites within the subject, tumors, and environments thereof, to become activated, expand, to exert various effector functions, including cytotoxic killing and secretion of various factors such as cytokines, to persist, including long-term, to differentiate, transition or engage in reprogramming into certain phenotypic states (such as effector, long-lived memory, less-differentiated, and effector states), to provide effective and robust recall responses following clearance and re-exposure to target ligand or antigen, and avoid or reduce exhaustion, anergy, terminal differentiation, and/or differentiation into a suppressive state.

[0148] In some aspects, the therapeutic effect of adoptive cell therapy may be limited by the development of toxicity in the subject to whom such cells are administered, which toxicity in some cases can be severe, at certain doses or exposure of administered cells. In some cases, while a higher dose of such cells can increase the therapeutic effect, for example, by increasing exposure to the cells such as by promoting expansion and/or persistence, they may also result in an even greater risk of developing a toxicity or a more severe toxicity. In some aspects, some of the administered cells can contain cells that expand or proliferate rapidly, which also may contribute to a risk of developing a toxicity or a more severe toxicity. Also, in some cases,

subjects with a higher disease burden also may be at a greater risk for developing a toxicity or a more severe toxicity. Certain available methods for dosing subjects cell therapy may not always be entirely satisfactory. Increasing a dose of cells or promoting expansion or proliferation of administered cells in the subject can be related to higher response rates, but also an increase in development of toxicity.

[0149] The provided methods offer advantages over available approaches in determining the dose of the cell therapy. The provided methods permit administering a dose to a subject that is or is suspected of being within a therapeutic dosage range and/or window, which generally is a range and/or window that achieves or is likely to achieve a desired level of the engineered cells in the subject. The provided methods permit dosing of cells that can achieve or can be associated with associated with a high or specified desired degree of likelihood of a treatment outcome such as a favorable outcome or response and/or a durable response or outcome, and also associated with a relatively low or minimized or desired degree of likelihood of risk of developing a toxic outcome or toxicity following administration to the subject of the cell therapy.

[0150] The provided methods also offer advantages over available approaches by permitting modulation, modification and/or alteration of the activity, function, proliferation and/or expansion of the cells in the cell therapy if the subject is determined to be not likely to achieve a response and/or a durable response, thereby optimizing the response without substantially increasing the risk of toxicity. In some embodiments, pharmacokinetic parameters, patient attributes, tumor burden and/or expression of biomarkers, such as inflammatory markers can be used to determine likelihood of response and/or any need for modulating, modifying or altering the therapy, to achieve greater response or more durable response, without substantially increasing the risk of toxicity.

[0151] Also provided are methods of treating and/or ameliorating a toxicity that may be related to administration of a cell therapy. In some aspects, the methods involve administering a treatment regimen to treat and/or ameliorate the toxicity. The provided methods offer an advantage of permitting systematic management of toxicities that may be associated with immunotherapies and/or adoptive cell therapies.

[0152] In some embodiments, the therapeutic dosage range and/or window achieves or is likely to achieve a desired level of the engineered cells, e.g., CAR T cells, that, in some aspects, is a peak level, which generally refers to the maximum number, concentration or percentage of the cells observed or measured in the relevant sample, fluid, tissue, organ or other location

following treatment or within a certain period following treatment. In some aspects, the level may be a number, concentration or percentage (such as number of the cells per weight or volume or area or total cell number) or exposure of the subject or tissue or organ or fluid or location to the cells, at a given time or over a period of time. In some aspects, the level is an area under the curve (AUC) with respect to a plot of the number or percentage or other readout of the relevant cells in the tissue or sample or fluid or organ or other location, over a given period of time following treatment or administration of the cells or initiation thereof.

[0153] In some examples, the level is expressed as CAR+ cell concentration (e.g., CAR+ cells/microliter (μ l)) in the blood, AUC of a curve of CAR+ cells/volume (e.g., CAR+ cells/microliter) over a period of time, maximum or peak CAR+ cells/volume (e.g., CAR+ cells/microliter) in the blood following treatment, or CAR+ cells/microliter of blood at day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21, or week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 or more post-treatment or initiation thereof. In some embodiments, the desired level is within, or is a level within, a determined therapeutic range. In some examples, the level is expressed as copies of the nucleic acid sequence (e.g., transgene sequence) encoding the CAR or a nucleic acid sequence operably connected to the CAR-encoding sequences, per mass of DNA (e.g., copies/ μ g of DNA); AUC of the curve of copies/ μ g of DNA over time, maximum or peak copies/ μ g of DNA following treatment, or copies/ μ g of DNA at day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21, or week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 or more post-treatment or initiation thereof. In some embodiments, the desired level is within, or is a level within, a determined therapeutic range.

[0154] In some embodiments, the therapeutic range is a therapeutic range and/or window associated with a high or specified desired degree of likelihood of a treatment outcome such as a favorable outcome or response and/or a durable response or outcome, and also associated with a relatively low or minimized or desired degree of likelihood of risk of developing a toxic outcome or toxicity following administration to the subject of the cell therapy, e.g., the engineered cells. In some aspects, the toxicity or toxic outcome is cytokine release syndrome (CRS) or neurotoxicity (NT). In some aspects, the toxicity or toxic outcome is any CRS or grade 1 or higher CRS or any neurotoxicity or grade 1 or higher neurotoxicity. In some aspects, the toxicity or toxic outcome is severe CRS or grade 3 or higher CRS or severe neurotoxicity or grade 3 or higher neurotoxicity. In some cases, risk of toxicity is correlated to disease burden, dose of cells, expansion of cells, and the pharmacokinetic (PK) of the cells, e.g., cell exposure or peak cell concentration. Yet, at the same time to maximize response, in some cases, a higher or

greater dose of cells, exposure of cells or peak concentration of cells is required. In some aspects, however, it is found herein that probability of durable response, e.g., response that persists after a period of time from initiation of therapy, can increase with higher or greater dose of cells, exposure of cells or peak concentration of cells, up to a certain dose, exposure or concentration; then can decrease. It is found herein, from probability curves for toxicity (e.g. CRS or neurotoxicity, severe CRS or severe neurotoxicity) and response (e.g. marrow response) and/or durable response generated from a population of subjects treated with CAR+ T cells, that there is a therapeutic range and/or window, e.g. widest range between curves, at which a dose can be determined to maximize estimated probability of response or durable response and minimize estimated risk of toxicity. In some embodiments, such probability curves can be used in methods to choose or to determine a dose of cells to administer to a subject. In some embodiments, such probability curves can be used in methods to modify the dose of cells and/or to modulate the expansion and/or activity of cells, e.g., by administering an agent and/or intervention that affects cell expansion, activity and/or function.

[0155] In some embodiment, the provided methods include administering to the subject a dose of cells engineered with a chimeric antigen receptor (CAR), wherein the dose is sufficient to achieve peak CAR+ cells/ μ l within a determined therapeutic range and/or an exposure (e.g., AUC) within a determined therapeutic range, wherein the therapeutic range is determined based upon the estimated probability of a response outcome (e.g. marrow response) and/or durable response, e.g., response at 3 months, and the estimated probability of a toxic outcome (e.g. grade 3-5 neurotoxicity).

[0156] In some embodiments, the estimated probability is determined from a probability curve generated based on results or outcomes from a population of subjects, such as at least 10, 25, 50, 100, 150, 300, 400, 500 or more subjects. In some embodiments, the population of subjects is diseased subjects, such as subjects having a disease or condition, such as a tumor or cancer. In some embodiments, the population of subjects is or includes subjects that are likely to or are candidates or who are or have been receiving treatment with genetically engineered cells, e.g. CAR-T cells, for treating the disease or condition. In some embodiments, the subject has a sarcoma, a carcinoma or a lymphoma, optionally a non-Hodgkin lymphoma (NHL), diffuse large B cell lymphoma (DLBCL), leukemia, chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML) and myeloma. In some embodiments, the subject has CLL. In some embodiments, a first probability curve is generated for risk of a toxic outcome (e.g., CRS or neurotoxicity, such as grade 3-5 neurotoxicity) and a

second probability curve is generated for a response outcome (e.g. marrow response). In some embodiments, a first probability curve is generated for risk of a toxic outcome (e.g., CRS or neurotoxicity, such as grade 3-5 neurotoxicity) and a second probability curve is generated for durable response outcome. In some embodiments, the probability curves are transformed or provided as a Sigmoidal curve.

[0157] In some embodiments, the estimated probability of toxicity (e.g. CRS or neurotoxicity, such as grade 3-5 CRS or neurotoxicity) and/or estimated probability of response (e.g. marrow response) or durable response (e.g., response at 3 months) is correlated to peak CAR+ cell concentration (cells/ μ l) in a biological sample, such as in blood. In some embodiments, the CAR+ cells are or comprise T cells, e.g., are or comprise CD3+ T cells. In some embodiments, the T cells are CD4+ or CD8+ T cells. In some embodiments, the administered composition comprises CD4+ and CD8+ CAR+ T cells and the probability curves are generated separately for the CD4+ cells and for the CD8+ cells and/or for CD3+ cells.

[0158] In some embodiments, the provided methods include a method of dosing a subject comprising administering to the subject a dose of cells engineered with a recombinant receptor, such as an antigen receptor, e.g. chimeric antigen receptor (CAR), wherein the dose is sufficient to achieve peak CAR+ cells/ μ l within a determined therapeutic range, wherein the therapeutic range is determined based upon the estimated probability of a response outcome (e.g. marrow response) and/or durable response (e.g., response at 3 months) and the estimated probability of a toxic outcome (e.g. CRS or neurotoxicity, such as grade 3-5 neurotoxicity). In some embodiments, the estimated probability of causing toxicity is less than 35%, less than 30%, less than 25%, less than 20%, less than 15%, less than 10% or less than 5% on the toxicity probability curve. In some embodiments, the estimated probability of achieving a response is greater than 65%, 70%, 75%, 80%, 85%, 90%, 95% or more. In some embodiments, the estimated probability of achieving a durable response, e.g., a response at 3 months, is greater than 65%, 70%, 75%, 80%, 85%, 90%, 95% or more. In some embodiments, the toxicity is CRS, such as any CRS, such as grade 1 or higher CRS, or neurotoxicity, such as any neurotoxicity, such as grade 1 or higher neurotoxicity. In some embodiments, the severe toxicity is severe CRS or grade 3 or higher CRS or severe neurotoxicity or grade 3 or higher neurotoxicity. In some cases, the response is a marrow response. In some embodiments, response is assessed using IgH deep sequencing. In some embodiments, the toxicity outcome is severe neurotoxicity or grade 3 or higher neurotoxicity, such as grade 3-5 neurotoxicity.

[0159] Also provided, is a method of dosing by administering, to a subject having a disease

or condition (e.g. tumor or cancer), a dose of cells, and monitoring the subject post-infusion for peak CAR+ cells/ μ l, such as at one or more various time points, e.g. at or about or greater than 3 days, 7 days, 14 days, 28 days, 2 months, 3 months, 4 months, 5 months, 6 months, 1 year, 2 years or more after infusion with the cell therapy, or AUC over time, such as up to one or more time point after administration, e.g., up to or up to about or greater than 3 days, 7 days, 14 days, 28 days, 2 months, 3 months, 4 months, 5 months, 6 months, 1 year, 2 years or more after infusion with the cell therapy. In some embodiments, the method can include determining or assessing the probability the peak CAR+ cells/ μ l are in the therapeutic range, such as determined from a toxicity probability curve and/or response probability curve and/or a durable response probability curve. In some embodiments, if the peak CAR+ cells/ μ l or AUC is not in the therapeutic range, the method further involves administering a compound or agent to enhance or boost CAR+ cell expansion *in vivo* such that the peak CAR+ expansion is within the therapeutic range, such as determined by the provided methods and/or to reduce, inhibit, prevent and/or delay CAR+ T cell activity and/or expansion.

[0160] Also provided, is a method of dosing, to a subject having a disease or condition (e.g. tumor or cancer), by administering to the subject a sub-optimal dose of cells, wherein the dose is insufficient to achieve peak CAR+ cells/ μ l within a determined therapeutic range. In some embodiments, the method further involves administering a compound or agent to enhance or boost CAR+ cell expansion *in vivo* such that the peak CAR+ expansion is within the therapeutic range, such as determined by the provided methods.

[0161] In some embodiments, the method further involves administering to the subject a second dose of cells based on the response and toxicity probability curves for peak CD3+, CD4+ and/or CD8+ CAR+T cell concentration (cells/ μ l) and/or AUC, e.g., peak CD8+ CAR+ T cell concentrations. In some embodiments, the method further involves administering to the subject a tumor microenvironment (TME) targeting agent based on the response and toxicity probability curves for peak CD3+, CD4+ and/or CD8+ CAR+T cell concentrations (cells/ μ l) and/or AUC, e.g., peak CD8+ CAR+ T cell concentrations. In some aspects, the method allows the selection of a dosing range that achieves a more durable response and/or remission. Also provided are methods that involve assessing, determining or monitoring pharmacokinetic parameters, such as maximum (peak) plasma concentration (C_{max}) and area under the curve (*i.e.* the area under the curve generated by plotting time versus plasma concentration of the therapeutic agent CAR+ T cells; AUC) of administered cells in the subject. In some embodiments, such assessments can be used to determine whether the administered cells are within a therapeutic range or window. In

some embodiments, such assessments can be used as an indicator to modulate, modify and/or alter therapy, e.g., by administering agents capable of modulating the expansion, proliferation and/or activity of the administered CAR+ T cells, administer additional and/or modified doses, and/or administer alternative therapy. In some embodiments, also provided are methods of administering a therapeutic agent accordingly. In some embodiments, such assessments can be used to monitor the progress of the therapy and/or to assess the effect of modulated therapy. In some embodiments, such measurements can be used to assess the likelihood of a response or a durable response.

[0162] Also provided are methods that involve assessing, determining or monitoring other parameters, such as patient attributes, tumor burden and/or expression of biomarkers, such as inflammatory markers. In some embodiments, the assessment can be performed using a sample from the subject obtained prior to administration of the cell therapy or initiation thereof. In some embodiments, the assessment can be performed using a sample from the subject obtained after administration of the cell therapy or initiation thereof. In some embodiments, such assessments can be used to determine whether the administered cells are likely to be, or is likely to correlate with or associate with being, within a therapeutic range or window. In some embodiments, such assessments can be used as an indicator to modulate, modify and/or alter therapy, e.g., by administering agents capable of modulating the expansion, proliferation and/or activity of the administered CAR+ T cells, administer additional and/or modified doses, and/or administer alternative therapy. In some embodiments, also provided are methods of administering a therapeutic agent accordingly. In some embodiments, such assessments can be used to monitor the progress of the therapy and/or to assess the effect of modulated therapy. In some embodiments, such measurements can be used to assess the likelihood of a response or a durable response.

II. TOXICITY AND RESPONSE PROBABILITY CURVES

[0163] In some embodiments, probability curves from a population of subjects as described are generated and correlated with the risk of toxic outcome (e.g. CRS or neurotoxicity, e.g., grade 2-5 CRS or grade 3-5 neurotoxicity) or response (e.g. marrow response), and/or durability of response (e.g., month 3 response). In some embodiments, the information regarding toxic outcome and response outcome as described above are combined and/or correlated with data collected regarding peak cell levels and/or concentrations, or exposure (e.g., AUC) in the subject. In some embodiments, the information about toxic outcome and response outcome are

collected from a cohort of subjects, each correlated with cell level data (*e.g.*, peak number or concentration of CAR+ T cells), and independently assessed. In some embodiments, for example, the toxic outcome data are collected and assessed with CAR+ cell numbers to construct a toxicity probability curve. In some cases, the response outcome data, including data for durable response outcomes, are collected and assessed with CAR+ cell numbers to construct a response probability curve and/or a durable response probability curve.

[0164] In some embodiments, the resulting toxicity and response and/or durable response probability curves can be jointly assessed, such as assessed in parallel or at around the same time or substantially the same time, to inform the dosing decisions or adaptive treatments of subjects.

[0165] In some embodiments, toxic outcome and response outcome are used to construct an estimated probability curve of response and an estimated probability of developing toxicity based on the number, concentration and/or exposure of CAR+ T cells in the blood. In some cases, the estimated probability of achieving a response is greater than at or about 65%, greater than at or about 70%, greater than at or about 75%, greater than at or about 80%, greater than at or about 85%, greater than at or about 90%, greater than at or about 95% or more. In some cases, the estimated probability of achieving a durable response, *e.g.*, a 3- or 6-month durable response, is greater than at or about 65%, greater than at or about 70%, greater than at or about 75%, greater than at or about 80%, greater than at or about 85%, greater than at or about 90%, greater than at or about 95% or more. In some cases, the estimated probability of causing or resulting in toxicity is 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% or less than at or about 5% on the toxicity probability curve.

[0166] In some embodiments, the methods involve administering a sufficient number or dose of cells to achieve a peak CAR+ cell concentration in the subject that is within a determined target therapeutic range or window. In some embodiments, the methods involve administering a sufficient number or dose of cells to achieve a peak CAR+ cell concentration in a majority of subjects so treated by the method, or greater than or greater than at or about 50%, 60%, 70%, 75%, 80%, 85%, 90% or 95% or more, such as greater than 75% of the subjects so treated by the method, is within a determined target therapeutic range or window. In the provided methods, one or more therapeutic outcomes or events associated with toxicity (toxic outcome) and one or more therapeutic outcomes or events associated with efficacy (response outcome, including durable response outcome) of the therapeutic agent is assessed and dosing decisions are made in accord with the provided methods. In some embodiments, the information

regarding toxic outcome and response outcome are combined and/or correlated with data collected regarding peak cell levels, concentrations and/or exposure in the subject. In some embodiments, the information about toxic outcome and response outcome are collected from a cohort of subjects, each correlated with cell level data, and independently assessed. In some embodiments, for example, the toxic outcome data are collected and assessed to construct a toxicity probability curve and the response outcome data are collected and assessed to construct a response probability curve. In some embodiments, durable response outcome data (e.g., durable response at 3, 6, 9 or 12 months) are collected and assessed to construct a durable response probability curve.

[0167] In some embodiments, the toxicity and response probability curves can be jointly assessed, such as assessed in parallel or at around the same time or substantially the same time, to inform the dosing decisions or adaptive treatments of subjects.

[0168] In some embodiments, the toxic outcome and response outcome are monitored at a time at which a toxicity outcome and a response outcome are present. The particular time at which such outcome may be present will depend on the particular therapeutic agent and is known to a skilled artisan, such as a physician or clinician, or is within the level of such a skilled artisan to determine. In some embodiments, the time at which a toxic outcome or response outcome is assessed is within or within about a period of time in which a symptom of toxicity or efficacy is detectable in a subject or at such time in which an adverse outcome associated with non-response or toxicity is not detectable in the subject. In some embodiments, the time period is near or substantially near to when the toxic outcome and/or response outcome has peaked in the subject. In some embodiments, the time period includes time required for assessing durability of response, e.g., durable response at 3, 6, 9 or 12 months after first administration of the cells.

[0169] In some embodiments, the toxic outcome or response outcome can be assessed in the subject at a time that is within or about within 120 days after initiation of the first dose of the therapeutic agent to the subject, within or within about 90 days after initiation of the first dose, within or within about 60 days after initiation of the first dose of the therapeutic agent or within or within about 30 days after initiation of the first dose to a subject. In some embodiments, the toxic outcome or response can be assessed in the subject within or within at or about 6 days, 12 days, 16 days, 20 days, 24 days, 28 days, 32 days, 36 days, 40 days, 44 days, 48 days, 52 days, 56 days, 60 days, 64 days, 68 days, 72 days, 76 days, 80 days, 84 days, 88 days, 92 days, 96 days or 100 days after initiation of the first dose to a subject.

[0170] In some embodiments, the toxic outcome or response outcome is present or can be assessed or monitored at such time period where only a single dose of the therapeutic agent is administered. 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, provided in multiple individual compositions or infusions, over a specified period of time, which is no more than 3 days. Thus, in some contexts, the first 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 first 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.

[0171] The term “split dose” refers to a dose that is split so that it is administered over more than one day. This type of dosing is encompassed by the present methods and is considered to be a single dose.

[0172] As used herein, “first dose” is used to describe the timing of a given dose, which, in some cases can be the only dose or can be followed by one or more repeat or additional doses. The term does not necessarily imply that the subject has never before received a dose of a therapeutic agent even that the subject has not before received a dose of the same or substantially the same therapeutic agent.

[0173] In some embodiments, the toxic outcome or response outcome is present and/or can be assessed or monitored at such time period that is after a first cycle of administration of the therapeutic agent, after a second cycle of administration of the therapeutic agent, after a third cycle of administration of the therapeutic agent, or after a fourth cycle of administration of the therapeutic agent. In some embodiments, a cycle of administration can be a repeated schedule of a dosing regimen that is repeated over successive administrations. In some embodiments, a schedule of administration can be daily, every other day, or once a week for one week, two weeks, three weeks or four weeks (e.g. 28 days).

[0174] In some embodiments, the toxic outcome and response outcome can be assessed by monitoring one or more symptoms or events associated with a toxic outcome and one or more symptoms or events associated with a response outcome. In some embodiments, the disease or condition is a tumor or cancer.

A. Toxicity Outcome

[0175] In some embodiments, a toxic outcome in a subject to administration of a therapeutic agent (e.g. CAR T-cells) can be assessed or monitored. In some embodiments, the toxic outcome is or is associated with the presence of a toxic event, such as cytokine release syndrome (CRS), severe CRS (sCRS), macrophage activation syndrome, tumor lysis syndrome, fever of at least at or about 38 degrees Celsius for three or more days and a plasma level of C-reactive protein (CRP) of at least at or about 20 mg/dL, neurotoxicity (NT) and/or severe neurotoxicity (sNT). In some embodiments, the toxic outcome is a sign, or symptom, particular signs, and symptoms and/or quantities or degrees thereof which presence or absence may specify a particular extent, severity or level of toxicity in a subject. It is within the level of a skilled artisan to specify or determine a particular sign, symptom and/or quantities or degrees thereof that are related to an undesired toxic outcome of a therapeutic agent (e.g. CAR- T cells).

[0176] In some embodiments, the toxic outcome is an indicator associated with the toxic event. In some embodiments, the toxic outcome is the presence or absence of one or more biomarkers or the presence of absence of a level of one or more biomarkers. In some embodiments, the biomarker is a molecule present in the serum or other bodily fluid or tissue indicative of cytokine-release syndrome (CRS), severe CRS or CRS-related outcomes. In some embodiments, the biomarker is a molecule present in the serum or other bodily fluid or tissue indicative of neurotoxicity or severe neurotoxicity.

[0177] In some embodiments, the subject exhibits toxicity or a toxic outcome if a toxic event, such as CRS-related outcomes, e.g. if a serum level of an indicator of CRS or other biochemical indicator of the toxicity is more than at or about 10 times, more than at or about 15 times, more than at or about 20 times, more than at or about 25 times, more than at or about 50 times, more than at or about 75 times, more than at or about 100 times, more than at or about 125 times, more than at or about 150 times, more than at or about 200 times, or more than at or about 250 times the baseline or pre-treatment level, such as the serum level of the indicator immediately prior to administration of the first dose of the therapeutic agent.

[0178] 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.

[0179] 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. 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.

[0180] 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 or tumor burden 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.

[0181] Exemplary signs or symptoms associated with CRS include fever, rigors, chills, hypotension, dyspnea, acute respiratory distress syndrome (ARDS), encephalopathy, aspartate transaminase (AST)/alanine transaminase (ALT) 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 signs or 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).

[0182] In some embodiments, signs or symptoms 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 (e.g. IFN γ or IL-6); 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 (PO $_2$) 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.

[0183] 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.

[0184] In some embodiments, one or more inflammatory markers, e.g., 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-2R α , granulocyte macrophage colony stimulating factor (GM-CSF), or macrophage inflammatory protein (MIP). In some embodiments, IFN- γ , TNF- α , and IL-6 are monitored.

[0185] In some embodiments, the presence of one or more biomarkers is indicative of the grade of, severity or extent of a toxic event, such as CRS or neurotoxicity. In some embodiments, the toxic outcome is a particular grade, severity or extent of a toxic event, such as a particular grade, severity or extent of CRS or neurotoxicity. In some embodiments, the presence of a toxic event about a certain grade, severity or extent can be a dose-limiting toxicity. In some embodiments, the absence of a toxic event or the presence of a toxic event below a certain grade, severity or extent can indicate the absence of a dose-limiting toxicity.

[0186] 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 whose

treatment-induced elevation can correlate well with both pretreatment tumor burden and sCRS symptoms. Other guidelines on the diagnosis and management of CRS are known (see e.g., Lee et al, Blood. 2014;124(2):188-95). 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 (e.g., fever, nausea, fatigue, headache, myalgias, malaise)
2 Moderate	Require and respond to moderate intervention: <ul style="list-style-type: none"> • 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: <ul style="list-style-type: none"> • Oxygen requirement \geq 40%, or • Hypotension requiring high dose of a single vasopressor (e.g., norepinephrine \geq 20 μg/kg/min, dopamine \geq 10 μg/kg/min, phenylephrine \geq 200 μg/kg/min, or epinephrine \geq 10 μg/kg/min), or • Hypotension requiring multiple vasopressors (e.g., vasopressin + one of the above agents, or combination vasopressors equivalent to \geq 20 μg/kg/min norepinephrine), or • Grade 3 organ toxicity or Grade 4 transaminitis (by CTCAE v4.0)
4 Life-threatening	Life-threatening: <ul style="list-style-type: none"> • Requirement for ventilator support, or • Grade 4 organ toxicity (excluding transaminitis)
5 Fatal	Death

[0187] 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	N/A	FiO ₂ < 40%	FiO ₂ \geq 40%	Needs ventilator

to reach SaO ₂ > 90%				support
Organ toxicity	N/A	Grade 2	Grade 3 or transaminitis	Grade 4 (excluding transaminitis)

[0188] In some embodiments, high-dose vasopressor therapy include 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 = [norepinephrine ($\mu\text{g}/\text{min}$)] + [dopamine ($\mu\text{g}/\text{kg}/\text{min}$) $\div 2$] + [epinephrine ($\mu\text{g}/\text{min}$)] + [phenylephrine ($\mu\text{g}/\text{min}$) $\div 10$]	

[0189] In some embodiments, the toxic outcome is severe CRS. In some embodiments, the toxic outcome is the absence of severe CRS (e.g. moderate or mild CRS). In some embodiments, severe CRS includes CRS with a grade of 3 or greater, such as set forth in **Table 1** and **Table 2**. In some embodiments, severe CRS includes CRS with a grade of 2 or higher, such as grades 2, 3, 4 or 5 CRS.

[0190] 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 $\geq 15 \text{ mg}/\text{dL}$ 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.

[0191] In some aspects, the toxic outcome is or is associated with neurotoxicity. In some embodiments, signs or 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)). 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**. In some embodiments, severe neurotoxicity includes neurotoxicity with a grade of 2 or higher, such as grades 2, 3, 4 or 5 neurotoxicity.

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	Symptoms that are life-threatening, requiring urgent intervention

Table 4: Exemplary Grading Criteria for neurotoxicity	
Grade	Description of Symptoms
Life-threatening	
5 Fatal	Death

[0192] 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.

B. Response Outcome

[0193] In some embodiments, a response outcome in a subject to administration of a therapeutic agent can be monitored or assessed. In some embodiments, the response outcome is no response. In some embodiments, the response outcome is a partial response (PR). In some embodiments, the response outcome is a complete response (CR). In some embodiments, response outcome is assessed by monitoring the disease burden in the subject. In some embodiments, the presence of no response, a partial response or a clinical or complete response can be assessed.

[0194] In some embodiments, a partial response (PR) or complete response (CR) is one in which the therapeutic agent reduces or prevents the expansion or burden of the disease or condition in the subject. For example, where the disease or condition is a tumor, reduced disease burden exists or is present if there is a reduction in the tumor size, bulk, metastasis, percentage of blasts in the bone marrow or molecularly detectable cancer and/or an improvement prognosis or survival or other symptom associated with tumor burden compared to prior to treatment with the therapeutic agent (e.g. CAR T cells).

[0195] In some embodiments, the administration effectively treats the subject despite the subject having become resistant to another therapy. In some embodiments, at least 35%, at least 40% or at least 50% of subjects treated according to the method achieve complete response (CR); and/or at least 50%, at least 60% or at least 70% of the subjects treated according to the method achieve objective response rate (ORR). In some embodiments, at least or about at least 50% of subjects, at least or about at least 60% of the subjects, at least or about at least 70% of

the subjects, at least or about at least 80% of the subjects or at least or about at least 90% of the subjects treated according to the method achieve CR and/or achieve an objective response (OR). In some embodiments, criteria assessed for effective treatment includes overall response rate or objective response rate (ORR), complete response (CR), duration of response (DOR), progression-free survival (PFS), and/or overall survival (OS).

[0196] In some embodiments, at least 40% or at least 50% of subjects treated according to the methods provided herein achieve complete remission (CR), exhibit progression-free survival (PFS) and/or overall survival (OS) of greater than at or about 3 months, 6 months or 12 months or greater than 13 months or approximately 14 months; on average, subjects treated according to the method exhibit a median PFS or OS of greater than at or about 6 months, 12 months, or 18 months; and/or the subject exhibits PFS or OS following therapy for at least at or about 6, 12, 18 or more months.

[0197] In some aspects, response rates in subjects, such as subjects with NHL, are based on the Lugano criteria. (Cheson et al., (2014) JCO 32(27):3059-3067; Johnson et al., (2015) Radiology 2:323–338; Cheson, B.D. (2015) Chin Clin Oncol 4(1):5). In some aspects, response assessment utilizes any of clinical, hematologic, and/or molecular methods. In some aspects, response assessed using the Lugano criteria involves the use of positron emission tomography (PET)–computed tomography (CT) and/or CT as appropriate. PET-CT evaluations may further comprise the use of fluorodeoxyglucose (FDG) for FDG-avid lymphomas. In some aspects, where PET-CT will be used to assess response in FDG-avid histologies, a 5-point scale may be used. In some respects, the 5-point scale comprises the following criteria: 1, no uptake above background; 2, uptake \leq mediastinum; 3, uptake $>$ mediastinum but \leq liver; 4, uptake moderately $>$ liver; 5, uptake markedly higher than liver and/or new lesions; X, new areas of uptake unlikely to be related to lymphoma.

[0198] In some aspects, a complete response as described using the Lugano criteria involves a complete metabolic response and a complete radiologic response at various measureable sites. In some aspects, these sites include lymph nodes and extralymphatic sites, wherein a CR is described as a score of 1, 2, or 3 with or without a residual mass on the 5-point scale, when PET-CT is used. In some aspects, in Waldeyer's ring or extranodal sites with high physiologic uptake or with activation within spleen or marrow (e.g., with chemotherapy or myeloid colony-stimulating factors), uptake may be greater than normal mediastinum and/or liver. In this circumstance, complete metabolic response may be inferred if uptake at sites of initial involvement is no greater than surrounding normal tissue even if the tissue has high physiologic

uptake. In some aspects, response is assessed in the lymph nodes using CT, wherein a CR is described as no extralymphatic sites of disease and target nodes/nodal masses must regress to ≤ 1.5 cm in longest transverse diameter of a lesion (LDi). Further sites of assessment include the bone marrow wherein PET-CT-based assessment should indicate a lack of evidence of FDG-avid disease in marrow and a CT-based assessment should indicate a normal morphology, which if indeterminate should be IHC negative. Further sites may include assessment of organ enlargement, which should regress to normal. In some aspects, nonmeasured lesions and new lesions are assessed, which in the case of CR should be absent (Cheson et al., (2014) JCO 32(27):3059-3067; Johnson et al., (2015) Radiology 2:323–338; Cheson, B.D. (2015) Chin Clin Oncol 4(1):5).

[0199] In some aspects, a partial response (PR) as described using the Lugano criteria involves a partial metabolic and/or radiological response at various measureable sites. In some aspects, these sites include lymph nodes and extralymphatic sites, wherein a PR is described as a score of 4 or 5 with reduced uptake compared with baseline and residual mass(es) of any size, when PET-CT is used. At interim, such findings can indicate responding disease. At the end of treatment, such findings can indicate residual disease. In some aspects, response is assessed in the lymph nodes using CT, wherein a PR is described as $\geq 50\%$ decrease in sum of product dimensions (SPD) of up to 6 target measureable nodes and extranodal sites. If a lesion is too small to measure on CT, 5 mm \times 5 mm is assigned as the default value; if the lesion is no longer visible, the value is 0 mm \times 0 mm; for a node > 5 mm \times 5 mm, but smaller than normal, actual measurements are used for calculation. Further sites of assessment include the bone marrow wherein PET-CT-based assessment should indicate residual uptake higher than uptake in normal marrow but reduced compared with baseline (diffuse uptake compatible with reactive changes from chemotherapy allowed). In some aspects, if there are persistent focal changes in the marrow in the context of a nodal response, consideration should be given to further evaluation with MRI or biopsy, or an interval scan. In some aspects, further sites may include assessment of organ enlargement, where the spleen must have regressed by $> 50\%$ in length beyond normal. In some aspects, nonmeasured lesions and new lesions are assessed, which in the case of PR should be absent/normal, regressed, but no increase. No response/stable disease (SD) or progressive disease (PD) can also be measured using PET-CT and/or CT based assessments. (Cheson et al., (2014) JCO 32(27):3059-3067; Johnson et al., (2015) Radiology 2:323–338; Cheson, B.D. (2015) Chin Clin Oncol 4(1):5).

[0200] In some respects, progression-free survival (PFS) is described as the length of time

during and after the treatment of a disease, such as cancer, that a subject lives with the disease but it does not get worse. In some aspects, objective response (OR) is described as a measurable response. In some aspects, objective response rate (ORR) is described as the proportion of patients who achieved CR or PR. In some aspects, overall survival (OS) is described as the length of time from either the date of diagnosis or the start of treatment for a disease, such as cancer, that subjects diagnosed with the disease are still alive. In some aspects, event-free survival (EFS) is described as the length of time after treatment for a cancer ends that the subject remains free of certain complications or events that the treatment was intended to prevent or delay. These events may include the return of the cancer or the onset of certain symptoms, such as bone pain from cancer that has spread to the bone, or death.

[0201] In some embodiments, the measure of duration of response (DOR) includes the time from documentation of tumor response to disease progression. In some embodiments, the parameter for assessing response can include durable response, e.g., response that persists after a period of time from initiation of therapy and/or long-lasting positive response to therapy. In some embodiments, durable response is indicated by the response rate at approximately 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18 or 24 months after initiation of therapy. In some embodiments, the response is durable for greater than 3 months or greater than 6 months. In some embodiments, durable response is response measured at month 3 after administration of therapy, e.g., a 3-month response. In some embodiments, durable response is response measured at month 6 after administration of therapy, e.g., a 6-month response.

[0202] In some aspects, the RECIST criteria is used to determine objective tumor response; in some aspects, in solid tumors. (Eisenhauer et al., European Journal of Cancer 45 (2009) 228-247.) In some aspects, the RECIST criteria is used to determine objective tumor response for target lesions. In some respects, a complete response as determined using RECIST criteria is described as the disappearance of all target lesions and any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm. In other aspects, a partial response as determined using RECIST criteria is described as at least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters. In other aspects, progressive disease (PD) is described as at least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm (in some aspects the appearance of one or more new lesions is also considered progression). In other aspects, stable disease (SD) is

described as neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

[0203] In some embodiments, the disease or condition is a tumor and a reduction in disease burden is a reduction in tumor size. In some embodiments, the disease burden reduction is indicated by a reduction in one or more factors, such as load or number of disease cells in the subject or fluid or organ or tissue thereof, the mass or volume of a tumor, or the degree or extent of metastases. In some embodiments, disease burden, e.g. tumor burden, can be assessed or monitored for the extent of morphological disease and/or minimal residual disease.

[0204] In some embodiments, the burden of a disease or condition in the subject is detected, assessed, or measured. Disease burden may be detected in some aspects by detecting the total number of disease or disease-associated cells, e.g., tumor cells, in the subject, or in an organ, tissue, or bodily fluid of the subject, such as blood or serum. In some embodiments, disease burden, e.g. tumor burden, is assessed by measuring the mass of a solid tumor and/or the number or extent of metastases. In some aspects, survival of the subject, survival within a certain time period, extent of survival, presence or duration of event-free or symptom-free survival, or relapse-free survival, is assessed. In some embodiments, any symptom of the disease or condition is assessed. In some embodiments, the measure of disease or condition burden is specified.

[0205] In some embodiments, disease burden can encompass a total number of cells of the disease in the subject or in an organ, tissue, or bodily fluid of the subject, such as the organ or tissue of the tumor or another location, e.g., which would indicate metastasis. For example, tumor cells may be detected and/or quantified in the blood or bone marrow in the context of certain hematological malignancies. Disease burden can include, in some embodiments, the mass of a tumor, the number or extent of metastases and/or the percentage of blast cells present in the bone marrow.

[0206] In some embodiments, a subject has leukemia. The extent of disease burden can be determined by assessment of residual leukemia in blood or bone marrow.

[0207] In some aspects, response rates in subjects, such as subjects with chronic lymphocytic leukemia (CLL), are based on the International Workshop on Chronic Lymphocytic Leukemia (IWCLL) response criteria (Hallek, et al., Blood 2008, Jun 15; 111(12): 5446-5456). In some aspects, these criteria are described as follows: complete remission (CR), which in some aspects requires the absence of peripheral blood clonal lymphocytes by immunophenotyping, absence of lymphadenopathy, absence of hepatomegaly or splenomegaly, absence of

constitutional symptoms and satisfactory blood counts; complete remission with incomplete marrow recovery (CRi), which in some aspects is described as CR above, but without normal blood counts; partial remission (PR), which in some aspects is described as $\geq 50\%$ fall in lymphocyte count, $\geq 50\%$ reduction in lymphadenopathy or $\geq 50\%$ reduction in liver or spleen, together with improvement in peripheral blood counts; progressive disease (PD), which in some aspects is described as $\geq 50\%$ rise in lymphocyte count to $> 5 \times 10^9/L$, $\geq 50\%$ increase in lymphadenopathy, $\geq 50\%$ increase in liver or spleen size, Richter's transformation, or new cytopenias due to CLL; and stable disease, which in some aspects is described as not meeting criteria for CR, CRi, PR or PD.

[0208] In some embodiments, the subjects exhibits a CR or OR if, within 1 month of the administration of the dose of cells, lymph nodes in the subject are less than at or about 20 mm in size, less than at or about 10 mm in size or less than at or about 10 mm in size.

[0209] In some embodiments, an index clone of the CLL is not detected in the bone marrow of the subject (or in the bone marrow of greater than 50%, 60%, 70%, 80%, 90% or more of the subjects treated according to the methods. In some embodiments, an index clone of the CLL is assessed by IgH deep sequencing. In some embodiments, the index clone is not detected at a time that is at or about or at least at or about 1, 2, 3, 4, 5, 6, 12, 18 or 24 months following the administration of the cells.

[0210] In some embodiments, a response outcome exists if there is a reduction in the percent of blasts in the bone marrow compared to the percent of blasts in the bone marrow prior to treatment with the therapeutic agent. In some embodiments, reduction of disease burden exists if there is a decrease or reduction of at least or at least about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more in the number or percentage of blasts in the bone marrow compared to the number or percent of blasts in the bone marrow prior to treatment.

[0211] In some embodiments, the subject exhibits a response if the subject does not exhibit morphologic disease (non-morphological disease) or does not exhibit substantial morphologic disease. In some embodiments, a subject exhibits morphologic disease if there are greater than or equal to 5% blasts in the bone marrow, for example, as detected by light microscopy. In some embodiments, a subject exhibits complete or clinical remission if there are less than 5% blasts in the bone marrow.

[0212] In some embodiments, a subject has leukemia. The extent of disease burden can be determined by assessment of residual leukemia in blood or bone marrow.

[0213] In some embodiments, a subject exhibits morphologic disease if there are greater

than or equal to 5% blasts in the bone marrow, for example, as detected by light microscopy, such as greater than or equal to 10% blasts in the bone marrow, greater than or equal to 20% blasts in the bone marrow, greater than or equal to 30% blasts in the bone marrow, greater than or equal to 40% blasts in the bone marrow or greater than or equal to 50% blasts in the bone marrow. In some embodiments, a subject exhibits complete or clinical remission if there are less than 5% blasts in the bone marrow.

[0214] In some embodiments, a subject exhibits reduced or decreased disease burden if they exhibited morphological disease prior to treatment and exhibit complete remission (e.g., fewer than 5% blasts in bone marrow) with or without molecular disease (e.g., minimum residual disease (MRD) that is molecularly detectable, e.g., as detected by flow cytometry or quantitative PCR) after treatment. In some embodiments, a subject exhibits reduced or decreased disease burden if they exhibited molecular disease prior to treatment and do not exhibit molecular disease after treatment.

[0215] In some embodiments, a subject may exhibit complete remission, but a small proportion of morphologically undetectable (by light microscopy techniques) residual leukemic cells are present. A subject is said to exhibit minimum residual disease (MRD) if the subject exhibits less than 5% blasts in the bone marrow and exhibits molecularly detectable cancer. In some embodiments, molecularly detectable cancer can be assessed using any of a variety of molecular techniques that permit sensitive detection of a small number of cells. In some aspects, such techniques include PCR assays, which can determine unique Ig/T-cell receptor gene rearrangements or fusion transcripts produced by chromosome translocations. In some embodiments, flow cytometry can be used to identify cancer cell based on leukemia-specific immunophenotypes. In some embodiments, molecular detection of cancer can detect as few as 1 leukemia or blast cell in 100,000 normal cells or 1 leukemia or blast cell in 10,000 normal cells. In some embodiments, a subject exhibits MRD that is molecularly detectable if at least or greater than 1 leukemia cell in 100,000 cells is detected, such as by PCR or flow cytometry. In some embodiments, the disease burden of a subject is molecularly undetectable or MRD⁻, such that, in some cases, no leukemia cells are able to be detected in the subject using PCR or flow cytometry techniques.

[0216] In some embodiments, an index clone of the leukemia, e.g. CLL, is not detected in the bone marrow of the subject (or in the bone marrow of greater than 50%, 60%, 70%, 80%, 90% or more of the subjects treated according to the methods. In some embodiments, an index clone of the leukemia, e.g. CLL, is assessed by IGH deep sequencing. In some embodiments,

the index clone is not detected at a time that is at or about or at least at or about 1, 2, 3, 4, 5, 6, 12, 18 or 24 months following the administration of the cells.

[0217] In some aspects MRD is detected by flow cytometry. Flow cytometry can be used to monitor bone marrow and peripheral blood samples for cancer cells. In particular aspects, flow cytometry is used to detect or monitor the presence of cancer cells in bone marrow. In some aspects, multiparameter immunological detection by flow cytometry is used to detect cancer cells (see for example, Coustan-Smith et al., (1998) *Lancet* 351:550-554). In some aspects, multiparameter immunological detection by mass cytometry is used to detect cancer cells. In some examples, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45 or 50 parameters can be used to detect cancer cells. The antigens used for detection are selected based on the cancer being detected (Foon and Todd (1986) *Blood* 68:1-31).

[0218] In some examples, bone marrow is harvested by bone marrow aspirates or bone marrow biopsies, and lymphocytes are isolated for analysis. Monoclonal and/or polyclonal antibodies conjugated to a fluorochrome (*e.g.*, fluorescein isothiocyanate (FITC), phycoerythrin, peridinin chlorophyll protein, or biotin) can be used to detect epitopes, such as terminal deoxynucleotidyl transferase (TdT), CD3, CD10, CD11c, CD13, CD14, CD33, CD19, CD20, CD21, CD22, CD23, CD34, CD45, CD56, CD79b, IgM, and/or KORSA3544, on isolated lymphocytes. Labeled cells can then be detected using flow cytometry, such as multiparameter flow cytometry, or mass cytometry, to detect multiple epitopes.

[0219] Lymphoid cells can be identified and gated based on a light-scatter dot plot and then secondarily gated to identify cell populations expressing the immunophenotypic features of interest. Exemplary epitopes are set forth in **Table 5** below. Other immunologic classification of leukemias and lymphomas are provided by Foon and Todd (*Blood* (1986) 68(1): 1-31). In some aspects, flow cytometric assessment of MRD can be achieved by quantifying live lymphocytes bearing one or more CLL immunophenotypes (*e.g.*, low forward/side scatter; CD3^{neg}; CD5⁺; CD14^{neg}; CD19⁺; CD23⁺; CD45⁺; CD56^{neg}).

Table 5. Exemplary Immunophenotype and Cytogenetics Characteristics

Disease	Immunophenotype	Cytogenetics
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Table 5. Exemplary Immunophenotype and Cytogenetics Characteristics		
Disease	Immunophenotype	Cytogenetics
Chronic Lymphocytic Leukemia (CLL)	Pan-B+; CD5+; CD23+; CD79b/CD22 weak; FMC7-; sIg weak	Trisomy12 del(13)(q14.3) del 11q22-q23 del 17p13 (p53) t(11;14)(q13;q32) BCL1/IgH rearrangement t(14;19)(q32;q13) IgH deletion (14q32) del(6q) +8q24 +3 +18 del 6q21
Small lymphocytic lymphoma (SLL)	Pan-B+; CD5+; CD23+; CD10-; sIgM+ faint	del(6)(q21-23)
Lymphoplasmacytic lymphoma	Pan-B+; CD5-; CD10-; cyIgM+	t(9;14)(p13;q32) PAX5/IgH
Follicle centre cell lymphoma	Pan-B+; CD10+/-; CD5-; sIg+	t(14;18)(q32;q21) / BCL2 Rearr
Diffuse large cell lymphoma	CD19+; CD22+; CD10-/+; SIg+	t(14;18) and p53 mutations t(3;V)(q27;V)/ BCL6 Rearr variants c-MYC Rearr
Burkitt's lymphoma	Pan-B+; TdT-; CD10+; CD5-; sIgM+	t(8;14)(q24;q32) or variants / c-MYC Rearr
Burkitt-like lymphoma	Pan-B+; TdT-; CD10-/+ CD5-; sIg+	t(8;14) or variants t(8;14)+ t(14;18)
Mantle cell lymphoma	Pan-B +; CD5+; CD23-; CD10-/+; sIgM+ bright	t(11;14)(q13;q32) / BCL1 Rearr
Marginal zone B-cell lymphoma (MZBCL)	pan-B+; CD5-/+; CD10-; CD23-; CD11c+/-; cyIg + (40% of the cells), sIgM+ bright; sIgD-	t(11;18)(q21;q21) / PI2 / MLT fusion: Extra-nodal low-grade MALT lymphoma; indolent disease t(1;14)(p21;q32): Extra-nodal MALT lymphoma del(7)(q22-31): Splenic MZBCL /+3q: Nodal, extra-nodal and splenic MZBCL

Table 5. Exemplary Immunophenotype and Cytogenetics Characteristics

Disease	Immunophenotype	Cytogenetics
+: positive in >90% of the cases +/-: positive in more than 50% of the cases -/+: positive in less than 50% of cases -: positive in <10% of the cases Pan-B markers: e.g., CD19, CD20, CD79a sIg: surface immunoglobulins cyIg: cytoplasmic immunoglobulins		

[0220] In some aspects, deep sequencing of the immunoglobulin heavy chain (IGH) locus of harvested B cells can be used to detect minimal residual disease (MRD). Clonal presence of a particular IgG rearrangement can provide a marker to detect the presence of B cell malignancies, such as CLL or NHL and/or residual presence of malignant cells thereof. In some aspects cells such as a population containing or suspected of containing B cells are harvested and isolated from blood. In some aspects, cells are harvested and isolated from bone marrow, e.g., from bone marrow aspirates or bone marrow biopsies and/or from other biological samples. In some aspects, polymerase chain reaction (PCR) amplification of the complementarity determining region 3 (CDR3) is achieved using primers to highly conserved sequences within the V and J regions of the gene locus, which may be used to identify clonal populations of cells for purposes of assessing minimal residual disease. Other methods for detecting clonal populations, such as single cell sequencing approaches, including those providing information regarding number of cells of a particular lineage and/or expressing a particular variable chain such as variable heavy chain or binding site thereof, such as a clonal population, may be used. In some aspects, the IGH DNA is amplified using a degenerate primers or primers recognizing regions of variable chains shared among different cell clones, such as those recognizing consensus V and degenerate consensus J region of the IGH sequence. An exemplary sequence of the V region is ACACGGCCTCGTGTATTACTGT (SEQ ID NO: 57). An exemplary degenerate consensus sequence of the J region is ACCTGAGGAGACGGTGACC (SEQ ID NO:58).

[0221] The PCR product or sequencing result in some aspects is specific to the rearranged allele and serves as a clonal marker for MRD detection. Following PCR amplification of the CDR3 region, PCR products can be sequenced to yield patient-specific oligonucleotides constructed as probes for allele-specific PCR for sensitive detection of MRD following treatment of B-cell malignancies with CAR-T cell therapy, e.g. CD19 CAR- T cell therapy. In examples where a PCR product is not generated using the consensus primers, V region family-specific primers for the framework region 1 can be used instead.

[0222] In some aspects, persistence of PCR-detectable tumor cells such as cells of the B cell malignancy such as the NHL or CLL, such as detectable IGH sequences corresponding to the malignant or clonal IGH sequences, after treatment is associated with increased risk of relapse. In some aspects, patients who are negative for malignant IGH sequences following treatment (in some aspects, even in the context of other criteria indicating progressive disease or only a partial response, such as persistence of enlarged lymph nodes or other criteria that may in some contexts be associated with disease or lack of complete response) may be deemed to have increased likelihood of PFS or to enter into CR or durable CR or prolonged survival, compared to patients with persistent malignant IGH sequences. In some embodiments, such prognostic and staging determinations are particularly relevant for treatments in which clearance of malignant cells is observed within a short period of time following administration of the therapy, e.g., in comparison to resolution of other clinical symptoms such as lymph node size or other staging criteria. For example, in some such aspects, absence of detectable IGH or minimal residual disease in a sample such as the bone marrow may be a preferred readout for response or likelihood of response or durability thereof, as compared to other available staging or prognostic approaches. In some aspects, results from MRD, e.g., IGH deep sequencing information, may inform further intervention or lack thereof. For example, the methods and other provided embodiments in some contexts provide that a subject deemed negative for malignant IGH may in some aspects be not further treated or not be further administered a dose of the therapy provided, or that the subject be administered a lower or reduced dose. Conversely, it may be provided or specified that a subject exhibiting MRD via IGH deep sequencing be further treated, e.g., with the therapy initially administered at a similar or higher dose or with a further treatment.

[0223] In some embodiments the response outcome is the absence of a CR or the presence of a complete response in which the subject achieves or exhibits minimal residual disease or molecular detectable disease status. In some embodiments, the response outcome is the presence of a CR with molecularly detectable disease or the presence of a CR without molecularly detectable disease. In some embodiments, subjects are assessed for disease burden using methods as described herein, such as methods that assess blasts in bone marrow or molecular disease by flow cytometry or qPCR methods.

[0224] In some embodiments of the methods provided herein, response is determined by complete remission or complete response (CR) and/or objective response (OR); and/or the subject exhibits CR, OR, lymph nodes of less than at or about 20 mm in size, within 1 month of

the administration of the dose of cells; and/or an index clone of the disease or condition, such as the CLL or NHL, is not detected in the bone marrow of the subject (or in the bone marrow of greater than 50% of subjects treated according to the methods), optionally as assessed by IgH deep sequencing, optionally at a time that is at or about or at least at or about 1, 2, 3, 4, 5, 6, 12, 18, or 24 months following the administration of the cell dose.

C. Determining Pharmacokinetics (PK) of Engineered Cells, e.g. Peak Cell Levels

[0225] In some embodiments, the method includes assessment of the exposure, number, concentration, persistence and proliferation of the T cells, *e.g.*, T cells administered for the T cell based therapy. In some embodiments, the method includes assessment of the exposure, number or level of engineered T cells, *e.g.*, T cells administered for the T cell based therapy, or subset thereof, such as CD3+ cells, CD4+ cells, CD8+ cells, CD3+ CAR+ cells, CD4+ CAR+ cells or CD8+ CAR+ cells. In some embodiments, the exposure, or prolonged expansion and/or persistence of the cells, and/or changes in cell phenotypes or functional activity of the cells, *e.g.*, cells administered for immunotherapy, *e.g.* T cell therapy, in the methods provided herein, can be measured by assessing the characteristics of the T cells *in vitro* or *ex vivo*. In some embodiments, such assays can be used to determine or confirm the function of the T cells used for the immunotherapy, *e.g.* T cell therapy, before or after administering the cell therapy provided herein.

[0226] In some aspects, the exposure, number, concentration, persistence and proliferation relate to pharmacokinetic parameters. In some cases, pharmacokinetics can be assessed by measuring such parameters as the maximum (peak) plasma concentration (C_{\max}), the peak time (*i.e.* when maximum plasma concentration (C_{\max}) occurs; T_{\max}), the minimum plasma concentration (*i.e.* the minimum plasma concentration between doses of a therapeutic agent, *e.g.*, CAR+ T cells; C_{\min}), the elimination half-life ($T_{1/2}$) and area under the curve (*i.e.* the area under the curve generated by plotting time versus plasma concentration of the therapeutic agent CAR+ T cells; AUC), following administration. The concentration of a particular therapeutic agent, *e.g.*, CAR+ T cells, in the plasma following administration can be measured using any method known in the art suitable for assessing concentrations of the therapeutic agents, *e.g.*, CAR+ T cells, in samples of blood, or any methods described herein. For example, nucleic acid-based methods, such as quantitative PCR (qPCR) or flow cytometry-based methods, or other assays, such as an immunoassay, ELISA, or chromatography/mass spectrometry-based assays can be used.

[0227] In some embodiments, the pharmacokinetics (PK) of administered cells, e.g., CAR⁺ T cell composition, are determined to assess the availability, e.g., bioavailability, of the administered cells. In some embodiments, the determined pharmacokinetic parameters of the administered cells include maximum (peak) plasma concentrations (C_{\max}), such as C_{\max} of CD3⁺ CAR⁺ cells, CD4⁺ CAR⁺ cells and or CD8⁺ CAR⁺ T cells; the time point at which C_{\max} is achieved (T_{\max}), such as the T_{\max} of CD3⁺ CAR⁺ cells, CD4⁺ CAR⁺ cells and or CD8⁺ CAR⁺ T cells, and or area under the curve (AUC), such as the AUC₀₋₂₈, of CD3⁺ CAR⁺ cells, CD4⁺ CAR⁺ cells and or CD8⁺ CAR⁺ T cells. In some embodiments, the pharmacokinetic parameter is peak CD3⁺ CAR⁺ T cell concentration (C_{\max} CD3⁺ CAR⁺ T cells), or CD8⁺ CAR⁺ T cell concentration (C_{\max} CD8⁺ CAR⁺ T cells). In some embodiments, the pharmacokinetic parameter is AUC₀₋₂₈, of CD3⁺ CAR⁺ T cells, (AUC₀₋₂₈ CD3⁺ CAR⁺ T cells), or AUC₀₋₂₈, of CD8⁺ CAR⁺ T cells, (AUC₀₋₂₈ CD8⁺ CAR⁺ T cells),

[0228] In some embodiments, “exposure” can refer to the body exposure of a therapeutic agent, e.g., CAR⁺ T cells in the plasma (blood or serum) after administration of the therapeutic agent over a certain period of time. In some embodiments exposure can be set forth as the area under the therapeutic agent concentration-time curve (AUC) as determined by pharmacokinetic analysis after administration of a dose of the therapeutic agent, e.g., CAR⁺ T cells. In some cases, the AUC is expressed in cells*days/ μ L, for cells administered in cell therapy, or in corresponding units thereof. In some embodiments, the AUC is measured as an average AUC in a patient population, such as a sample patient population, e.g., the average AUC from one or more patient(s). In some embodiments, systemic exposure refers to the area under the curve (AUC) within a certain period of time, e.g., from day 0 to day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 21, 28 days or more, or week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more, or month 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18, 24, 48 or more. In some embodiments, the AUC is measured as an AUC from day 0 to day 28 (AUC₀₋₂₈) after administration of the therapeutic agent, e.g., CAR⁺ T cells, including all measured data and data extrapolated from measured pharmacokinetic (PK) parameters, such as an average AUC from a patient population, such as a sample patient population. In some embodiments, to determine exposure over time, e.g., AUC for a certain period of time, such as AUC₀₋₂₈, a therapeutic agent concentration-time curve is generated, using multiple measurements or assessment of parameters, e.g., cell concentrations, over time, e.g., measurements taken every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 21 or 28 days or more.

[0229] In some embodiments, the presence and/or amount of cells expressing the

recombinant receptor (*e.g.*, CAR-expressing cells administered for T cell based therapy) in the subject following the administration of the T cells and before, during and/or after the administration of the therapy is detected. In some aspects, nucleic acid-based methods, such as quantitative PCR (qPCR), is used to assess the quantity of cells expressing the recombinant receptor (*e.g.*, CAR-expressing cells administered for T cell based therapy) in the blood or serum or organ or tissue sample (*e.g.*, disease site, *e.g.*, tumor sample) of the subject. In some aspects, persistence is quantified as copies of DNA or plasmid encoding the receptor, *e.g.*, CAR, per microgram of DNA, or as the number of receptor-expressing, *e.g.*, CAR-expressing, cells per microliter of the sample, *e.g.*, of blood or serum, or per total number of peripheral blood mononuclear cells (PBMCs) or white blood cells or T cells per microliter of the sample. 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 receptor, 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 embodiments, the primers can be specific for regulatory elements, such as the woodchuck hepatitis virus post-transcriptional regulatory element (WPRE). In some examples, the presence and/or amount of cells expressing the recombinant receptor is expressed as copies of the nucleic acid sequence (*e.g.*, transgene sequence) encoding the CAR or a nucleic acid sequence operably connected to the CAR-encoding sequences, per mass of DNA (*e.g.*, copies/ μ g of DNA); AUC of the curve of copies/ μ g of DNA over time, maximum or peak copies/ μ g of DNA following treatment, or copies/ μ g of DNA at day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21, or week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 or more post-treatment or initiation thereof.

[0230] In some embodiments, the cells are detected in the subject at or at least at 4, 14, 15, 27, or 28 days following the administration of the T cells, *e.g.*, CAR-expressing T cells. In some aspects, the cells are detected at or at least at 2, 4, or 6 weeks following, or 3, 6, or 12, 18, or 24, or 30 or 36 months, or 1, 2, 3, 4, 5, or more years, following the administration of the T cells, *e.g.*, CAR-expressing T cells.

[0231] In some embodiments, the peak levels and/or AUC are assessed and/or the sample is obtained from the subject at a time that is at least 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days or 21 days after initiation of administration of the genetically engineered cells. In some embodiments the peak levels and/or AUC are assessed and/or the sample is obtained from the subject at a time that is between or

between about 11 to 22 days, 12 to 18 days or 14 to 16 days, each inclusive, after initiation of administration of the genetically engineered cells.

[0232] The exposure, *e.g.*, number or concentration of cells, *e.g.* T cells administered for T cell therapy, indicative of expansion and/or persistence, may be stated in terms of maximum numbers or concentration of the cells to which the subject is exposed, duration of detectable cells or cells above a certain number or percentage, area under the curve (AUC) for number or concentration of cells over time, and/or combinations thereof and indicators thereof. Such outcomes may be assessed using known methods, such as qPCR to detect copy number of nucleic acid encoding the recombinant receptor compared to total amount of nucleic acid or DNA in the particular sample, *e.g.*, blood, serum, plasma or tissue, such as a tumor sample, and/or flow cytometric assays detecting cells expressing the receptor generally using antibodies specific for the receptors. Cell-based assays may also be used to detect the number or percentage or concentration of functional cells, such as cells capable of binding to and/or neutralizing and/or inducing responses, *e.g.*, cytotoxic responses, against cells of the disease or condition or expressing the antigen recognized by the receptor.

[0233] In some aspects, increased exposure of the subject to the cells includes increased expansion of the cells. In some embodiments, the receptor expressing cells, *e.g.* CAR-expressing cells, expand in the subject following administration of the T cells, *e.g.*, CAR-expressing T cells.

[0234] In some embodiments, cells expressing the receptor are detectable in the serum, plasma, blood or tissue, *e.g.*, tumor sample, of the subject, *e.g.*, by a specified method, such as qPCR or flow cytometry-based detection method, at least 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60 or more days following administration of the T cells, *e.g.*, CAR-expressing T cells, for at least at or about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 or more weeks following the administration of the T cells, *e.g.*, CAR-expressing T cells.

[0235] In some aspects, at least at or about 1×10^2 , at least at or about 1×10^3 , at least at or about 1×10^4 , at least at or about 1×10^5 , or at least at or about 1×10^6 or at least at or about 5×10^6 or at least at or about 1×10^7 or at least at or about 5×10^7 or at least at or about 1×10^8 recombinant receptor-expressing, *e.g.*, CAR-expressing cells, and/or at least 10, 25, 50, 100, 200, 300, 400, or 500, or 1000 receptor-expressing cells per microliter, *e.g.*, at least 10 per microliter, are detectable or are present in the subject or fluid, plasma, serum, tissue, or compartment thereof, such as in the blood, *e.g.*, peripheral blood, or disease site, *e.g.*, tumor,

thereof. In some embodiments, such a number or concentration of cells is detectable in the subject for at least at or about 20 days, at least at or about 40 days, or at least at or about 60 days, or at least at or about 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months, or at least 2 or 3 years, following administration of the T cells, *e.g.*, CAR-expressing T cells. Such cell numbers may be as detected by flow cytometry-based or quantitative PCR-based methods and extrapolation to total cell numbers using known methods. *See, e.g.*, Brentjens *et al.*, *Sci Transl Med.* 2013 5(177), Park *et al.*, *Molecular Therapy* 15(4):825–833 (2007), Savoldo *et al.*, *JCI* 121(5):1822–1826 (2011), Davila *et al.*, (2013) *PLoS ONE* 8(4):e61338, Davila *et al.*, *Oncoimmunology* 1(9):1577–1583 (2012), Lamers, *Blood* 2011 117:72–82, Jensen *et al.*, *Biol Blood Marrow Transplant* 2010 September; 16(9): 1245–1256, Brentjens *et al.*, *Blood* 2011 118(18):4817–4828.

[0236] In some aspects, the copy number of nucleic acid encoding the recombinant receptor, *e.g.*, vector copy number, per 100 cells, for example in the peripheral blood or bone marrow or other compartment, as measured by immunohistochemistry, PCR, and/or flow cytometry, is at least 0.01, at least 0.1, at least 1, or at least 10, at about 1 week, about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, or at least about 6 weeks, or at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months or at least 2 or 3 years following administration of the cells, *e.g.*, CAR-expressing T cells. In some embodiments, the copy number of the vector expressing the receptor, *e.g.* CAR, per microgram of genomic DNA is at least 100, at least 1000, at least 5000, or at least 10,000, or at least 15,000 or at least 20,000 at a time about 1 week, about 2 weeks, about 3 weeks, or at least about 4 weeks following administration of the T cells, *e.g.*, CAR-expressing T cells or at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months or at least 2 or 3 years following such administration.

[0237] In some aspects, the receptor, *e.g.* CAR, expressed by the cells, is detectable by quantitative PCR (qPCR) or by flow cytometry in the subject, plasma, serum, blood, tissue and/or disease site thereof, *e.g.*, tumor site, at a time that is at least at or about 3 months, at least at or about 6 months, at least at or about 12 months, at least at or about 1 year, at least at or about 2 years, at least at or about 3 years, or more than 3 years, following the administration of the cells, *e.g.*, following the initiation of the administration of the T cells. In some embodiments, the area under the curve (AUC) for concentration of receptor- (*e.g.*, CAR-) expressing cells in a fluid, plasma, serum, blood, tissue, organ and/or disease site, *e.g.* tumor site, of the subject over time following the administration of the T cells, *e.g.*, CAR-expressing T cells, is measured.

[0238] Also provided are methods of assessing likelihood of a response or a durable response. In some embodiments, the methods involve detecting, in a biological sample from a subject, peak levels of one or more inflammatory marker and/or peak levels of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR), wherein the subject has been previously administered a dose of the genetically engineered cells for treating a disease or condition. In some embodiments, the methods involve comparing, individually, the peak levels to a threshold value, thereby determining a likelihood that a subject will achieve a durable response to the administration of the genetically engineered cells.

[0239] In some embodiments, the subject is likely to achieve a response or a durable response if the peak levels of the one or more inflammatory marker is below a threshold value and the subject is not likely to achieve a durable response if the peak levels of the one or more inflammatory marker is above a threshold value. In some embodiments, the subject is likely to achieve a durable response if the peak level of the genetically engineered cells is within a therapeutic range between a lower threshold value and an upper threshold value and the subject is not likely to achieve a durable response if the peak level of the genetically engineered cells is below the lower threshold value or is above the upper threshold value.

III. METHOD OF TREATMENT

[0240] In some embodiments, provided are methods of treatment. In some embodiments, the methods include administering an immunotherapy and/or a cell therapy. In some embodiments, the methods involve administration of genetically engineered cells, e.g., cells engineered to express a recombinant receptor such as a chimeric antigen receptor (CAR). In some aspects, also provided are methods of administering any of the engineered cells or compositions containing engineered cells to a subject, such as a subject that has a disease or disorder. In some aspects, also provided are uses of the engineered cells or compositions containing engineered cells for treatment of a disease or disorder. In some aspects, also provided are uses of the engineered cells or compositions containing engineered cells for the manufacture of a medicament for the treatment of a disease or disorder. In some aspects, also provided are the engineered cells or compositions containing engineered cells, for use in treatment of a disease or disorder, or for administration to a subject having a disease or disorder.

[0241] The engineered cells expressing a recombinant receptor, such as a chimeric antigen receptor (CAR), or compositions comprising the same are useful in a variety of therapeutic, diagnostic and prophylactic indications. For example, the engineered cells or compositions

comprising the engineered cells are useful in treating a variety of diseases and disorders in a subject. Such methods and uses include therapeutic methods and uses, for example, involving administration of the engineered cells, or compositions containing the same, to a subject having a disease, condition, or disorder, such as a tumor or cancer. In some embodiments, the engineered cells or compositions comprising the same are administered in an effective amount to effect treatment of the disease or disorder. Uses include uses of the engineered cells or compositions in such methods and treatments, and in the preparation of a medicament in order to carry out such therapeutic methods. In some embodiments, the methods are carried out by administering the engineered cells, or compositions comprising the same, to the subject having or suspected of having the disease or condition. In some embodiments, the methods thereby treat the disease or condition or disorder in the subject.

[0242] In some embodiments, the methods include administering a dose of cells, e.g., CAR+ expressing cells, to a subject such that the cells are within a target therapeutic range or window. In some embodiments, whether the cells in the subject is within a target therapeutic range or window can be determined or assessed by monitoring parameters, e.g., pharmacokinetic parameters, such as peak cell concentration (C_{\max}). In some aspects, the provided methods also include a method of determining a dose of a subject, or a method of dosing a subject, based on an assessment of the parameters, e.g., pharmacokinetic parameters, such as peak cell concentration (C_{\max}), patient attributes and/or biomarkers.

[0243] In some embodiments, a dose of cells expressing a recombinant receptor are administered to a subject to treat or prevent diseases, conditions, and disorders, including cancers. In some embodiments, the cells, populations, and compositions are administered to a subject or patient having the particular disease or condition to be treated, e.g., via adoptive cell therapy, such as adoptive T cell therapy. In some embodiments, cells and compositions, such as engineered compositions and end-of-production compositions following incubation and/or other processing steps, are administered to a subject, such as a subject having or at risk for the disease or condition. In some aspects, the methods thereby treat, e.g., ameliorate one or more symptom of, the disease or condition, such as by lessening tumor burden in a cancer expressing an antigen recognized by an engineered T cell.

[0244] Methods for administration of cells for adoptive cell therapy are known and may be used in connection with the provided methods and compositions. For example, adoptive T cell therapy methods are described, e.g., in US Patent Application Publication No. 2003/0170238 to Gruenberg et al; US Patent No. 4,690,915 to Rosenberg; Rosenberg (2011) Nat Rev Clin Oncol.

8(10):577-85). See, e.g., Themeli et al. (2013) Nat Biotechnol. 31(10): 928-933; Tsukahara et al. (2013) Biochem Biophys Res Commun 438(1): 84-9; Davila et al. (2013) PLoS ONE 8(4): e61338.

[0245] The disease or condition that is treated can be any in which expression of an antigen is associated with and/or involved in the etiology of a disease condition or disorder, e.g. causes, exacerbates or otherwise is involved in such disease, condition, or disorder. Exemplary diseases and conditions can include diseases or conditions associated with malignancy or transformation of cells (e.g. cancer), autoimmune or inflammatory disease, or an infectious disease, e.g. caused by a bacterial, viral or other pathogen. Exemplary antigens, which include antigens associated with various diseases and conditions that can be treated, are described above. In particular embodiments, the chimeric antigen receptor or transgenic TCR specifically binds to an antigen associated with the disease or condition.

[0246] Among the diseases, conditions, and disorders are tumors, including solid tumors, hematologic malignancies, and melanomas, and including localized and metastatic tumors, infectious diseases, such as infection with a virus or other pathogen, e.g., HIV, HCV, HBV, CMV, and parasitic disease, and autoimmune and inflammatory diseases. In some embodiments, the disease or condition is a tumor, cancer, malignancy, neoplasm, or other proliferative disease or disorder. Such diseases include but are not limited to leukemia, lymphoma, e.g., chronic lymphocytic leukemia (CLL), acute-lymphoblastic leukemia (ALL), non-Hodgkin's lymphoma, acute myeloid leukemia, multiple myeloma, refractory follicular lymphoma, mantle cell lymphoma, indolent B cell lymphoma, B cell malignancies, cancers of the colon, lung, liver, breast, prostate, ovarian, skin, melanoma, bone, and brain cancer, ovarian cancer, epithelial cancers, renal cell carcinoma, pancreatic adenocarcinoma, Hodgkin lymphoma, cervical carcinoma, colorectal cancer, glioblastoma, neuroblastoma, Ewing sarcoma, medulloblastoma, osteosarcoma, synovial sarcoma, and/or mesothelioma. In some embodiments, the subject has acute-lymphoblastic leukemia (ALL). In some embodiments, the subject has a B-cell malignancy. In some embodiments, the subject has a non-Hodgkin's lymphoma.

[0247] In some embodiments, the disease or condition is an infectious disease or condition, such as, but not limited to, viral, retroviral, bacterial, and protozoal infections, immunodeficiency, Cytomegalovirus (CMV), Epstein-Barr virus (EBV), adenovirus, BK polyomavirus. In some embodiments, the disease or condition is an autoimmune or inflammatory disease or condition, such as arthritis, e.g., rheumatoid arthritis (RA), Type I

diabetes, systemic lupus erythematosus (SLE), inflammatory bowel disease, psoriasis, scleroderma, autoimmune thyroid disease, Grave's disease, Crohn's disease, multiple sclerosis, asthma, and/or a disease or condition associated with transplant.

[0248] In some embodiments, the antigen associated with the disease or disorder is selected from the group consisting of $\alpha\text{v}\beta\text{6}$ integrin (avb6 integrin), B cell maturation antigen (BCMA), B7-H3, B7-H6, carbonic anhydrase 9 (CA9, also known as CAIX or G250), a cancer-testis antigen, cancer/testis antigen 1B (CTAG, also known as NY-ESO-1 and LAGE-2), carcinoembryonic antigen (CEA), a cyclin, cyclin A2, C-C Motif Chemokine Ligand 1 (CCL-1), CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD133, CD138, CD171, chondroitin sulfate proteoglycan 4 (CSPG4), epidermal growth factor protein (EGFR), type III epidermal growth factor receptor mutation (EGFR vIII), epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), ephrinB2, ephrine receptor A2 (EPHa2), estrogen receptor, Fc receptor like 5 (FCRL5; also known as Fc receptor homolog 5 or FCRH5), fetal acetylcholine receptor (fetal AchR), a folate binding protein (FBP), folate receptor alpha, ganglioside GD2, O-acetylated GD2 (OGD2), ganglioside GD3, glycoprotein 100 (gp100), glypican-3 (GPC3), G Protein Coupled Receptor 5D (GPRC5D), Her2/neu (receptor tyrosine kinase erb-B2), Her3 (erb-B3), Her4 (erb-B4), erbB dimers, Human high molecular weight-melanoma-associated antigen (HMW-MAA), hepatitis B surface antigen, Human leukocyte antigen A1 (HLA-A1), Human leukocyte antigen A2 (HLA-A2), IL-22 receptor alpha (IL-22R α), IL-13 receptor alpha 2 (IL-13R α 2), kinase insert domain receptor (kdr), kappa light chain, L1 cell adhesion molecule (L1-CAM), CE7 epitope of L1-CAM, Leucine Rich Repeat Containing 8 Family Member A (LRRC8A), Lewis Y, Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, MAGE-A10, mesothelin (MSLN), c-Met, murine cytomegalovirus (CMV), mucin 1 (MUC1), MUC16, natural killer group 2 member D (NKG2D) ligands, melan A (MART-1), neural cell adhesion molecule (NCAM), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME), progesterone receptor, a prostate specific antigen, prostate stem cell antigen (PSCA), prostate specific membrane antigen (PSMA), Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1), survivin, Trophoblast glycoprotein (TPBG also known as 5T4), tumor-associated glycoprotein 72 (TAG72), Tyrosinase related protein 1 (TRP1, also known as TYRP1 or gp75), Tyrosinase related protein 2 (TRP2, also known as dopachrome tautomerase, dopachrome delta-isomerase or DCT), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor receptor 2 (VEGFR2), Wilms Tumor 1 (WT-1), a pathogen-specific or pathogen-expressed

antigen, or an antigen associated with a universal tag, and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV or other pathogens.

[0249] Antigens targeted by the receptors in some embodiments include antigens associated with a B cell malignancy, such as any of a number of known B cell marker. In some embodiments, the antigen is or includes CD20, CD19, CD22, ROR1, CD45, CD21, CD5, CD33, Igkappa, Iglambda, CD79a, CD79b or CD30. In some embodiments, the antigen is or includes a pathogen-specific or pathogen-expressed antigen. In some embodiments, the antigen is a viral antigen (such as a viral antigen from HIV, HCV, HBV, etc.), bacterial antigens, and/or parasitic antigens.

[0250] In some embodiments, the antigen associated with the disease or disorder is selected from the group consisting of orphan tyrosine kinase receptor ROR1, Her2, L1-CAM, CD19, CD20, CD22, mesothelin, CEA, and hepatitis B surface antigen, anti-folate receptor, CD23, CD24, CD30, CD33, CD38, CD44, EGFR, EGP-2, EGP-4, 0EPHa2, ErbB2, 3, or 4, FBP, fetal acetylcholine e receptor, GD2, GD3, HMW-MAA, IL-22R-alpha, IL-13R-alpha2, kdr, kappa light chain, Lewis Y, L1-cell adhesion molecule, MAGE-A1, mesothelin, MUC1, MUC16, PSCA, NKG2D Ligands, NY-ESO-1, MART-1, gp100, oncofetal antigen, ROR1, TAG72, VEGF-R2, carcinoembryonic antigen (CEA), prostate specific antigen, PSMA, Her2/neu, estrogen receptor, progesterone receptor, ephrinB2, CD123, CS-1, c-Met, GD-2, and MAGE A3, CE7, Wilms Tumor 1 (WT-1), a cyclin, such as cyclin A1 (CCNA1), and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV or other pathogens.

[0251] In some embodiments, the cell therapy, e.g., adoptive T cell therapy, is carried out by autologous transfer, in which the cells are isolated and/or otherwise prepared from the subject who is to receive the cell therapy, or from a sample derived from such a subject. Thus, in some aspects, the cells are derived from a subject, e.g., patient, in need of a treatment and the cells, following isolation and processing are administered to the same subject.

[0252] In some embodiments, the cell therapy, e.g., adoptive T cell therapy, is carried out by allogeneic transfer, in which the cells are isolated and/or otherwise prepared from a subject other than a subject who is to receive or who ultimately receives the cell therapy, e.g., a first subject. In such embodiments, the cells then are administered to a different subject, e.g., a second subject, of the same species. In some embodiments, the first and second subjects are genetically identical. In some embodiments, the first and second subjects are genetically similar. In some embodiments, the second subject expresses the same HLA class or supertype as the first subject.

[0253] The 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, subconjunctival injection, sub-Tenon's injection, retrobulbar injection, peribulbar injection, or posterior juxtасleral 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. In some embodiments, it is administered by multiple bolus administrations of the cells, for example, over a period of no more than 3 days, or by continuous infusion administration of the cells.

[0254] For the prevention or treatment of disease, the appropriate dosage may depend on the type of disease to be treated, the type of cells or recombinant receptors, the severity and course of the disease, whether the cells are administered for preventive or therapeutic purposes, previous therapy, the subject's clinical history and response to the cells, and the discretion of the attending physician. The compositions and cells are in some embodiments suitably administered to the subject at one time or over a series of treatments.

[0255] In some embodiments, the cells are administered as part of a combination treatment, such as simultaneously with or sequentially with, in any order, another therapeutic intervention, such as an antibody or engineered cell or receptor or agent, such as a cytotoxic or therapeutic agent. The cells in some embodiments are co-administered with one or more additional therapeutic agents or in connection with another therapeutic intervention, either simultaneously or sequentially in any order. In some contexts, the cells are co-administered with another therapy sufficiently close in time such that the cell populations enhance the effect of one or more additional therapeutic agents, or vice versa. In some embodiments, the cells are administered prior to the one or more additional therapeutic agents. In some embodiments, the cells are administered after the one or more additional therapeutic agents. In some embodiments, the one or more additional agents include a cytokine, such as IL-2, for example, to enhance persistence. In some embodiments, the methods comprise administration of a chemotherapeutic agent.

[0256] In some embodiments, the methods comprise administration of a chemotherapeutic agent, e.g., a conditioning chemotherapeutic agent, for example, to reduce tumor burden prior to the administration.

[0257] Preconditioning subjects with immunodepleting (e.g., lymphodepleting) therapies in

some aspects can improve the effects of adoptive cell therapy (ACT).

[0258] 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 initiation of the cell therapy. For example, the subject may be administered a preconditioning agent at least 2 days prior, such as at least 3, 4, 5, 6, or 7 days prior, to the initiation of the cell therapy. In some embodiments, the subject is administered a preconditioning agent no more than 7 days prior, such as no more than 6, 5, 4, 3, or 2 days prior, to the initiation of the cell therapy.

[0259] In some embodiments, the subject is preconditioned with cyclophosphamide at a dose between at or about 20 mg/kg and at or about 100 mg/kg, such as between at or about 40 mg/kg and at or about 80 mg/kg. In some aspects, the subject is preconditioned with or with at or 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.

[0260] In some embodiments, where the lymphodepleting agent comprises fludarabine, the subject is administered fludarabine at a dose between or between about 1 mg/m² and 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 30 mg/m², or at or about 24 mg/m² and at or about 26 mg/m². In some instances, the subject is administered 25 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.

[0261] 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 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.

[0262] Following administration of the cells, the biological activity of the engineered cell populations in some embodiments is measured, e.g., by any of a number of known methods.

Parameters to assess include specific binding of an engineered or natural T cell or other immune cell to antigen, in vivo, e.g., by imaging, or ex vivo, e.g., by ELISA or flow cytometry. In certain embodiments, the ability of the engineered cells to destroy target cells can be measured using any suitable method known in the art, such as cytotoxicity assays described in, for example, Kochenderfer et al., *J. Immunotherapy*, 32(7): 689-702 (2009), and Herman et al. *J. Immunological Methods*, 285(1): 25-40 (2004). In certain embodiments, the biological activity of the cells is measured by assaying expression and/or secretion of one or more cytokines, such as CD107a, IFN γ , IL-2, and TNF. In some aspects the biological activity is measured by assessing clinical outcome, such as reduction in tumor burden or load.

[0263] In certain embodiments, the engineered cells are further modified in any number of ways, such that their therapeutic or prophylactic efficacy is increased. For example, the engineered CAR or TCR expressed by the population can be conjugated either directly or indirectly through a linker to a targeting moiety. The practice of conjugating compounds, e.g., the CAR or TCR, to targeting moieties is known in the art. See, for instance, Wadwa et al., *J. Drug Targeting* 3: 111 (1995), and U.S. Patent 5,087,616.

A. Dosing

[0264] In some embodiments, the subject is administered a dose that achieves or is likely to achieve the therapeutic range and/or window of CAR+ T cells. The method, in some embodiments, involves administering a dose of cells in an amount that is or is likely to achieve a peak CAR+ cell number in the blood within a range in which the peak CAR+ cell numbers have less than a certain estimated probability of causing toxicity. The method, in some embodiments, involves administering a dose of cells in an amount that is or is likely to achieve a peak CAR+ cell number in the blood within a range in which the peak CAR+ cell numbers have more than a certain estimated probability of causing response or durable response. In some cases, the amount of cells is an amount effective to treat the disease or condition, such as therapeutically effective or prophylactically effective amount. In some cases, the estimated probability of achieving a response is greater than at or about 65%, greater than at or about 70%, greater than at or about 75%, greater than at or about 80%, greater than at or about 85%, greater than at or about 90%, greater than at or about 95% or more. In some cases, the estimated probability of causing toxicity is 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% or less than at or about 5% on the toxicity probability curve. In some embodiments, the dose of

cells is both above the desired estimated probability of achieving a response and below the desired estimated probability of causing toxicity.

[0265] In some embodiments, the amount or dose of cells that is administered is based upon assessment of parameters, e.g., pharmacokinetic parameters, and estimated probability of response and/or toxicity, e.g., as described in Section II.

[0266] In some embodiments, the methods involve administering a sufficient number or dose of cells to achieve a peak CAR+ cell concentration in the subject that is within a determined target therapeutic range or window. In some embodiments, the methods involve administering a sufficient number or dose of cells to achieve a peak CAR+ cell concentration in a majority of subjects so treated by the method, or greater than or greater than at or about 50%, 60%, 70%, 75%, 80%, 85%, 90% or 95% or more, such as greater than 75% of the subjects so treated by the method, is within a determined target therapeutic range or window.

[0267] In some embodiments, the therapeutic window or range is determined as described above, e.g., in Section II. In some embodiments, the therapeutic range is based upon the range of peak CD3+ CAR+ T cells, or a CD8+CAR+ T cell subset thereof, in the blood among one or more subjects previously treated with the genetically engineered cells that is associated with an estimated probability of response of greater than or greater than about 65%, 70%, 75%, 80%, 85%, 90%, 95% or more, and an estimated probability of a toxicity of less than or less than about 30%, 25%, 20%, 15%, 10%, 5% or less.

[0268] In some embodiments, the therapeutic window or range is determined based on specific range of numbers and/or concentrations of cells, e.g., CD3+, CD4+ or CD8+ T cells. In some embodiments, an exemplary peak CD3+CAR+ T cell concentration in the blood that can achieve a therapeutic window, is or includes between at or approximately 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 20, 30, 40, 50 CD3+ CAR+ T cells per microliter in the blood and at or approximately 200, 300, 400, 500, 600, 700 or 750 CD3+ CAR+ T cells per microliter in the blood. In some embodiments, an exemplary peak CD8+CAR+ T cell concentration in the blood that can achieve a therapeutic window, is or includes between at or approximately 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 20, 30, 40, 50 CD8+ CAR+ T cells per microliter in the blood and at or approximately 200, 300, 400, 500, 600, 700 or 750 CD8+ CAR+ T cells per microliter in the blood.

[0269] In some embodiments, the target therapeutic range or window is a peak CD3+CAR+ T cell concentration of between at or about 10 cells per microliter and at or about 500 cells per microliter in the blood following administration. In some embodiments, the target therapeutic

range or window is a peak CD8+CAR+ T cell concentration of between at or about 2 cells per microliter and at or about 200 cells per microliter in the blood following administration.

[0270] In some embodiments, provided are methods of dosing a subject that involves administering, to a subject having a disease or condition, a dose of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR), wherein the dose comprises a number of the genetically engineered cells that is sufficient to achieve peak CAR+ cells in the blood within a determined therapeutic range in the subject, or in a majority of subjects so treated by the method or in greater than 75% of the subjects so treated by the method, wherein the therapeutic range is: (i) based upon the range of peak CD3+ CAR+ T cells, or a CD8+CAR+ T cell subset thereof, in the blood among one or more subjects previously treated with the genetically engineered cells that is associated with an estimated probability of response of greater than or greater than about 65% and an estimated probability of a toxicity of less than or less than about 30%; or (ii) peak CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between at or about 10 cells per microliter and at or about 500 cells per microliter; or (iii) peak CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between at or about 2 cells per microliter and at or about 200 cells per microliter.

[0271] In some embodiments, provided are methods of dosing a subject that involves (a) administering, to a subject having a disease or condition, a sub-optimal dose of genetically engineered cells comprising T cells engineered with a chimeric antigen receptor (CAR), wherein the dose comprises a number of the genetically engineered cells that is insufficient to achieve peak CAR+ cells in the blood within a determined therapeutic range in the subject, or in a majority of subjects so treated by the method or in greater than 75% of the subjects so treated by the method, ; and (b) subsequent to administering the genetically engineered cells, administering an agent to enhance CAR+ cell expansion or proliferation in the subject to achieve peak CAR+ T cells in the blood within the therapeutic range, wherein the therapeutic range is: (i) based upon the range of peak CD3+ CAR+ T cells, or a CD8+CAR+ T cell subset thereof, in the blood among one or more subjects previously treated with the genetically engineered cells that is associated with an estimated probability of response of greater than or greater than about 65% and an estimated probability of a toxicity of less than or about 30%; or (ii) peak CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between at or about 10 cells per microliter and at or about 500 cells per microliter; or (iii) peak CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells,

that is between at or about 2 cells per microliter and at or about 200 cells per microliter. In some embodiments, the subject is administered a dose that can achieve the target therapeutic range or window. In some embodiments, the dose is less than or less than about 1×10^7 CAR-expressing cells, less than or less than about 5×10^6 CAR-expressing cells, less than or less than about 2.5×10^6 CAR-expressing cells, less than or less than about 1×10^6 CAR-expressing cells, less than or less than about 5×10^5 CAR-expressing cells, less than or less than about 2.5×10^5 CAR-expressing cells, less than or less than about 1×10^5 CAR-expressing cells.

[0272] 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, provided in multiple individual compositions or infusions, over a specified period of time, which is 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.

[0273] Thus, in some aspects, the cells of the dose are administered in a single pharmaceutical composition. In some embodiments, the cells of the dose are administered in a plurality of compositions, collectively containing the cells of the first dose.

[0274] The term “split dose” refers to a dose that is split so that it is administered over more than one day. This type of dosing is encompassed by the present methods and is considered to be a single dose.

[0275] Thus, the dose in some aspects may be administered as a split dose. For example, in some embodiments, the dose may be administered to the subject over 2 days or over 3 days. Exemplary methods for split dosing include administering 25% of the dose on the first day and administering the remaining 75% of the dose on the second day. In other embodiments, 33% of the first dose may be administered on the first day and the remaining 67% administered on the second day. In some aspects, 10% of the dose is administered on the first day, 30% of the dose is administered on the second day, and 60% of the dose is administered on the third day. In some embodiments, the split dose is not spread over more than 3 days.

[0276] In some embodiments, cells of the dose may be administered by administration of a plurality of compositions or solutions, such as a first and a second, optionally more, each

containing some cells of the dose. In some aspects, the plurality of compositions, each containing a different population and/or sub-types of cells, are administered separately or independently, optionally within a certain period of time. For example, the populations or sub-types of cells can include CD8⁺ and CD4⁺ T cells, respectively, and/or CD8⁺- and CD4⁺-enriched populations, respectively, e.g., CD4⁺ and/or CD8⁺ T cells each individually including cells genetically engineered to express the recombinant receptor. In some embodiments, the administration of the dose comprises administration of a first composition comprising a dose of CD8⁺ T cells or a dose of CD4⁺ T cells and administration of a second composition comprising the other of the dose of CD4⁺ T cells and the CD8⁺ T cells.

[0277] In some embodiments, the administration of the composition or dose, e.g., administration of the plurality of cell compositions, involves administration of the cell compositions separately. In some aspects, the separate administrations are carried out simultaneously, or sequentially, in any order. In some embodiments, the dose comprises a first composition and a second composition, and the first composition and second composition are administered 0 to 12 hours apart, 0 to 6 hours apart or 0 to 2 hours apart. In some embodiments, the initiation of administration of the first composition and the initiation of administration of the second composition are carried out no more than 2 hours, no more than 1 hour, or no more than 30 minutes apart, no more than 15 minutes, no more than 10 minutes or no more than 5 minutes apart. In some embodiments, the initiation and/or completion of administration of the first composition and the completion and/or initiation of administration of the second composition are carried out no more than 2 hours, no more than 1 hour, or no more than 30 minutes apart, no more than 15 minutes, no more than 10 minutes or no more than 5 minutes apart.

[0278] In some composition, the first composition, e.g., first composition of the dose, comprises CD4⁺ T cells. In some composition, the first composition, e.g., first composition of the dose, comprises CD8⁺ T cells. In some embodiments, the first composition is administered prior to the second composition.

[0279] In some embodiments, the dose or composition of cells includes a defined or target ratio of CD4⁺ cells expressing a recombinant receptor to CD8⁺ cells expressing a recombinant receptor and/or of CD4⁺ cells to CD8⁺ cells, which ratio optionally is approximately 1:1 or is between at or approximately 1:3 and at or approximately 3:1, such as approximately 1:1. In some aspects, the administration of a composition or dose with the target or desired ratio of different cell populations (such as CD4⁺:CD8⁺ ratio or CAR+CD4⁺:CAR+CD8⁺ ratio, e.g., 1:1) involves the administration of a cell composition containing one of the populations and then

administration of a separate cell composition comprising the other of the populations, where the administration is at or approximately at the target or desired ratio.

[0280] In some embodiments, one or more consecutive or subsequent dose of cells can be administered to the subject. In some embodiments, the consecutive or subsequent dose of cells is administered greater than or greater than about 7 days, 14 days, 21 days, 28 days or 35 days after initiation of administration of the first dose of cells. The consecutive or subsequent dose of cells can be more than, approximately the same as, or less than the first dose. In some embodiments, administration of the T cell therapy, such as administration of the first and/or second dose of cells, can be repeated.

[0281] In some embodiments, a dose of cells is administered to subjects in accord with the provided methods. In some embodiments, the size or timing of the doses is determined as a function of the particular disease or condition in the subject. It is within the level of a skilled artisan to empirically determine the size or timing of the doses for a particular disease. Dosages may vary depending on attributes particular to the disease or disorder and/or patient and/or other treatments.

[0282] In some aspects, the time between the administration of the first dose and the administration of the consecutive dose is at or about 9 to at or about 35 days, at or about 14 to at or about 28 days, or at or about 15 to at or about 27 days. In some embodiments, the administration of the consecutive dose is at a time point more than at or about 14 days after and less than at or about 28 days after the administration of the first dose. In some aspects, the time between the first and consecutive dose is at or 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 at or about 14 and less than at or about 28 days following administration of a prior dose. In some embodiments, the additional dose is administered less than at or 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 at or about 14 days following the prior dose and/or no dose is administered more than at or about 28 days after the prior dose.

[0283] In some embodiments, the dose of cells, e.g., recombinant receptor-expressing cells, comprises two doses (e.g., a double dose), comprising a first dose of the T cells and a consecutive dose of the T cells, wherein one or both of the first dose and the second dose comprises administration of the split dose of T cells.

[0284] 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.*, 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), 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 450 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 PBMCs or total cells administered.

[0285] In some embodiments, the cell therapy comprises administration of a dose comprising a number of cells that is at least or at least about or is or is about 0.1×10^6 cells/kg body weight of the subject, 0.2×10^6 cells/kg, 0.3×10^6 cells/kg, 0.4×10^6 cells/kg, 0.5×10^6 cells/kg, 1×10^6 cell/kg, 2.0×10^6 cells/kg, 3×10^6 cells/kg or 5×10^6 cells/kg.

[0286] In some embodiments, the cell therapy comprises administration of a dose comprising a number of cells is between at or about 0.1×10^6 cells/kg body weight of the subject and at or about 1.0×10^7 cells/kg, between at or about 0.5×10^6 cells/kg and at or about 5×10^6 cells/kg, between at or about 0.5×10^6 cells/kg and at or about 3×10^6 cells/kg, between at or about 0.5×10^6 cells/kg and at or about 2×10^6 cells/kg, between at or about 0.5×10^6 cells/kg and at or about 1×10^6 cell/kg, between at or about 1.0×10^6 cells/kg body weight of the subject and at or about 5×10^6 cells/kg, between at or about 1.0×10^6 cells/kg and at or about 3×10^6

cells/kg, between at or about 1.0×10^6 cells/kg and at or about 2×10^6 cells/kg, between at or about 2.0×10^6 cells/kg body weight of the subject and at or about 5×10^6 cells/kg, between at or about 2.0×10^6 cells/kg and at or about 3×10^6 cells/kg, or between at or about 3.0×10^6 cells/kg body weight of the subject and at or about 5×10^6 cells/kg, each inclusive.

[0287] In some embodiments, the dose of cells comprises between at or about 2×10^5 of the cells/kg and at or about 2×10^6 of the cells/kg, such as between at or about 4×10^5 of the cells/kg and at or about 1×10^6 of the cells/kg or between at or about 6×10^5 of the cells/kg and at or about 8×10^5 of the cells/kg. In some embodiments, the dose of cells comprises no more than 2×10^5 of the cells (*e.g.* antigen-expressing, such as CAR-expressing 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, nor 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 2×10^5 of the cells (*e.g.* antigen-expressing, such as CAR-expressing cells) per kilogram body weight of the subject (cells/kg), such as at least or at least about or at or about 3×10^5 cells/kg, at least or at least about or at or about 4×10^5 cells/kg, at least or at least about or at or about 5×10^5 cells/kg, at least or at least about or at or about 6×10^5 cells/kg, at least or at least about or at or about 7×10^5 cells/kg, at least or at least about or at or about 8×10^5 cells/kg, at least or at least about or at or about 9×10^5 cells/kg, at least or at least about or at or about 1×10^6 cells/kg, or at least or at least about or at or about 2×10^6 cells/kg.

[0288] In some embodiments, the cells are administered at a desired dosage, which in some aspects includes a desired dose or number of cells or cell type(s) and/or a desired ratio of cell types. Thus, the dosage of cells in some embodiments is based on a total number of cells (or number per kg body weight) and a desired ratio of the individual populations or sub-types, such as the CD4⁺ to CD8⁺ ratio. In some embodiments, the dosage of cells is based on a desired total number (or number per kg of body weight) of cells in the individual populations or of individual cell types. In some embodiments, the dosage is based on a combination of such features, such as a desired number of total cells, desired ratio, and desired total number of cells in the individual populations.

[0289] In some embodiments, the populations or sub-types of cells, such as CD8⁺ and CD4⁺ T cells, are administered at or within a tolerated difference of a desired dose of total cells, such

as a desired dose of T cells. In some aspects, the desired dose is a desired number of cells or a desired number of cells per unit of body weight of the subject to whom the cells are administered, *e.g.*, cells/kg. In some aspects, the desired dose is at or above a minimum number of cells or minimum number of cells per unit of body weight. In some aspects, among the total cells, administered at the desired dose, the individual populations or sub-types are present at or near a desired output ratio (such as CD4⁺ to CD8⁺ ratio), *e.g.*, within a certain tolerated difference or error of such a ratio.

[0290] In some embodiments, the cells are administered at or within a tolerated difference of a desired dose of one or more of the individual populations or sub-types of cells, such as a desired dose of CD4⁺ cells and/or a desired dose of CD8⁺ cells. In some aspects, the desired dose is a desired number of cells of the sub-type or population, or a desired number of such cells per unit of body weight of the subject to whom the cells are administered, *e.g.*, cells/kg. In some aspects, the desired dose is at or above a minimum number of cells of the population or sub-type, or minimum number of cells of the population or sub-type per unit of body weight.

[0291] Thus, in some embodiments, the dosage is based on a desired fixed dose of total cells and a desired ratio, and/or based on a desired fixed dose of one or more, *e.g.*, each, of the individual sub-types or sub-populations. Thus, in some embodiments, the dosage is based on a desired fixed or minimum dose of T cells and a desired ratio of CD4⁺ to CD8⁺ cells, and/or is based on a desired fixed or minimum dose of CD4⁺ and/or CD8⁺ cells.

[0292] In some embodiments, the cells are administered at or within a tolerated range of a desired output ratio of multiple cell populations or sub-types, such as CD4⁺ and CD8⁺ cells or sub-types. In some aspects, the desired ratio can be a specific ratio or can be a range of ratios. for example, in some embodiments, the desired ratio (*e.g.*, ratio of CD4⁺ to CD8⁺ cells) is between at or about 5:1 and at or about 5:1 (or greater than about 1:5 and less than about 5:1), or between at or about 1:3 and at or about 3:1 (or greater than about 1:3 and less than about 3:1), such as between at or about 2:1 and at or about 1:5 (or greater than about 1:5 and less than about 2:1, such as at or about 5:1, 4.5:1, 4:1, 3.5:1, 3:1, 2.5:1, 2:1, 1.9:1, 1.8:1, 1.7:1, 1.6:1, 1.5:1, 1.4:1, 1.3:1, 1.2:1, 1.1:1, 1:1, 1:1.1, 1:1.2, 1:1.3, 1:1.4, 1:1.5, 1:1.6, 1:1.7, 1:1.8, 1:1.9: 1:2, 1:2.5, 1:3, 1:3.5, 1:4, 1:4.5, or 1:5. In some aspects, the tolerated difference is within about 1%, about 2%, about 3%, about 4% about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50% of the desired ratio, including any value in between these ranges.

[0293] 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.

[0294] In some embodiments, for example, where the subject is a human, the dose includes fewer than about 5×10^8 total recombinant receptor (e.g., CAR)-expressing cells, T cells, or peripheral blood mononuclear cells (PBMCs), e.g., in the range of at or about 1×10^6 to at or about 5×10^8 such cells, such as at or about 2×10^6 , 5×10^6 , 1×10^7 , 5×10^7 , 1×10^8 , or 5×10^8 total such cells, or the range between any two of the foregoing values. In some embodiments, for example, where the subject is a human, the dose includes more than at or about 1×10^6 total recombinant receptor (e.g., CAR)-expressing cells, T cells, or peripheral blood mononuclear cells (PBMCs) and fewer than at or about 2×10^9 total recombinant receptor (e.g., CAR)-expressing cells, T cells, or peripheral blood mononuclear cells (PBMCs), e.g., in the range of at or about 2.5×10^7 to at or about 1.2×10^9 such cells, such as at or about 2.5×10^7 , 5×10^7 , 1×10^8 , 1.5×10^8 , 3×10^8 , 4.5×10^8 , 8×10^8 , or 1.2×10^9 total such cells, or the range between any two of the foregoing values.

[0295] In some embodiments, the dose of genetically engineered cells comprises from at or about 1×10^5 to at or about 5×10^8 total CAR-expressing T cells, from at or about 1×10^5 to at or about 2.5×10^8 total CAR-expressing T cells, from at or about 1×10^5 to at or about 1×10^8 total CAR-expressing T cells, from at or about 1×10^5 to at or about 5×10^7 total CAR-expressing T cells, from at or about 1×10^5 to at or about 2.5×10^7 total CAR-expressing T cells, from at or about 1×10^5 to at or about 1×10^7 total CAR-expressing T cells, from at or about 1×10^5 to at or about 5×10^6 total CAR-expressing T cells, from at or about 1×10^5 to at or about 2.5×10^6 total CAR-expressing T cells, from at or about 1×10^5 to at or about 1×10^6 total CAR-expressing T cells, from at or about 1×10^6 to at or about 5×10^8 total CAR-expressing T cells, from at or about 1×10^6 to at or about 2.5×10^8 total CAR-expressing T cells, from at or about 1×10^6 to at or about 1×10^8 total CAR-expressing T cells, from at or about 1×10^6 to at or about 5×10^7 total CAR-expressing T cells, from at or about 1×10^6 to at or about 2.5×10^7 total CAR-expressing T cells, from at or about 1×10^6 to at or about 1×10^7 total CAR-expressing T cells, from at or about 1×10^6 to at or about 5×10^6 total CAR-expressing T cells, from at or about 1×10^6 to at or about 2.5×10^6 total CAR-expressing T cells, from at or about 2.5×10^6 to at or about 5×10^8 total CAR-expressing T cells, from at or about 2.5×10^6 to at or about 2.5×10^8 total CAR-expressing T cells, from at or about 2.5×10^6 to at or about 1×10^8 total CAR-expressing T cells, from at or about 2.5×10^6 to at or about 5×10^7 total CAR-

expressing T cells, from at or about 2.5×10^6 to at or about 2.5×10^7 total CAR-expressing T cells, from at or about 2.5×10^6 to at or about 1×10^7 total CAR-expressing T cells, from at or about 2.5×10^6 to at or about 5×10^6 total CAR-expressing T cells, from at or about 5×10^6 to at or about 5×10^8 total CAR-expressing T cells, from at or about 5×10^6 to at or about 2.5×10^8 total CAR-expressing T cells, from at or about 5×10^6 to at or about 1×10^8 total CAR-expressing T cells, from at or about 5×10^6 to at or about 5×10^7 total CAR-expressing T cells, from at or about 5×10^6 to at or about 2.5×10^7 total CAR-expressing T cells, from at or about 5×10^6 to at or about 1×10^7 total CAR-expressing T cells, from at or about 1×10^7 to at or about 5×10^8 total CAR-expressing T cells, from at or about 1×10^7 to at or about 2.5×10^8 total CAR-expressing T cells, from at or about 1×10^7 to at or about 1×10^8 total CAR-expressing T cells, from at or about 1×10^7 to at or about 5×10^7 total CAR-expressing T cells, from at or about 1×10^7 to at or about 2.5×10^7 total CAR-expressing T cells, from at or about 2.5×10^7 to at or about 5×10^8 total CAR-expressing T cells, from at or about 2.5×10^7 to at or about 2.5×10^8 total CAR-expressing T cells, from at or about 2.5×10^7 to at or about 1×10^8 total CAR-expressing T cells, from at or about 2.5×10^7 to at or about 5×10^7 total CAR-expressing T cells, from at or about 5×10^7 to at or about 5×10^8 total CAR-expressing T cells, from at or about 5×10^7 to at or about 2.5×10^8 total CAR-expressing T cells, from at or about 5×10^7 to at or about 1×10^8 total CAR-expressing T cells, from at or about 1×10^8 to at or about 5×10^8 total CAR-expressing T cells, from at or about 1×10^8 to at or about 2.5×10^8 total CAR-expressing T cells, from at or about 2.5×10^8 to at or about 5×10^8 total CAR-expressing T cells.

[0296] In some embodiments, the dose of genetically engineered cells comprises at least or at least about 1×10^5 CAR-expressing cells, at least or at least about 2.5×10^5 CAR-expressing cells, at least or at least about 5×10^5 CAR-expressing cells, at least or at least about 1×10^6 CAR-expressing cells, at least or at least about 2.5×10^6 CAR-expressing cells, at least or at least about 5×10^6 CAR-expressing cells, at least or at least about 1×10^7 CAR-expressing cells, at least or at least about 2.5×10^7 CAR-expressing cells, at least or at least about 5×10^7 CAR-expressing cells, at least or at least about 1×10^8 CAR-expressing cells, at least or at least about 1.5×10^8 CAR-expressing cells, at least or at least about 2.5×10^8 CAR-expressing cells, at least or at least about 3×10^8 CAR-expressing cells, at least or at least about 4.5×10^8 CAR-expressing cells, or at least or at least about 5×10^8 CAR-expressing cells.

[0297] In some embodiments, the cell therapy comprises administration of a dose comprising a number of cell from or from about 1×10^5 to or to about 5×10^8 total recombinant

receptor-expressing cells, total T cells, or total peripheral blood mononuclear cells (PBMCs), from or from about 5×10^5 to or to about 1×10^7 total recombinant receptor-expressing cells, total T cells, or total peripheral blood mononuclear cells (PBMCs) or from or from about 1×10^6 to or to about 1×10^7 total recombinant receptor-expressing cells, total T cells, or total peripheral blood mononuclear cells (PBMCs), each inclusive. In some embodiments, the cell therapy comprises administration of a dose of cells comprising a number of cells at least or at least about 1×10^5 total recombinant receptor-expressing cells, total T cells, or total peripheral blood mononuclear cells (PBMCs), such at least or at least 1×10^6 , at least or at least about 1×10^7 , at least or at least about 1×10^8 of such cells. In some embodiments, the number is with reference to the total number of CD3+ or CD8+, in some cases also recombinant receptor-expressing (e.g. CAR+) cells. In some embodiments, the cell therapy comprises administration of a dose comprising a number of cell from or from about 1×10^5 to 5×10^8 CD3+ or CD8+ total T cells or CD3+ or CD8+ recombinant receptor-expressing cells, from or from about 5×10^5 to 1×10^7 CD3+ or CD8+ total T cells or CD3+ or CD8+ recombinant receptor-expressing cells, or from or from about 1×10^6 to 1×10^7 CD3+ or CD8+ total T cells or CD3+ or CD8+ recombinant receptor-expressing cells, each inclusive. In some embodiments, the cell therapy comprises administration of a dose comprising a number of cell from or from about 1×10^5 to 5×10^8 total CD3+/CAR+ or CD8+/CAR+ cells, from or from about 5×10^5 to 1×10^7 total CD3+/CAR+ or CD8+/CAR+ cells, or from or from about 1×10^6 to 1×10^7 total CD3+/CAR+ or CD8+/CAR+ cells, each inclusive.

[0298] In some embodiments, the T cells of the dose include CD4+ T cells, CD8+ T cells or CD4+ and CD8+ T cells.

[0299] In some embodiments, for example, where the subject is human, the CD8+ T cells of the dose, including in a dose including CD4+ and CD8+ T cells, includes between at or about 1×10^6 and at or about 5×10^8 total recombinant receptor (e.g., CAR)-expressing CD8+ cells, e.g., in the range of from at or about 5×10^6 to at or about 1×10^8 such cells, such as 1×10^7 , 2.5×10^7 , 5×10^7 , 7.5×10^7 , 1×10^8 , 1.5×10^8 , 3×10^8 , 4.5×10^8 , or 5×10^8 total such cells, or the range between any two of the foregoing values. In some embodiments, the patient is administered multiple doses, and each of the doses or the total dose can be within any of the foregoing values. In some embodiments, the dose of cells comprises the administration of from or from about 1×10^7 to or to about 0.75×10^8 total recombinant receptor-expressing CD8+ T cells, from or from about 1×10^7 to or to about 5×10^7 total recombinant receptor-expressing CD8+ T cells, from or from about 1×10^7 to or to about 0.25×10^8 total recombinant receptor-

expressing CD8+ T cells, each inclusive. In some embodiments, the dose of cells comprises the administration of at or about 1×10^7 , 2.5×10^7 , 5×10^7 , 7.5×10^7 , 1×10^8 , 1.5×10^8 , 2.5×10^8 , 3×10^8 , 4.5×10^8 , or 5×10^8 total recombinant receptor-expressing CD8+ T cells.

[0300] 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, 1 year or more.

[0301] 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, e.g. chemotherapy, disease burden in the subject, such as tumor load, bulk, size, or degree, extent, or type of metastasis, stage, and/or likelihood or incidence of the subject developing toxic outcomes, e.g., CRS, macrophage activation syndrome, tumor lysis syndrome, neurotoxicity, and/or a host immune response against the cells and/or recombinant receptors being administered.

IV. METHODS OF MONITORING, ASSESSMENT AND MODULATING THERAPY

[0302] In some embodiments, provided are methods of treatment. In some embodiments, the methods include administering an immunotherapy and/or a cell therapy. In some embodiments, the methods involve administration of genetically engineered cells, e.g., cells engineered to express a recombinant receptor such as a chimeric antigen receptor (CAR). In some embodiments, the methods include administering a dose of cells, e.g., CAR+ expressing cells, to a subject such that the cells are within a target therapeutic range or window. In some embodiments, the methods also involve monitoring parameters such as numbers or level e.g., pharmacokinetic parameters, such as peak cell concentration (C_{max}), to determine whether the cells in the subject is within the therapeutic range or window. In some embodiments, if the cells are not within the therapeutic range or window, the treatment can be modified, e.g., by administering additional doses, altering subsequent or additional doses, and/or by administering an agent that can modulate CAR+ T cell expansion, proliferation and/or activity. In some aspects, the provided methods also include a method of determining a dose of a subject, or a method of dosing a subject, based on an assessment of the parameters such as numbers or level, e.g., pharmacokinetic parameters, such as peak cell concentration (C_{max}), patient attributes and/or biomarkers.

[0303] In some aspects, provided are methods of modulating a therapy, e.g., a cell therapy such as a T cell therapy with recombinant receptor-expressing cells. In some embodiments, the cell therapy is modulated by administering to the subject receiving cell therapy an agent to the

subject capable of modulating CAR+ T cell expansion, proliferation, expansion, survival, activity and/or function,, e.g., increases or decreases CAR+ T cell expansion, proliferation, survival and/or activity.

[0304] In some embodiments, the agent is administered after assessment of pharmacokinetic parameters, e.g., number, level or peak CAR+ T cell concentration, exposure (e.g., AUC) and/or cell level or concentration. In some embodiments, the agent is administered after assessment of other parameters, such as patient attributes, factors, characteristics and/or expression of biomarkers, that is associated with and/or correlated with pharmacokinetic parameters, response, durable response and/or development of toxicity.

[0305] In some embodiments, provided are methods of treatment that involves administering, to a subject having a disease or condition, a dose of genetically engineered cells comprising T cells expressing a recombinant receptor, such as a chimeric antigen receptor (CAR) for treating the disease or condition. In some embodiments, the method involves after administering the dose of genetically engineered cells, monitoring pharmacokinetic parameters, e.g., CAR+ T cells, in the blood of the subject to assess if the cells are within a therapeutic range or window. In some embodiments, the method involves administering an agent to the subject capable of modulating, optionally increasing or decreasing, CAR+ T cell expansion, proliferation and/or activity, in the subject if the genetically engineered cells are not within the therapeutic range.

[0306] In some embodiments, also provided are methods of treatment that involves monitoring, in the blood of a subject, the presence of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR) to assess if the cells are within a therapeutic range, wherein the subject has been previously administered a dose of the genetically engineered cells for treating a disease or condition. In some embodiments, the methods also involve administering an agent capable of modulating, optionally increasing or decreasing, CAR+ T cell expansion, proliferation and/or activity, in the subject if the genetically engineered cells are not within the therapeutic range.

[0307] In some aspects, if the number, level or peak number of CAR+ T cells in the blood of the subject is less than the lowest number of number, level or peak CAR+ T cells in the therapeutic range, an agent is administered to the subject that is capable of increasing CAR+ T cell expansion or proliferation. In some aspects, if the number, level or peak number of CAR+ T cells in the blood of the subject is greater than the highest number of number, level or peak CAR+ T cells in the therapeutic range, an agent is administered to the subject that is capable of

decreasing CAR+ T cell expansion or proliferation.

[0308] In some embodiments, also provided are methods of modulating activity of engineered cells. In some embodiments, the methods involves assessing the level, amount or concentration of a parameter, such as a volumetric measure of tumor burden or an inflammatory marker, in a sample from the subject is at or above a threshold level. In some embodiments, the sample does not comprise genetically engineered T cells expressing a chimeric antigen receptor (CAR) and/or is obtained from the subject prior to receiving administration of genetically engineered T cells expressing a CAR. In some embodiments, a subject is selected for administration of an agent capable of decreasing expansion or proliferation of genetically engineered T cells expressing a CAR. In some embodiments, the agent capable of decreasing expansion or proliferation of genetically engineered T cells expressing a CAR is administered to the subject.

[0309] In some embodiments, also provided are methods of modulating activity of engineered cells, that involves administering to a subject an agent capable of decreasing expansion or proliferation of genetically engineered T cells expressing a chimeric antigen receptor (CAR) in a subject, wherein the subject is one in which the level, amount or concentration of a parameter, e.g., a volumetric measure of tumor burden or an inflammatory marker in a sample from the subject is at or above a threshold level.

[0310] In some embodiments, the provided methods involve administration of a genetically engineered cell, e.g., a T cell engineered to express a recombinant receptor, e.g., CAR. In some embodiments, an agent capable of modulating, e.g., increasing or decreasing, CAR+ T cell expansion, proliferation and/or activity, is administered prior to or concurrently with initiation of administration of a dose of genetically engineered cells comprising T cells expressing a chimeric antigen receptor. In some aspects, prior to administering the agent, the selected subject is at risk of developing a toxicity following administration of the genetically engineered cells. In some embodiments, the administration of the agent is sufficient to achieve number, level or peak CAR+ T cells in a therapeutic range or window in the subject. In some embodiments, the administration of the agent is sufficient to achieve number, level or peak CAR+ T cell concentrations, in the blood in a majority of subjects so treated by the method, or greater than or greater than about 50%, 60%, 70%, 75%, 80%, 85%, 90% or 95% or more, such as greater than or greater than about 75% of the subjects so treated by the method, is within a determined target therapeutic range or window.

[0311] In some embodiments, also provided are methods of dosing a subject. In some

embodiments, the methods involve administering, to a subject having a disease or condition, a sub-optimal dose of genetically engineered cells comprising T cells engineered with a chimeric antigen receptor (CAR), wherein the dose comprises a number of the genetically engineered cells that is insufficient to achieve number, level or peak CAR+ cells in the blood within a determined therapeutic range in the subject, or in a majority of subjects so treated by the method or in greater than 75% of the subjects so treated by the method. In some embodiments, the methods involve administering an agent to enhance CAR+ cell expansion or proliferation in the subject to achieve number, level or peak CAR+ T cells in the blood within the therapeutic range or window, subsequent to administering the genetically engineered cells. In some embodiments, the dose of genetically engineered cells is less than or less than about 1×10^7 CAR-expressing cells, less than or less than about 5×10^6 CAR-expressing cells, less than or less than about 2.5×10^6 CAR-expressing cells, less than or less than about 1×10^6 CAR-expressing cells, less than or less than about 5×10^5 CAR-expressing cells, less than or less than about 2.5×10^5 CAR-expressing cells, less than or less than about 1×10^5 CAR-expressing cells.

[0312] In some embodiments, following administration of the agent, the method achieves an increased frequency of number, level or peak CAR+ cells in the blood within a determined therapeutic range in the subject, compared to a method involving administration of the same dose of genetically engineered cells but without the agent; or number, level or peak CAR+ cells in the blood within a determined therapeutic range in the subject, or in a majority of subjects so treated by the method or in greater than 75% of the subjects so treated by the method.

[0313] In some embodiments, the therapeutic range or window is determined as described herein, e.g., in Section II or elsewhere. In some embodiments, the therapeutic range is based upon the range of number, level or peak CD3+ CAR+ T cells, or a CD8+CAR+ T cell subset thereof, in the blood among one or more subjects previously treated with the genetically engineered cells that is associated with an estimated probability of response of greater than or greater than about 65%, 70%, 75%, 80%, 85%, 90%, 95% or more, and an estimated probability of a toxicity of less than or less than about 30%, 25%, 20%, 15%, 10%, 5% or less.

[0314] In some embodiments, the therapeutic window or range is determined based on specific range of numbers and/or concentrations of cells, e.g., CD3+, CD4+ or CD8+ T cells. In some embodiments, an exemplary number, level or peak CD3+CAR+ T cell concentration in the blood that can achieve a therapeutic window, is or includes between at or approximately 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50 CD3+ CAR+ T cells per microliter in the blood and at or approximately 200, 300, 400, 500, 600, 700 or 750, CD3+ CAR+ T cells per microliter

in the blood. In some embodiments, an exemplary number, level or peak CD8+CAR+ T cell concentration in the blood that can achieve a therapeutic window, is or includes between at or approximately 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50 CD8+ CAR+ T cells per microliter in the blood and at or approximately 200, 300, 400, 500, 600, 700 or 750, CD8+ CAR+ T cells per microliter in the blood.

[0315] In some embodiments, the methods also involve monitoring the CAR+ T cells in the blood of the subject after administering the dose of genetically engineered cells.

[0316] In some embodiments, the subject is monitored for CAR+ T cells in the blood at a time that is at least 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days or 21 days after initiation of administration of the genetically engineered cells. In some embodiments, the subject is monitored for CAR+ T cells in the blood at a time that is between or between about 11 to 22 days, 12 to 18 days or 14 to 16 days, each inclusive, after initiation of administration of the genetically engineered cells.

[0317] Also provided are methods of assessing likelihood of a response or a durable response, and methods of administering a therapeutic agent accordingly. In some embodiments, the methods involve detecting, in a biological sample from a subject, peak levels of one or more inflammatory marker and/or peak levels of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR), wherein the subject has been previously administered a dose of the genetically engineered cells for treating a disease or condition. In some embodiments, the methods involve comparing, individually, the peak levels to a threshold value, thereby determining a likelihood that a subject will achieve a durable response to the administration of the genetically engineered cells.

[0318] In some embodiments, the subject is likely to achieve a response or a durable response if the peak levels of the one or more inflammatory marker is below a threshold value and the subject is not likely to achieve a durable response if the peak levels of the one or more inflammatory marker are above a threshold value. In some embodiments, the subject is likely to achieve a durable response if the peak level of the genetically engineered cells is within a therapeutic range between a lower threshold value and an upper threshold value and the subject is not likely to achieve a durable response if the peak level of the genetically engineered cells is below the lower threshold value or is above the upper threshold value.

[0319] In some embodiments, the threshold value is a value that: is within 25%, within 20%, within 15%, within 10%, or within 5% above the average value of the volumetric measure or inflammatory marker and/or is within a standard deviation above the average value of the

volumetric measure or the inflammatory marker in a plurality of control subjects. In some embodiments, the threshold value is a value that: is above the highest value of the volumetric measure or inflammatory marker, optionally within 50%, within 25%, within 20%, within 15%, within 10%, or within 5% above such highest fold change, measured in at least one subject from among a plurality of control subjects. In some embodiments, the threshold value is a value that: is above the highest value of the volumetric measure or inflammatory marker as measured among more than 75%, 80%, 85%, 90%, or 95%, or 98% of subjects from a plurality of control subjects. In some embodiments, the plurality of control subjects are a group of subjects prior to receiving a dose of the genetically engineered cells, wherein: each of the control subjects of the group exhibited a peak CAR+ T cells in the blood greater than the highest peak CAR+ T cells in the therapeutic range; each of the control subjects of the group went on to develop at toxicity, optionally a neurotoxicity or cytokine release syndrome (CRS), a grade 2 or grade 3 or higher neurotoxicity or a grade 3 or higher CRS, after receiving a dose of the engineered cells for treating the same disease or condition; each of the control subjects of the group did not develop a response, optionally a complete response (CR) or partial response (PR), following administration of the dose of genetically engineered cells; and/or each of the control subjects of the group did not develop a durable response, optionally for at or about or greater than or about 3 months or at or about or greater than or about 6 months, following administration of the dose of genetically engineered cells.

[0320] In some embodiments, the methods also involve administering an agent or an alternative therapy, based on the assessment of the likelihood of achieving a response or a durable response. In some embodiments, if the subject is determined not likely to achieve a response or durable response, the subject is selected for treatment with a therapeutic agent or with an alternative therapeutic treatment other than the genetically engineered cells. In some embodiments, if the subject is determined not likely to achieve a response or durable response, a therapeutic agent or an alternative therapeutic treatment other than the genetically engineered cells is administered to the subject.

[0321] In some embodiments, also provided are methods of treatment that involves selecting a subject for administration of a therapeutic agent and/or alternative therapeutic treatment. In some embodiments, the methods involve selecting a subject having received administration of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR) in which: peak levels of one or more inflammatory markers in a sample from the subject is above a threshold value; and/or peak level of T cells comprising a chimeric antigen receptor (CAR) in

a sample from the subject is below a lower threshold value or is above an upper threshold value.

[0322] In some embodiments, the response is a complete response (CR), objective response (OR) or partial response (PR). In some embodiments, the response is durable for at or greater than 3 months, 4 months, 5 months, or 6 months.

[0323] In some embodiments, the peak levels are assessed and/or the sample is obtained from the subject at a time that is at least 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days or 21 days after initiation of administration of the genetically engineered cells. In some embodiments, the peak levels are assessed and/or the sample is obtained from the subject at a time that is between or between about 11 to 22 days, 12 to 18 days or 14 to 16 days, each inclusive, after initiation of administration of the genetically engineered cells.

[0324] In some embodiments, the peak level is or includes the peak level of one or more inflammatory markers, e.g., C reactive protein (CRP), IL-2, IL-6, IL-10, IL-15, TNF-alpha, MIP-1alpha, MIP-1beta, MCP-1, CXCL10 or CCL13.

[0325] In some embodiments, the peak level of one or more inflammatory marker is assessed and the threshold value is within 25%, within 20%, within 15%, within 10% or within 5% and/or is within a standard deviation of the median or mean of the peak level of the inflammatory marker as determined among a group of control subjects having received administration of the genetically engineered cells, wherein each of the subjects of the group did not achieve a durable response, optionally a CR and/or PR, optionally at or greater than 3 months or 6 months following administration of the genetically engineered cells. In some embodiments, the control subjects exhibited stable disease (SD) or progressive disease (PD) following administration of the genetically engineered cells, optionally at or greater than 3 months or 6 months following administration of the genetically engineered cells. In some embodiments, the peak level is a peak level of CAR+ T cells, or a CD8+ T cell subset thereof.

[0326] In some embodiments, the lower threshold value and upper threshold value is the lower and upper end, respectively, of a therapeutic range of peak CD3+ CAR+ T cells, or a CD8+CAR+ T cell subset thereof, in the blood among one or more subjects previously treated with the genetically engineered cells that is associated with an estimated probability of response of greater than or greater than about 65% and an estimated probability of a toxicity of less than or about 30%. In some embodiments, the therapeutic range is the range in which the estimated probability of toxicity is less than at or about 20%, less than at or about 15%, less than at or about 10% or less than at or about 5% and the estimated probability of achieving a response is

greater than at or about 65%, greater than at or about 70%, greater than at or about 75%, greater than at or about 80%, greater than at or about 85%, greater than at or about 90%, greater than at or about 95% or more.

[0327] In some embodiments, the probability of response is based on a response that is a complete response (CR), an objective response (OR) or a partial response (PR), optionally wherein the response is durable, optionally durable for at or at least 3 months or at or at least 6 months.

[0328] In some embodiments, number, level or peak CAR+ T cells is determined as the number of CAR+ T cells per microliter in the blood of the subject. In some embodiments, the upper threshold value is between at or about 300 cells per microliter and at or about 1000 cells per microliter, or between at or about 400 cells per microliter and at or about 600 cells per microliter, or is at or about 300 cells per microliter, 400 cells per microliter, 500 cells per microliter, 600 cells per microliter, 700 cells per microliter, 800 cells per microliter, 900 cells per microliter or 1000 cells per microliter; or the lower threshold value is less than or less than about 10 cells per microliter, 9 cells per microliter, 8 cells per microliter, 7 cells per microliter, 6 cells per microliter, 5 cells per microliter, 4 cells per microliter, 3 cells per microliter, 2 cells per microliter or 1 cell per microliter.

[0329] In some embodiments of the methods provided herein, among a plurality of subjects treated, the method achieves an increase in the percentage of subjects achieving a durable response, optionally a complete response (CR) or objective response (OR) or a partial response (PR), optionally that is durable for at or greater than 3 months or at or greater than 6 months, compared to a method that does not comprise administering the agent. In some embodiments, the increase is greater than or greater than about 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold or more.

[0330] In some embodiments, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40% or at least 50% of subjects treated according to the method achieve a complete response (CR) that is durable for at or greater than 3 months or at or greater than 6 months; and/or at least 25%, at least 30%, at least 40%, at least 50%, at least 60% or at least 70% of the subjects treated according to the method achieve objective response (OR) that is durable for at or greater than 3 months or at or greater than 6 months. In some embodiments, greater than or greater than about 50%, greater than or greater than about 60%, greater than or greater than about 70%, or greater than or greater than about 80% of the subjects treated according to the method do not exhibit a grade 3 or greater cytokine release syndrome (CRS)

and/or do not exhibit a grade 2 or greater or grade 3 or greater neurotoxicity; or greater than or greater than about 40%, greater than or greater than about 50% or greater than or greater than about 55% of the subjects treated according to the method do not exhibit any neurotoxicity or CRS.

[0331] In some embodiments, the parameters, such as attributes, factors, characteristic of the patient and/or the disease or condition, and/or expression of biomarkers, are assessed prior to administration of the therapy, e.g., cell therapy. In some embodiments, the parameters, such as attributes, factors, characteristic of the patient and/or the disease or condition, and/or expression of biomarkers, are assessed after administration of the therapy, e.g., cell therapy. In some embodiments, the parameters include levels or measurements, e.g., peak levels, of attributes, factors, characteristic of the patient and/or the disease or condition, and/or expression of biomarkers, that can be assessed after administration of the therapy, e.g., cell therapy.

[0332] In some embodiments, the parameter is a parameter related to tumor burden, e.g., a measurement of tumor burden. In some aspects, the methods also involve further monitoring the subject for possible symptoms of toxicity based on the risk of toxicity determined by assessment of the presence or absence of the biomarker and/or comparison of the biomarkers to a reference value or threshold level of the biomarker.

[0333] In some embodiments, the parameter is SPD and in some cases, development of toxicity, e.g., CRS or NT, is correlated with the SPD value that is above a threshold value. In some embodiments, the volumetric measure is SPD, and the threshold value is or is about 30 cm², is or is about 40 cm², is or is about 50 cm², is or is about 60 cm², or is or is about 70 cm². In some embodiments, the volumetric measure is SPD and the threshold value is or is about 30 cm², is or is about 40 cm², is or is about 50 cm², is or is about 60 cm², or is or is about 70 cm².

[0334] In some embodiments, the parameter is LDH and in some cases, development of toxicity, e.g., CRS or NT, is correlated with the LDH value that is above a threshold value. In some embodiments, the inflammatory marker is LDH and the threshold value is or is about 300 units per liter, is or is about 400 units per liter, is or is about 500 units per liter or is or is about 600 units per liter.

A. Pharmacokinetic Parameters

[0335] In some cases, the provided embodiments involve administering an agent capable of modulating CAR+ T cell expansion, proliferation, and/or activity to the subject, based on assessment of pharmacokinetic (PK) parameters. In some embodiments, the pharmacokinetic

parameters include any of those described herein, e.g., in Section II.C. In some embodiments, the pharmacokinetic parameters include maximum (peak) plasma concentration (C_{\max}), the peak time (*i.e.* when maximum plasma concentration (C_{\max}) occurs; T_{\max}), the minimum plasma concentration (*i.e.* the minimum plasma concentration between doses of a therapeutic agent, e.g., CAR+ T cells; C_{\min}), the elimination half-life ($T_{1/2}$) and area under the curve (*i.e.* the area under the curve generated by plotting time versus plasma concentration of the therapeutic agent CAR+ T cells; AUC), following administration.

[0336] In some embodiments, if the assessed pharmacokinetic parameters indicate that the dose of cells administered is not within or falls outside a therapeutic range and/or window, the subject can be administered an agent capable of modulating CAR+ T cell expansion, proliferation, and/or activity to the subject. In some embodiments, the therapeutic range and/or window is any described herein and/or is associated with any pharmacokinetic parameters described herein.

[0337] In some embodiments, if a pharmacokinetic parameter, e.g., peak number of CAR+ T cells in the blood of the subject, is less than the lowest number of the pharmacokinetic parameter, e.g., peak number of CAR+ T cells in the blood of the subject in the therapeutic range, an agent is administered to the subject that increases CAR+ T cell expansion, proliferation, and/or activity.

[0338] In some embodiments, if a pharmacokinetic parameter, e.g., peak number of CAR+ T cells in the blood of the subject, is more than the highest number of the pharmacokinetic parameter, e.g., peak number of CAR+ T cells in the blood of the subject in the therapeutic range, an agent is administered to the subject that decreases CAR+ T cell expansion, proliferation, and/or activity.

[0339] In some embodiments, the agent is administered after assessment of pharmacokinetic parameters, e.g., peak CAR+ T cell concentration, exposure (e.g., AUC) and/or cell level or concentration.

[0340] In some aspects, the provided embodiments involve assessing and/or monitoring pharmacokinetic parameters, e.g., number or concentration of CAR+ T cells in the blood. In some embodiments, the methods involve monitoring CAR+ T cell numbers and/or concentration in the blood of the subject to assess if the cells are within a therapeutic range and/or window. In some embodiments, the methods involve administering an agent to the subject capable of modulating CAR+ T cell expansion, optionally increasing or decreasing CAR+ T cell expansion, in the subject, if the subjects are not within the therapeutic range.

[0341] In some embodiments, the therapeutic range and/or window is determined and/or based upon any criteria based on the assessment of the parameters described herein. In some embodiments, the therapeutic range and/or window is based upon the range of peak CD3+ CAR+ T cells, or a CD8+CAR+ T cell subset thereof, in the blood among one or more subjects previously treated with the genetically engineered cells that is associated with an estimated probability of response of greater than or greater than about 65% and an estimated probability of a toxicity of less than or about 30%; or peak CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between at or about 10 cells per microliter and at or about 500 cells per microliter; or peak CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between at or about 2 cells per microliter and at or about 200 cells per microliter.

B. Patient Attributes and Biomarkers

[0342] In some cases, the provided embodiments involve assessing parameters, such as attributes, factors, characteristic of the patient and/or the disease or condition, and/or expression of biomarkers. In some embodiments, the assessed parameters are associated with and/or correlated with pharmacokinetic parameters, response, durable response and/or development of toxicity. In some embodiments, the parameters include patient factors or patient attributes. In some embodiments, the parameters include attributes, factors, characteristic of the disease or condition. In some embodiments, the parameters are assessed prior to treatment, e.g., prior to administration of the cell therapy. In some embodiments, the parameters are assessed after treatment, e.g., after administration of one or more doses of the cell therapy.

[0343] In some embodiments, the parameter is or includes pharmacokinetic parameters, e.g., maximum (peak) plasma concentration (C_{\max}), the peak time (*i.e.* when maximum plasma concentration (C_{\max}) occurs; T_{\max}), the minimum plasma concentration (*i.e.* the minimum plasma concentration between doses of a therapeutic agent, e.g., CAR+ T cells; C_{\min}), the elimination half-life ($T_{1/2}$) and area under the curve (*i.e.* the area under the curve generated by plotting time versus plasma concentration of the therapeutic agent CAR+ T cells; AUC; such as AUC_{0-28}).

[0344] In some embodiments, the parameter is or includes one or more factors indicative of the state of the patient and/or the disease or condition of the patient. In some embodiments, the parameter is indicative of tumor burden. In some embodiments, the factor indicative of tumor burden is a volumetric measure of tumor(s). In some embodiments, the volumetric measure is a measure of the lesion(s), such as the tumor size, tumor diameter, tumor volume, tumor mass,

tumor load or bulk, tumor-related edema, tumor-related necrosis, and/or number or extent of metastases. In some embodiments, the volumetric measure of tumor is a bidimensional measure. For example, in some embodiments, the area of lesion(s) are calculated as the product of the longest diameter and the longest perpendicular diameter of all measurable tumors. In some cases, the volumetric measure of tumor is a unidimensional measure. In some cases, the size of measurable lesions is assessed as the longest diameter. In some embodiments, the sum of the products of diameters (SPD), longest tumor diameters (LD), sum of longest tumor diameters (SLD), necrosis, tumor volume, necrosis volume, necrosis-tumor ratio (NTR), peritumoral edema (PTE), and edema-tumor ratio (ETR) is measured.

[0345] Exemplary methods for measuring and assessing tumor burden include those described in, e.g., Carceller et al., *Pediatr Blood Cancer*. (2016) 63(8):1400-1406 and Eisenhauer et al., *Eur J Cancer*. (2009) 45(2):228-247. In some embodiments, the volumetric is a sum of the products of diameters (SPD) measured by determining the sum of the products of the largest perpendicular diameters of all measurable tumors. In some aspects, the tumor or lesion are measured in one dimension with the longest diameter (LD) and/or by determining the sum of longest tumor diameters (SLD) of all measurable lesions. In some embodiments, the volumetric measure of tumor is a volumetric quantification of tumor necrosis, such as necrosis volume and/or necrosis-tumor ratio (NTR), see Monsky et al., *Anticancer Res*. (2012) 32(11): 4951-4961. In some aspects, the volumetric measure of tumor is a volumetric quantification of tumor-related edema, such as peritumoral edema (PTE) and/or edema-tumor ratio (ETR). In some embodiments, measuring can be performed using imaging techniques such as computed tomography (CT), positron emission tomography (PET), and/or magnetic resonance imaging (MRI) of the subject.

[0346] In some embodiments, the volumetric measure is SPD and in some cases, development of toxicity, e.g., CRS or NT, is correlated with the SPD value that is above a threshold value. In some embodiments, the volumetric measure is SPD, and the threshold value is or is about 30 cm², is or is about 40 cm², is or is about 50 cm², is or is about 60 cm², or is or is about 70 cm². In some embodiments, the volumetric measure is SPD and the threshold value is or is about 30 cm², is or is about 40 cm², is or is about 50 cm², is or is about 60 cm², or is or is about 70 cm².

[0347] In some embodiments, the volumetric measure of tumor is determined at a screening session, such as a routine assessment or blood draw to confirm and/or identify the condition or disease in the subject.

[0348] In some aspects, the parameter, e.g., measurements of tumor burden, correlates to and/or is associated with pharmacokinetic parameters. In some embodiments, the parameter, including pharmacokinetic parameters, is associated with response and/or durable response, and/or a risk for developing toxicity, e.g., CRS or neurotoxicity (NT).

[0349] In some embodiments, the parameter is or includes at least one or a panel of biomarkers. In some embodiments, expression and/or presence of the biomarker is associated with and/or correlated with pharmacokinetic parameters, response, durable response and/or development of toxicity. In some embodiments, the parameter is compared to a particular reference value, e.g., those associated with response and/or durable response, and/or a risk for developing toxicity, e.g., CRS or neurotoxicity (NT). In some embodiments, the methods also involve administering an agent capable of modulating CAR+ T cell expansion, proliferation, and/or activity, to the subject, based on the assessment of patient factors and/or biomarkers.

[0350] In some embodiments, the presence or absence of one or a panel of biomarkers and/or concentration, amount, level or activity associated with one or a panel of biomarkers can be assessed. In some cases, the parameters can be compared to a particular reference value, such as a threshold level, e.g., those associated with a risk for developing toxicity or those associated with a particular response, such as OR, CR or PR, or durable response, such as a response that is durable for 3 months, 6 months, 9 months 12 months or more, after the initial response. In some embodiments, the methods also involve selecting subjects for treatment with a cell therapy based on the assessment of the presence or absence of the biomarker and/or comparison of the biomarkers to a reference value or threshold level of the biomarker. In some embodiments, the methods also involve administering an agent or a therapy that can treat, prevent, delay and/or attenuate development of the toxicity, e.g., based on the assessment of the presence or absence of the biomarker and/or comparison of the biomarkers to a reference value or threshold level of the biomarker.

[0351] In some aspects, the embodiments involve obtaining a biological sample for detecting the parameter and/or assessing the presence of and/or or detecting the parameter. In some embodiments, the biological sample is obtained generally within 4 hours to 12 months of administration of the cell therapy, or a first administration or dose thereof, or after the initiation of any of the foregoing, such as generally within or within at or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 21, 28, 30, 60 or 90 or more days, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more weeks, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18, 24, 48 or more months, after administration of the cell therapy, or a first administration or dose thereof, or after the initiation

of any of the foregoing. In some embodiments, the parameter is assessed or measured in a subject prior to administration of the cell therapy or soon after administration of the cell therapy, or a first administration or dose thereof, or after the initiation of any of the foregoing, such as generally within 4 hours to 3 days of administration of the cell therapy, or a first administration or dose thereof, or after the initiation of any of the foregoing, such as generally within at or about 1 day, 2 days or 3 days after administration of the cell therapy, or a first administration or dose thereof, or after the initiation of any of the foregoing. In some embodiments, the parameter is assessed or measured. In some embodiments, the parameter is assessed generally within 4 hours to 12 months of administration of the cell therapy, or a first administration or dose thereof, or after the initiation of any of the foregoing, such as generally within or within at or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 21, 28, 30, 60 or 90 or more days, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more weeks, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18, 24, 48 or more months, after administration of the cell therapy, or a first administration or dose thereof, or after the initiation of any of the foregoing.

[0352] In some aspects, the parameter, e.g., patient factor and/or biomarker, correlates to and/or is associated with pharmacokinetic parameters. In some embodiments, the parameter, including pharmacokinetic parameters, is associated with response and/or durable response, and/or a risk for developing toxicity, e.g., CRS or neurotoxicity (NT).

[0353] In some embodiments, the parameter is a biomarker. In some embodiments, the parameter is or includes the expression of the biomarker and/or the number, concentration, and/or percentage of cells that express a particular biomarker. In some embodiments, the parameter includes biomarkers or each biomarker in a panel that comprises a plurality of biomarkers. In some embodiments, the biomarker is or comprises a cytokine and/or other serum or blood factor, such as any as described herein. In some embodiments, the biomarker or each biomarker in a panel is a cytokine, which, in some cases, can be a chemokine. In some embodiments, the biomarkers or each biomarker in a panel comprises a soluble receptor. In some embodiments, the biomarkers or each biomarker in a panel comprises a soluble serum protein. Exemplary biomarkers or panel of biomarkers is described herein.

[0354] In some aspects, a biological sample, e.g., blood sample or tissue sample from the subject, can be obtained for detecting the presence or absence of a biomarker, such as for detecting or measuring a parameter (*e.g.* concentration, amount, level or activity) of the biomarker and/or assessing the presence of a biomarker, for analysis, correlation and/or detection of particular outcomes and/or toxicities. In some embodiments, certain physiological

or biological parameters associated with a biomarker, including expression of biomarkers and/or clinical and laboratory parameters, can be assessed, from a biological sample, e.g., blood, from subjects before or after administration of the cell therapy. In some embodiments, expression biomarkers and/or clinical and laboratory parameters, can be assessed from a biological sample, e.g., blood, from subjects before administration of the cell therapy (pre-treatment). In some embodiments, expression biomarkers or analytes and/or clinical and laboratory parameters, can be assessed from a biological sample, e.g., blood, from subjects after administration of the cell therapy (post-treatment). In some embodiments, the concentration, amount, level or activity of biomarkers and/or clinical and laboratory parameters can be assessed at one or more time points before or after administration of the cell therapy. In some embodiments, the peak concentration, amount, level or activity of biomarkers and/or clinical and laboratory parameters during a specified period of time can also be determined.

[0355] In some embodiments, a biomarker (in some cases, also called an analyte), including parameters associated with a biomarker or an analyte, is an objectively measurable characteristic or a molecule expressed by or in a biological sample, including cells, that can be indicative of or associated with a particular state or phenomenon, such as a biological process, a therapeutic outcome, a cell phenotype or a diseased state. In some aspects, a biomarker or parameters associated with a biomarker can be measured or detected. For example, the presence or absence of expression of a biomarker, can be detected. In some aspects, the parameters such as concentration, amount, level or activity of the biomarker can be measured or detected. In some embodiments, the presence, absence, expression, concentration, amount, level and/or activity of the biomarker can be associated with, correlated to, indicative of and/or predictive of particular states, such as particular therapeutic outcomes or state of the subject. In some aspects, the presence, absence, expression, concentration, amount, level and/or activity of the biomarker, such as any described herein, can be used to assess the likelihood of a particular outcome or state, such as a particular therapeutic outcome, including response outcome or toxicity outcome. In some embodiments, exemplary biomarkers include cytokines, cell surface molecules, chemokines, receptors, soluble receptors, soluble serum proteins and/or degradation products. In some embodiments, biomarkers can also include particular attributes, factors, characteristic of the patient and/or the disease or condition or factors indicative of the state of the patient and/or the disease or condition of the patient (including disease burden), and/or clinical or laboratory parameters.

[0356] In some embodiments, the parameter is or includes levels and/or concentrations of a

blood analyte. In some embodiments, the parameter is or includes levels and/or concentrations of an inflammatory marker. In some embodiments, the blood analyte and/or inflammatory marker is or includes levels and/or concentrations of interleukin-7 (IL-7), IL-15, macrophage inflammatory protein (MIP-1 α). In some embodiments, the blood analyte and/or inflammatory marker is or includes levels and/or concentrations of IL-6, IL-10, IL-16, interferon gamma (IFN- γ), tumor necrosis factor alpha (TNF- α), MIP-1 α , MIP-1 β , Monocyte chemoattractant protein-1 (MCP-1), and C-X-C motif chemokine 10 (CXCL10). In some embodiments, the blood analyte and/or inflammatory marker is or includes levels and/or concentrations of ferritin, C-reactive protein (CRP), D-dimer (fibrin degradation product), IL-6, IL-10, IL-15, IL-16, TNF- α , MIP-1 α , and MIP-1 β . In some embodiments, the blood analyte and/or inflammatory marker is or includes levels and/or concentrations of LDH, Ferritin, CRP, IL-6, IL-8, IL-10, TNF- α , IFN- α 2, MCP-1 and/or MIP-1 β . In some embodiments, the blood analyte and/or inflammatory marker is or includes levels and/or concentrations of CRP, Serum Amyloid A1 (SAA-1), IL-2, IL-6, IL-10, IL-15, TNF- α , MIP-1 α , MIP-1 β , MCP-1, CXCL10 and C-C Motif Chemokine Ligand 13 (CCL13). In some embodiments, the blood analyte and/or inflammatory marker is or includes levels and/or concentrations of LDH, ferritin, CRP, D-dimer, SAA-1, IL-6, IL-10, IL-15, IL-16, TNF- α , IFN- γ and/or MIP-1 α .

[0357] In some embodiments, an inflammatory marker is or includes the level or presence of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), albumin, ferritin, β 2 microglobulin (β 2-M), or lactate dehydrogenase (LDH) is detected and assessed. In some embodiments, the inflammatory marker is assessed using an immune assay. For example, an enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), radioimmunoassay (RIA), surface plasmon resonance (SPR), Western Blot, Lateral flow assay, immunohistochemistry, protein array or immuno-PCR (iPCR) can be used to detect the inflammatory marker. In some embodiments, using the articles of manufacture include detecting an inflammatory marker indicative of tumor burden. In some cases, the assaying or assessing of an inflammatory marker is using flow cytometry. In some cases, the reagent is a soluble protein that binds the inflammatory marker. In some example, the reagent is a protein that binds C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), albumin, ferritin, β 2 microglobulin (β 2-M), or lactate dehydrogenase (LDH).

[0358] In some embodiments, the biomarker, e.g., inflammatory marker is or includes C-reactive protein (CRP). In some embodiments, CRP is assessed using an in vitro enzyme-linked immunosorbent assay to obtain a quantitative measurement of human CRP from a sample such

as serum, plasma, or blood. In some examples, CRP is detected using a human Enzyme- Linked Immunosorbent Assay (ELISA). In some embodiments, the biomarker, e.g. inflammatory marker is or includes erythrocyte sedimentation rate (ESR). In some embodiments, ESR is assessed by measuring the distance (in millimeters per hour) that red cells have fallen after separating from the plasma in a vertical pipette or tube. In some embodiments the biomarker is or includes albumin. In some aspects, albumin is assessed using a colorimetric test or an in vitro enzyme-linked immunosorbent assay. In some examples, albumin is detected using a human Enzyme- Linked Immunosorbent Assay (ELISA). In some embodiments, the biomarker, e.g., inflammatory marker is or includes ferritin or $\beta 2$ microglobulin. In some embodiments, ferritin or $\beta 2$ microglobulin is assessed using an immunoassay or detected using an ELISA. In some aspects, the biomarker, e.g., inflammatory marker is or includes lactate dehydrogenase (LDH), and LDH is assessed using a colorimetric test or an in vitro enzyme-linked immunosorbent assay.

[0359] In some embodiments, the one or more biomarkers include two or more biomarkers, e.g., cytokines, such as inflammatory cytokines, and/or patient attributes, e.g., tumor burden and/or expression of inflammatory markers. In some aspects, the two or more biomarkers are measured simultaneously from the same sample. In other aspects, the two or more biomarkers are measured or sequentially from the same sample or from different samples from the subject.

[0360] In some embodiments, the level, amount, concentration or other parameter of the biomarker or the panel of biomarkers are indicative of pharmacokinetic parameters of the cells, e.g., maximum (peak) plasma concentration (C_{\max}), the peak time (*i.e.* when maximum plasma concentration (C_{\max}) occurs; T_{\max}), the minimum plasma concentration (*i.e.* the minimum plasma concentration between doses of a therapeutic agent, e.g., CAR+ T cells; C_{\min}), the elimination half-life ($T_{1/2}$) and area under the curve (*i.e.* the area under the curve generated by plotting time versus plasma concentration of the therapeutic agent CAR+ T cells; AUC; such as AUC_{0-28}). In some embodiments, the level, amount, concentration of the biomarker or the panel of biomarkers are indicative of the risk of developing a toxicity, e.g., neurotoxicity, such as severe neurotoxicity and/or CRS, such as sCRS. In some embodiments, the level, amount, concentration of the biomarker or the panel of biomarkers are indicative of, correlate with and/or associate with the likelihood and/or probability of response, e.g., objective response (OR), complete response (CR) or partial response (PR), or durable response, e.g., 3-month response.

[0361] In some embodiments, the parameter is or includes levels, concentrations and/or numbers of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), albumin, ferritin, $\beta 2$

microglobulin (β 2-M), lactate dehydrogenase (LDH) and/or is an inflammatory cytokine. In some embodiments, the inflammatory marker is LDH. In some embodiments, the level, concentration and/or number of LDH is a surrogate for disease burden, e.g., for tumors or cancers, and may be useful for potential neurotoxicity risk assessment and/or risk-adapted dosing or adjustment of treatment of certain subjects. In some aspects, LDH levels may be assessed alone and/or in combination with another pre-treatment parameter, such as another measure or indicator of disease burden, such as a volumetric tumor measurement such as sum of product dimensions (SPD) or other CT-based or MRI-based volumetric measurement of disease burden, such as any described herein. In some aspects, one or more parameters indicative of disease burden are assessed, and in some contexts may indicate the presence, absence or degree of risk of developing neurotoxicity following the T cell therapy. In some aspects, the one or more parameters include LDH and/or a volumetric tumor measurement. In some embodiments, the parameter is SPD and/or LDH.

[0362] In some embodiments, the parameter is a patient attribute, factor and/or characteristic. In some embodiments, the parameter is a pre-treatment measurement, e.g., a baseline measurement, a pre-infusion measurement and/or a pre-lymphodepletion measurement. In some embodiments, the parameter is assessed before treatment, e.g., before administration of the cell therapy, or a first administration or dose thereof, or after the initiation of any of the foregoing. and/or lymphodepletion prior to cell therapy. In some embodiments, the parameter is assessed prior to lymphodepletion. In some embodiments, the parameter is assessed prior to administration of the cell therapy (e.g., pre-infusion), e.g., obtained up to 2 days, up to 7 days, up to 14 days, up to 21 days, up to 28 days, up to 35 days or up to 40 days prior to initiation of the administration of the engineered cells. In some embodiments, the reagents can be used prior to the administration of the cell therapy or after the administration of cell therapy, for diagnostic purposes, to assess parameters such as a patient attribute, factor and/or characteristics.

[0363] In some embodiments, the pre-treatment measurement is or includes the level and/or concentration of C-reactive protein (CRP), D-dimer (fibrin degradation product), Ferritin, IFN- α 2, IFN- γ , IL-6, IL-7, IL-8, IL-10, IL-15, IL-16, lactate dehydrogenase (LDH), macrophage inflammatory protein (MIP-1 α), MIP-1 β , MCP-1, SAA-1 and/or TNF- α .

[0364] In some embodiments, higher or lower pre-treatment measurement of one or more of the parameters is correlated to and/or is associated with higher or lower pharmacokinetic parameters, e.g., C_{\max} or AUC, of CAR+ T cells and/or higher or lower rate and/or incidence of toxicity, e.g., CRS or NT, such as severe CRS or severe NT. In some embodiments, higher or

lower pre-treatment measurement of one or more of the parameters is correlated to and/or is associated with higher or lower response, e.g., ORR including CR and PR, and/or higher or lower durability of response, e.g., 3-month response.

[0365] In some embodiments, higher pre-treatment measurement of one or more of the parameters is correlated to and/or is associated with higher pharmacokinetic parameters, e.g., C_{\max} or AUC, of CAR+ T cells and/or higher rate and/or incidence of toxicity, e.g., CRS or NT, such as severe CRS or severe NT.

[0366] In some embodiments, the parameter is or includes a post-treatment measurement, e.g., a peak or maximum measurement after administration of the therapy, e.g., cell therapy, and/or a post-infusion measurement and/or measurement after administration of the cell therapy, or a first administration or dose thereof, or after the initiation of any of the foregoing. In some embodiments, the peak measurement is or includes the peak or maximum value within a period of time after a certain amount of time after administration of the cell therapy and/or initiation thereof, such as within or within at or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 21, 28, 30, 60 or 90 or more days, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more weeks, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18, 24, 48 or more months, after administration of the cell therapy, or a first administration or dose thereof, or after the initiation of any of the foregoing.

[0367] In some embodiments, the parameter is or includes peak level and/or concentration of inflammatory markers, including cytokines or chemokines. In some embodiments, lower peak measurements of one or more of the parameters is correlated to and/or is associated with higher pharmacokinetic parameters, e.g., C_{\max} or AUC, of CAR+ T cells and/or higher rate and/or incidence of toxicity, e.g., CRS or NT, such as severe CRS or severe NT. In some embodiments, lower pre-treatment measurement of one or more of the parameters is correlated to and/or is associated with response, e.g., ORR including CR and PR, and/or lower durability of response, e.g., 3-month response.

[0368] In some embodiments, the parameter is or includes peak level and/or concentration of inflammatory markers, including cytokines or chemokines. In some embodiments, the parameter is or includes peak level and/or concentration of biomarkers, including C-C Motif Chemokine Ligand 13 (CCL13), C-reactive protein (CRP), C-X-C motif chemokine 10 (CXCL10), IL-2, IL-5, IL-6, IL-7, IL-8, IL-10, IL-15, IL-16, interferon gamma (IFN- γ), Lymphotoxin-alpha (LT- α), Monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein 1 alpha (MIP-1 α), MIP-1 β , Serum Amyloid A1 (SAA-1), Transforming growth factor beta (TGF- β) and tumor necrosis factor alpha (TNF- α). In some embodiments,

higher peak levels and/or concentrations of one or more of the parameters is correlated to and/or is associated with higher rate and/or incidence of toxicity, e.g., CRS or NT, such as severe CRS or severe NT. In some embodiments, lower peak levels and/or concentrations is correlated to and/or is associated with higher response, e.g., ORR including CR and PR, and/or higher durability of response, e.g., 3-month response.

[0369] In some embodiments, the biomarkers (e.g., analytes), include those associated with increased pharmacokinetic (PK) parameters of the cell, e.g., increased maximum serum concentration of cell (C_{\max}) or increased exposure (e.g., area under the curve (AUC)). In some embodiments, the biomarkers (e.g. analytes), including parameters thereof, include IL-7, IL-15, MIP-1 α and TNF- α .

[0370] In some embodiments, the biomarkers (e.g., analytes) include those associated with a response outcome, and/or a durable response. In some embodiments, the biomarkers (e.g. analytes), including parameters thereof, include LDH, ferritin, CRP, D-dimer, Serum Amyloid A1 (SAA-1), IL-6, IL-10, IL-15, IL-16, TNF- α , IFN- γ , MIP-1 α and C-X-C motif chemokine 10 (CXCL10).

[0371] In some embodiments, the threshold value is a value that: is within 25%, within 20%, within 15%, within 10%, or within 5% above the average value of the volumetric measure or inflammatory marker and/or is within a standard deviation above the average value of the volumetric measure or the inflammatory marker in a plurality of control subjects. In some embodiments, the threshold value is a value that: is above the highest value of the volumetric measure or inflammatory marker, optionally within 50%, within 25%, within 20%, within 15%, within 10%, or within 5% above such highest fold change, measured in at least one subject from among a plurality of control subjects. In some embodiments, the threshold value is a value that: is above the highest value of the volumetric measure or inflammatory marker as measured among more than 75%, 80%, 85%, 90%, or 95%, or 98% of subjects from a plurality of control subjects. In some embodiments, the plurality of control subjects are a group of subjects prior to receiving a dose of the genetically engineered cells, wherein: each of the control subjects of the group exhibited a peak CAR+ T cells in the blood greater than the highest peak CAR+ T cells in the therapeutic range; each of the control subjects of the group went on to develop at toxicity, optionally a neurotoxicity or cytokine release syndrome (CRS), a grade 2 or grade 3 or higher neurotoxicity or a grade 3 or higher CRS, after receiving a dose of the engineered cells for treating the same disease or condition; each of the control subjects of the group did not develop a response, optionally a complete response (CR) or partial response (PR), following

administration of the dose of genetically engineered cells; and/or each of the control subjects of the group did not develop a durable response, optionally for at or about or greater than or about 3 months or at or about or greater than or about 6 months, following administration of the dose of genetically engineered cells.

[0372] In some embodiments, the parameters, such as attributes, factors, characteristic of the patient and/or the disease or condition, and/or expression of biomarkers, can be assessed for particular subjects or in particular samples, and can be compared to a threshold value (also referred to in some cases as threshold level). In some aspects, such comparison can be used to calculate or assess the likelihood for response or risk of toxicity to a therapy, such as a cell therapy. In some aspects, parameters, such as attributes, factors, characteristic of the patient and/or the disease or condition, and/or expression of biomarkers, that are above or below a certain threshold value can be associated with, correlated with, predictive of or indicative of particular outcomes of a therapy, such as a response outcome or a toxicity outcome. In some embodiments, exemplary threshold values can be determined based on the mean or median values and values within a range or standard deviation of the mean or median values of the level, amount or concentration of the biomarker in a biological sample obtained from a group of subjects prior to receiving a cell therapy, wherein each of the subjects of the group went on to exhibit a particular outcome, such as a particular therapeutic outcome, including either exhibiting a response or not exhibiting a response; or either developing a toxicity or not developing a toxicity.

1. Exemplary Biomarkers Associated with Response Outcomes

[0373] In some embodiments, the biomarker is associated with, correlated to, indicative of and/or predictive of a particular outcome, such as a particular response outcome, such as an objective response (OR) a complete response (CR) or a partial response (PR), or durable response, such as an OR or CR or a PR that is durable at 3, 6, 9 months or more. In some embodiments, lower or reduced levels or increased levels of one or more of such biomarkers (e.g., biomarkers), such as compared to a reference value or threshold value, can be associated with the a response, such as an OR, CR or PR, or any response outcomes described herein, e.g., in Section II.B, optionally a durable response, such as a response that is durable for at least 3 months, 6 months or more.

[0374] In some embodiments, the biomarker is associated with, correlated to, indicative of and/or predictive of a particular outcome, such as a particular response or durable response

outcome, in a subject that has been administered a cell therapy, such as with a composition containing genetically engineered cells. In some embodiments, the presence, expression, level, amount or concentration of one or more biomarker in a biological sample obtained from a subject prior to the administration of cell therapy, can be associated with, correlated to, indicative of and/or predictive of a particular outcome, such as a particular response or durable response outcome. In some embodiments, presence, expression, level, amount or concentration of particular biomarkers can be correlated to a particular response or durable response outcome. In some embodiments, the response outcome can be any response outcomes described herein, e.g., in Section II.B.

[0375] In some embodiments, the methods include comparing, individually, the level, amount or concentration of the biomarker in the sample to a threshold value, thereby determining a likelihood that a subject will achieve a response to the cell therapy. In some embodiments, the methods include selecting a subject who is likely to respond to treatment based on the results of determining a likelihood that a subject will achieve a response to the cell therapy by comparing, individually, the level, amount or concentration of the biomarker in the sample to a threshold value. In some embodiments, the methods also include administering the cell therapy to the subject selected for treatment. In some embodiments, if the subject is determined as not likely to achieve a response or a durable response, further comprising administering an additional therapeutic agent to the subject.

[0376] In some embodiments, the biomarkers include those associated with a response outcome, and/or a durable response. In some embodiments, the biomarkers, including parameters thereof, include LDH, ferritin, CRP, D-dimer, Serum Amyloid A1 (SAA-1), IL-6, IL-10, IL-15, IL-16, TNF- α , IFN- γ , MIP-1 α and C-X-C motif chemokine 10 (CXCL10).

[0377] In some aspects, exemplary biomarkers or biomarkers that can be assessed or analyzed with respect to assessment of likelihood of response after administration of a cell therapy include one or more biomarker selected from ferritin, LDH, CXCL10, G-CSF, and IL-10. In some embodiments, for any of the foregoing biomarkers or biomarkers, the subject is likely to achieve a response if the level, amount or concentration of the one or more of the biomarker is below a threshold value and the subject is not likely to achieve a response if the level, amount or concentration of the one or more of the biomarker is above a threshold value. In some embodiments, the response is or comprises objective response. In some embodiments, the objective response is or comprises complete response (CR) or partial response (PR). In some aspects, reduced levels of ferritin, LDH, CXCL10, G-CSF, and IL-10, in a biological sample

from a subject obtained prior to administration of a cell therapy (pre-treatment), can be associated with achieving objective response, including complete response (CR) or partial response (PR).

[0378] In some embodiments, the threshold value is within 25%, within 20%, within 15%, within 10% or within 5% and/or is within a standard deviation below the median or mean level, amount or concentration of ferritin, LDH, CXCL10, G-CSF, or IL-10 in a biological sample obtained from a group of subjects prior to receiving a cell therapy, wherein each of the subjects of the group went on to achieve a response after administration of a recombinant-receptor-expressing therapeutic cell composition for treating the same disease or condition. In some embodiments, the threshold value is within 25%, within 20%, within 15%, within 10% or within 5% and/or is within a standard deviation above the median or mean level, amount or concentration of ferritin, LDH, CXCL10, G-CSF, or IL-10 in a biological sample obtained from a group of subjects prior to receiving a cell therapy, wherein each of the subjects of the group went on to exhibit stable disease (SD) and/or progressive disease (PD) after administration of a recombinant-receptor-expressing therapeutic cell composition for treating the same disease or condition.

[0379] In some aspects, exemplary biomarkers or biomarkers that can be assessed or analyzed with respect to assessment of likelihood of durable response after administration of a cell therapy include one or more biomarker selected from LDH, ferritin, CRP, D-dimer, SAA-1, IL-6, IL-10, IL-15, IL-16, TNF- α , IFN- γ , MIP-1 α , CXCL-10, IL-8, MCP-1 and MIP-1 β . In some embodiments, for any of the foregoing biomarkers or biomarkers, the subject is likely to achieve a durable response if the level, amount or concentration of the one or more of the biomarker is below a threshold value and the subject is not likely to achieve a durable response if the level, amount or concentration of the one or more of the biomarker is above a threshold value. In some embodiments, the durable response is or comprises a complete response (CR) or partial response (PR) that is durable for at or greater than 3 months, 4 months, 5 months, or 6 months. In some embodiments, the durable response is or comprises a CR or PR that is durable for at least 3 months. In some aspects, reduced levels of LDH, ferritin, CRP, D-dimer, SAA-1, IL-6, IL-10, IL-15, IL-16, TNF- α , IFN- γ , MIP-1 α , CXCL-10, IL-8, MCP-1 and MIP-1 β , in a biological sample from a subject obtained prior to administration of a cell therapy (pre-treatment), can be associated with achieving durable response, such as a CR or PR that is durable for at least 3 months.

[0380] In some embodiments, the threshold value is within 25%, within 20%, within 15%,

within 10% or within 5% and/or is within a standard deviation below the median or mean level, amount or concentration of LDH, ferritin, CRP, D-dimer, SAA-1, IL-6, IL-10, IL-15, IL-16, TNF- α , IFN- γ , MIP-1 α , CXCL-10, IL-8, MCP-1 or MIP-1 β in a biological sample obtained from a group of subjects prior to receiving a cell therapy, wherein each of the subjects of the group went on to achieve a durable response after administration of a recombinant-receptor-expressing therapeutic cell composition for treating the same disease or condition.

[0381] In some embodiments, the threshold value is within 25%, within 20%, within 15%, within 10% or within 5% and/or is within a standard deviation above the median or mean level, amount or concentration of LDH, ferritin, CRP, D-dimer, SAA-1, IL-6, IL-10, IL-15, IL-16, TNF- α , IFN- γ , MIP-1 α , CXCL-10, IL-8, MCP-1 or MIP-1 β in a biological sample obtained from a group of subjects prior to receiving a cell therapy, wherein each of the subjects of the group did not achieve a durable response after administration of a recombinant-receptor-expressing therapeutic cell composition for treating the same disease or condition.

[0382] In some embodiments, the response is durable response, such as a CR or PR that is durable for at least 3 months.

[0383] In some embodiments, the threshold value for LDH is at or at about or below or below about 600 U/L, 500 U/L, 400 U/L, 300 U/L or 200 U/L.

[0384] In some embodiments, exemplary threshold value for ferritin is at or at about or below or below about 1000 μ g/L, 900 μ g/L, 800 μ g/L, 700 μ g/L, 600 μ g/L, 500 μ g/L, 400 μ g/L, 300 μ g/L or 200 μ g/L.

[0385] In some embodiments, exemplary threshold value for CRP is at or at about or below or below about 20 mg/L, 19 mg/L, 18 mg/L, 17 mg/L, 16 mg/L, 15 mg/L, 14 mg/L, 13 mg/L, 12 mg/L, 11 mg/L, 10 mg/L, 9 mg/L, 8 mg/L, 7 mg/L, 6 mg/L or 5 mg/L.

[0386] In some embodiments, exemplary threshold value for D-dimer is at or at about or below or below about 1000 μ g/L, 900 μ g/L, 800 μ g/L, 700 μ g/L, 600 μ g/L, 500 μ g/L, 400 μ g/L, 300 μ g/L or 200 μ g/L.

[0387] In some embodiments, exemplary threshold value for SAA-1 is at or at about or below or below about 100 mg/L, 90 mg/L, 80 mg/L, 70 mg/L, 60 mg/L, 50 mg/L, 40 mg/L, 30 mg/L or 20 mg/L.

[0388] In some embodiments, exemplary threshold value for IL-6 is at or at about or below or below about 6 pg/mL, 5 pg/mL, 4 pg/mL, 3 pg/mL or 2 pg/mL.

[0389] In some embodiments, exemplary threshold value for IL-10 is at or at about or below or below about 2 pg/mL, 1 pg/mL, 0.9 pg/mL, 0.8 pg/mL, 0.7 pg/mL, 0.6 pg/mL or 0.5 pg/mL.

[0390] In some embodiments, exemplary threshold value for IL-15 is at or at about or below or below about 7 pg/mL, 6 pg/mL, 5 pg/mL, 4 pg/mL or 3 pg/mL.

[0391] In some embodiments, exemplary threshold value for IL-16 is at or at about or below or below about 1000 pg/mL, 900 pg/mL, 800 pg/mL, 700 pg/mL or 600 pg/mL.

[0392] In some embodiments, exemplary threshold value for TNF- α is at or at about or below or below about 10 pg/mL, 9 pg/mL, 8 pg/mL, 7 pg/mL or 6 pg/mL.

[0393] In some embodiments, exemplary threshold value for IFN- γ is at or at about or below or below about 30 pg/mL, 20 pg/mL, 10 pg/mL, 9 pg/mL, 8 pg/mL or 7 pg/mL;

[0394] In some embodiments, exemplary threshold value for MIP-1 α is at or at about or below or below about 40 pg/mL, 30 pg/mL or 20 pg/mL; and/or

[0395] In some embodiments, exemplary threshold value for CXCL-10 is at or at about or below or below about 1500 pg/mL, 1000 pg/mL, 900 pg/mL, 800 pg/mL, 700 pg/mL, 600 pg/mL or 500 pg/mL.

[0396] In some aspects, exemplary biomarkers or biomarkers that can be assessed or analyzed with respect to assessment of likelihood of durable response after administration of a cell therapy include one or more biomarker selected from ferritin, CRP, LDH, CXCL10, IL-8, IL-10, IL-15, MCP-1, MIP-1 β and TNF- α . In some embodiments, for any of the foregoing biomarkers or biomarkers, the subject is likely to achieve a durable response if the level, amount or concentration of the one or more of the biomarker is below a threshold value and the subject is not likely to achieve a durable response if the level, amount or concentration of the one or more of the biomarker is above a threshold value. In some embodiments, the durable response is or comprises a complete response (CR) or partial response (PR) that is durable for at or greater than 3 months, 4 months, 5 months, or 6 months. In some embodiments, the durable response is or comprises a CR or PR that is durable for at least 3 months. In some aspects, reduced levels of ferritin, CRP, LDH, CXCL10, IL-8, IL-10, IL-15, MCP-1, MIP-1 β and TNF- α , in a biological sample from a subject obtained prior to administration of a cell therapy (pre-treatment), can be associated with achieving durable response, such as a CR or PR that is durable for at least 3 months.

[0397] In some embodiments, the threshold value is within 25%, within 20%, within 15%, within 10% or within 5% and/or is within a standard deviation below the median or mean level, amount or concentration of ferritin, CRP, LDH, CXCL10, IL-8, IL-10, IL-15, MCP-1, MIP-1 β or TNF- α in a biological sample obtained from a group of subjects prior to receiving a cell therapy, wherein each of the subjects of the group went on to achieve a durable response after

administration of a recombinant-receptor-expressing therapeutic cell composition for treating the same disease or condition.

[0398] In some embodiments, the threshold value is within 25%, within 20%, within 15%, within 10% or within 5% and/or is within a standard deviation above the median or mean level, amount or concentration of ferritin, CRP, LDH, CXCL10, IL-8, IL-10, IL-15, MCP-1, MIP-1 β or TNF- α in a biological sample obtained from a group of subjects prior to receiving a cell therapy, wherein each of the subjects of the group did not achieve a durable response after administration of a recombinant-receptor-expressing therapeutic cell composition for treating the same disease or condition.

[0399] In some aspects, exemplary biomarkers or biomarkers that can be assessed or analyzed with respect to assessment of likelihood of durable response after administration of a cell therapy include one or more biomarker selected from hemoglobin and albumin. In some embodiments, for any of the foregoing biomarkers or biomarkers, the subject is likely to achieve a durable response if the level, amount or concentration of the one or more of the biomarker is above a threshold value and the subject is not likely to achieve a durable response if the level, amount or concentration of the one or more of the biomarker is below a threshold value. In some embodiments, the durable response is or comprises a complete response (CR) or partial response (PR) that is durable for at or greater than 3 months, 4 months, 5 months, or 6 months. In some embodiments, the durable response is or comprises a CR or PR that is durable for at least 3 months. In some aspects, elevated levels of hemoglobin and albumin, in a biological sample from a subject obtained prior to administration of a cell therapy (pre-treatment), can be associated with achieving durable response, such as a CR or PR that is durable for at least 3 months.

[0400] In some embodiments, the threshold value is within 25%, within 20%, within 15%, within 10% or within 5% and/or is within a standard deviation above the median or mean level, amount or concentration of hemoglobin or albumin in a biological sample obtained from a group of subjects prior to receiving a cell therapy, wherein each of the subjects of the group went on to achieve a durable response after administration of a recombinant-receptor-expressing therapeutic cell composition for treating the same disease or condition.

[0401] In some embodiments, the threshold value is within 25%, within 20%, within 15%, within 10% or within 5% and/or is within a standard deviation below the median or mean level, amount or concentration of hemoglobin or albumin in a biological sample obtained from a group of subjects prior to receiving a cell therapy, wherein each of the subjects of the group did not

achieve a durable response after administration of a recombinant-receptor-expressing therapeutic cell composition for treating the same disease or condition.

2. Exemplary Biomarkers Associated with Toxicity Outcomes

[0402] In some embodiments, the biomarker is associated with, correlated to, indicative of and/or predictive of a particular outcome, such as development of a toxicity, in a subject that has been administered a cell therapy, such as with a composition containing genetically engineered cells. In some embodiments, the presence, expression, level, amount or concentration of one or more biomarker in a biological sample obtained from a subject prior to the administration of cell therapy, can be associated with, correlated to, indicative of and/or predictive of a particular outcome, such as development of a toxicity, such as any toxicity outcomes described herein, e.g., in Section II.A. In some embodiments, presence, expression, level, amount or concentration of particular biomarkers can be correlated to particular outcomes or toxicities, e.g., development of NT or CRS. In some embodiments, the toxicity is a toxicity potentially associated with cell therapy, such as any described herein, for example, in Section II.A. In some embodiments, the toxicity is neurotoxicity (NT) or cytokine release syndrome (CRS). In some embodiments, the toxicity is a severe NT or severe CRS. In some embodiments, the toxicity is grade 2 or higher NT or grade 2 or higher CRS. In some embodiments, the toxicity is grade 3 or higher NT or grade 3 or higher CRS.

[0403] In some embodiments, the methods include comparing, individually, the level, amount or concentration of the biomarker in the sample to a threshold value, thereby determining a risk of developing a toxicity after administration of the cell therapy. In some embodiments, the methods include identifying a subject who has a risk of developing a toxicity after administration of a cell therapy based by comparing, individually, the level, amount or concentration of the biomarker in the sample to a threshold value. In some embodiments, the methods also include following or based on the results of the assessment, administering to the subject the cell therapy, and, optionally, an agent or other treatment capable of treating, preventing, delaying, reducing or attenuating the development or risk of development of a toxicity. In some embodiments, the methods also involve monitoring the subject for symptoms of toxicity if the subject is administered a cell therapy and is identified as having a risk of developing a toxicity.

[0404] In some embodiments, if the subject is identified as having a risk of developing a toxicity, one or more of the following steps can be performed can be administered to the subject:

(a) (1) an agent or other treatment capable of treating, preventing, delaying, reducing or attenuating the development or risk of development of a toxicity and (2) the cell therapy, wherein administration of the agent is to be administered (i) prior to, (ii) within one, two, or three days of, (iii) concurrently with and/or (iv) at first fever following, the initiation of administration of the cell therapy to the subject; and/or (b) administering to the subject a cell therapy at a reduced dose or at a dose that is not associated with risk of developing toxicity or severe toxicity, or is not associated with a risk of developing a toxicity or severe toxicity in a majority of subjects, and/or a majority of subjects having a disease or condition that the subject has or is suspected of having, following administration of the cell therapy; and/or (c) administering to the subject a cell therapy in an inpatient setting and/or with admission to the hospital for one or more days, optionally wherein the cell therapy is otherwise to be administered to subjects on an outpatient basis or without admission to the hospital for one or more days.

[0405] In some embodiments, biomarkers or biomarkers, including parameters thereof, that can be assessed include Lactate dehydrogenase (LDH), ferritin, C-reactive protein (CRP), Interleukin-6 (IL-6), IL-7, IL-8, IL-10, IL-15, IL-16, tumor necrosis factor alpha (TNF- α), interferon alpha 2 (IFN- α 2), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein 1 alpha (MIP-1 α), macrophage inflammatory protein 1 beta (MIP-1 β), Eotaxin, Granulocyte-colony stimulating factor (G-CSF), IL-1 receptor alpha (IL-1R α), IL-1 β , IFN- γ -Inducible Protein 10 (IP-10), perforin, and D-dimer (fibrin degradation product). In some embodiments, the biomarkers, including parameters thereof, include LDH, ferritin, CRP, IL-6, IL-8, IL-10, TNF- α , IFN- α 2, MCP-1 and MIP-1 β . In some embodiments, the biomarkers, including parameters thereof, include ferritin, CRP, D-dimer, IL-6, IL-15, TNF- α and MIP-1 α . In some embodiments, the biomarkers, including parameters thereof, include ferritin, CRP, IL-10, IL-15, IL-16, TNF- α , or MIP-1 β . In some embodiments, elevated levels or increased levels of one or more of such biomarkers (e.g, biomarkers), such as compared to a reference value or threshold value, can be associated with the development of neurotoxicity, e.g. severe neurotoxicity or grade 3 or higher or grade 4 or 5 neurotoxicity. In some embodiments, elevated levels or increased levels of one or more of such biomarkers (e.g, biomarkers), such as compared to a reference value or threshold value, can be associated with the development of neurotoxicity, e.g. severe neurotoxicity or grade 3 or higher or grade 4 or 5 neurotoxicity.

[0406] In some aspects, exemplary biomarkers or biomarkers that can be assessed or analyzed with respect to assessment of the risk of developing a toxicity after administration of a

cell therapy include one or more biomarker selected from LDH, Ferritin, C-reactive protein (CRP), IL-6, IL-8, IL-10, TNF- α , IFN- α 2, MCP-1 and MIP-1 β . In some embodiments, for any of the foregoing biomarkers or biomarkers, the subject has a risk of developing a toxicity if the level, amount or concentration of the one or more of the biomarker is above a threshold value and the subject has a low risk of developing a toxicity if the level, amount or concentration of the one or more of the biomarker is below a threshold value. In some embodiments, the toxicity is neurotoxicity. In some aspects, elevated levels of LDH, Ferritin, C-reactive protein (CRP), IL-6, IL-8, IL-10, TNF- α , IFN- α 2, MCP-1 and MIP-1 β , in a biological sample from a subject obtained prior to administration of a cell therapy (pre-treatment), can be associated with a higher risk of developing a neurotoxicity.

[0407] In some embodiments, the threshold value is within 25%, within 20%, within 15%, within 30% or within 5% and/or is within a standard deviation above the median or mean level, amount or concentration of LDH, Ferritin, C-reactive protein (CRP), IL-6, IL-8, IL-10, TNF- α , IFN- α 2, MCP-1 or MIP-1 β in a biological sample obtained from a group of subjects prior to receiving a cell therapy, wherein each of the subjects of the group went on not develop any toxicity after receiving a recombinant-receptor-expressing therapeutic cell composition for treating the same disease or condition.

[0408] In some embodiments, the threshold value is within 25%, within 20%, within 15%, within 30% or within 5% and/or is within a standard deviation below the median or mean level, amount or concentration of LDH, Ferritin, C-reactive protein (CRP), IL-6, IL-8, IL-10, TNF- α , IFN- α 2, MCP-1 or MIP-1 β in a biological sample obtained from a group of subjects prior to receiving a cell therapy, wherein each of the subjects of the group went on to develop a toxicity after receiving a recombinant-receptor-expressing therapeutic cell composition for treating the same disease or condition.

[0409] In some embodiments, the toxicity is neurotoxicity.

[0410] In some embodiments, exemplary threshold value for LDH is at or at about or above or above about 300 U/L, 400 U/L, 500 U/L, 600 U/L or 700 U/L.

[0411] In some embodiments, exemplary threshold value for Ferritin is at or at about or above or above about 500 ng/mL, 600 ng/mL, 700 ng/mL, 800 ng/mL, 900 ng/mL, 1000 ng/mL or 1500 ng/mL.

[0412] In some embodiments, exemplary threshold value for CRP is at or at about or above or above about 20 mg/L, 30 mg/L, 40 mg/L, 50 mg/L, 60 mg/L, 70 mg/L or 80 mg/L.

[0413] In some embodiments, exemplary threshold value for IL-6 is at or at about or above

or above about 5 pg/mL, 6 pg/mL, 7 pg/mL, 8 pg/mL, 9 pg/mL, 10 pg/mL, 20 pg/mL or 30 pg/mL.

[0414] In some embodiments, exemplary threshold value for IL-8 is at or at about or above or above about 8 pg/mL, 9 pg/mL, 10 pg/mL, 20 pg/mL or 30 pg/mL.

[0415] In some embodiments, exemplary threshold value for IL-10 is at or at about or above or above about 20 pg/mL, 30 pg/mL, 40 pg/mL, 50 pg/mL, 60 pg/mL or 70 pg/mL.

[0416] In some embodiments, exemplary threshold value for TNF- α is at or at about or above or above about 20 pg/mL or 30 pg/mL.

[0417] In some embodiments, exemplary threshold value for IFN- α 2 is at or at about or above or above about 40 pg/mL, 50 pg/mL, 60 pg/mL, 70 pg/mL or 80 pg/mL.

[0418] In some embodiments, exemplary threshold value for MCP-1; and/or is at or at about or above or above about 200 pg/mL or 300 pg/mL.

[0419] In some embodiments, exemplary threshold value for MIP-1 β is at or at about or above or above about 40 pg/mL, 50 pg/mL, 60 pg/mL, 70 pg/mL or 80 pg/mL.

[0420] In some aspects, exemplary biomarkers or biomarkers that can be assessed or analyzed with respect to assessment of the risk of developing a toxicity after administration of a cell therapy include one or more biomarker selected from IL-8, IL-10 and CXCL10. In some embodiments, for any of the foregoing biomarkers or biomarkers, the subject has a risk of developing a toxicity if the level, amount or concentration of the one or more of the biomarker is above a threshold value and the subject has a low risk of developing a toxicity if the level, amount or concentration of the one or more of the biomarker is below a threshold value. In some embodiments, the toxicity is neurotoxicity. In some embodiments, the toxicity is severe neurotoxicity or a grade 3 or higher neurotoxicity. In some aspects, elevated levels of IL-8, IL-10 and CXCL10, in a biological sample from a subject obtained prior to administration of a cell therapy (pre-treatment), can be associated with a higher risk of developing a neurotoxicity, or a severe neurotoxicity or a grade 3 or higher neurotoxicity.

[0421] In some embodiments, the threshold value is within 25%, within 20%, within 15%, within 30% or within 5% and/or is within a standard deviation above the median or mean level, amount or concentration of IL-8, IL-10 or CXCL10 in a biological sample obtained from a group of subjects prior to receiving a cell therapy, wherein each of the subjects of the group went on not develop any toxicity after receiving a recombinant-receptor-expressing therapeutic cell composition for treating the same disease or condition.

[0422] In some embodiments, the threshold value is within 25%, within 20%, within 15%,

within 30% or within 5% and/or is within a standard deviation below the median or mean level, amount or concentration of IL-8, IL-10 or CXCL10 in a biological sample obtained from a group of subjects prior to receiving a cell therapy, wherein each of the subjects of the group went on to develop a toxicity after receiving a recombinant-receptor-expressing therapeutic cell composition for treating the same disease or condition.

[0423] In some aspects, exemplary biomarkers or biomarkers or a volumetric measure of tumor burden that can be assessed or analyzed with respect to assessment of the risk of developing a toxicity after administration of a cell therapy include one or more biomarker or volumetric measure of tumor burden selected from a sum of the products of diameters (SPD), LDH, Ferritin, C-reactive protein (CRP), D-dimer (fibrin degradation product), IL-6, IL-10, IL-15, IL-16 TNF- α , MIP-1 α and MIP-1 β . In some embodiments, for any of the foregoing biomarkers or biomarkers or volumetric measure of tumor burden, the subject has a risk of developing a toxicity if the level, amount or concentration of the one or more of the biomarker or the volumetric measure of tumor burden is above a threshold value and the subject has a low risk of developing a toxicity if the level, amount or concentration of the one or more of the biomarker or the volumetric measure of tumor burden is below a threshold value. In some embodiments, the toxicity is neurotoxicity. In some aspects, elevated levels or measure of a sum of the products of diameters (SPD), LDH, Ferritin, C-reactive protein (CRP), D-dimer (fibrin degradation product), IL-6, IL-10, IL-15, IL-16 TNF- α , MIP-1 α and MIP-1 β , in a biological sample from a subject obtained prior to administration of a cell therapy (pre-treatment), can be associated with a higher risk of developing a neurotoxicity (NT) or a cytokine release syndrome (CRS).

[0424] In some embodiments, the one or more biomarker or volumetric measure of tumor burden selected from LDH, SPD, IL-10, IL-15, IL-16, TNF- α and MIP-1 β , and the toxicity is neurotoxicity. In some embodiments, the one or more biomarker or volumetric measure of tumor burden selected from LDH, SPD, CRP, d-dimer, IL-6, IL-15, TNF- α and MIP-1 α , and the toxicity is CRS. In some aspects, elevated levels or measure of LDH, SPD, IL-10, IL-15, IL-16, TNF- α and MIP-1 β , in a biological sample from a subject obtained prior to administration of a cell therapy (pre-treatment), can be associated with a higher risk of developing a neurotoxicity (NT). In some aspects, elevated levels or measure of LDH, SPD, CRP, d-dimer, IL-6, IL-15, TNF- α and MIP-1 α , in a biological sample from a subject obtained prior to administration of a cell therapy (pre-treatment), can be associated with a higher risk of developing a cytokine release syndrome (CRS).

[0425] In some embodiments, the threshold value is within 25%, within 20%, within 15%, within 32% or within 5% and/or is within a standard deviation above the median or mean level, amount or concentration of LDH, Ferritin, C-reactive protein (CRP), D-dimer (fibrin degradation product), IL-6, IL-10, IL-15, IL-16 TNF- α , MIP-1 α or MIP-1 β , or the median or mean volumetric measure of tumor burden of a sum of the products of diameters (SPD), in a biological sample obtained from a group of subjects prior to receiving a cell therapy, wherein each of the subjects of the group went on not develop any toxicity after receiving a recombinant-receptor-expressing therapeutic cell composition for treating the same disease or condition.

[0426] In some embodiments, the threshold value is within 25%, within 20%, within 15%, within 32% or within 5% and/or is within a standard deviation below the median or mean level, amount or concentration of LDH, Ferritin, C-reactive protein (CRP), D-dimer (fibrin degradation product), IL-6, IL-10, IL-15, IL-16 TNF- α , MIP-1 α or MIP-1 β , or the median or mean volumetric measure of tumor burden of a sum of the products of diameters (SPD), in a biological sample obtained from a group of subjects prior to receiving a cell therapy, wherein each of the subjects of the group went on to develop a toxicity after receiving a recombinant-receptor-expressing therapeutic cell composition for treating the same disease or condition.

[0427] In some embodiments, the toxicity is neurotoxicity and exemplary threshold value for LDH is at or at about or above or above about 300 U/L, 400 U/L, 500 U/L or 600 U/L.

[0428] In some embodiments, the toxicity is neurotoxicity and exemplary threshold value for SPD is at or at about or above or above about 30 cm², 40 cm², 50 cm², 60 cm², 70 cm², 80 cm² or 90cm².

[0429] In some embodiments, the toxicity is neurotoxicity and exemplary threshold value for IL-10 is at or at about or above or above about 0.8 pg/mL, 0.9 pg/mL, 1 pg/mL, 2 pg/mL, 3 pg/mL or 4 pg/mL.

[0430] In some embodiments, the toxicity is neurotoxicity and exemplary threshold value for IL-15 is at or at about or above or above about 3 pg/mL, 4 pg/mL, 5 pg/mL, 6 pg/mL or 7 pg/mL.

[0431] In some embodiments, the toxicity is neurotoxicity and exemplary threshold value for IL-16 is at or at about or above or above about 600 pg/mL, 700 pg/mL, 800 pg/mL, 900 pg/mL or 1000 pg/mL.

[0432] In some embodiments, the toxicity is neurotoxicity and exemplary threshold value for TNF- α is at or at about or above or above about 6 pg/mL, 7 pg/mL, 8 pg/mL, 9 pg/mL or 10 pg/mL.

[0433] In some embodiments, the toxicity is neurotoxicity and exemplary threshold value for MIP-1 β is at or at about or above or above about 70 pg/mL, 80 pg/mL, 90 pg/mL or 100 pg/mL.

[0434] In some embodiments, the toxicity is CRS and exemplary threshold value for LDH is at or at about or above or above about 300 U/L, 400 U/L, 500 U/L or 600 U/L.

[0435] In some embodiments, the toxicity is CRS the and threshold value for SPD is at or at about or above or above about 20 cm², 30 cm², 40 cm² or 50 cm².

[0436] In some embodiments, the toxicity is CRS and exemplary threshold value for ferritin is at or at about or above or above about 300 ng/mL, 400 ng/mL, 500 ng/mL, 600 ng/mL, 700 ng/mL, 800 ng/mL, 900 ng/mL or 1000 ng/mL.

[0437] In some embodiments, the toxicity is CRS and exemplary threshold value for CRP is at or at about or above or above about 20 mg/L, 30 mg/L or 40 mg/L.

[0438] In some embodiments, the toxicity is CRS and exemplary threshold value for d-dimer is at or at about or above or above about 300 pg/mL, 400 pg/mL, 500 pg/mL, 600 pg/mL, 700 pg/mL, 800 pg/mL, 900 pg/mL or 1000 pg/mL.

[0439] In some embodiments, the toxicity is CRS and exemplary threshold value for IL-6 is at or at about or above or above about 2 pg/mL, 3 pg/mL, 4 pg/mL, 5 pg/mL, 6 pg/mL, 7 pg/mL, 8 pg/mL or 9 pg/mL.

[0440] In some embodiments, the toxicity is CRS and exemplary threshold value for IL-15 is at or at about or above or above about 3 pg/mL, 4 pg/mL, 5 pg/mL, 6 pg/mL, 7 pg/mL, 8 pg/mL, 9 pg/mL or 10 pg/mL.

[0441] In some embodiments, the toxicity is CRS and exemplary threshold value for TNF- α is at or at about or above or above about 7 pg/mL, 8 pg/mL, 9 pg/mL, 10 pg/mL or 15 pg/mL.

[0442] In some embodiments, the toxicity is CRS and exemplary threshold value for MIP-1 α is at or at about or above or above about 20 pg/mL, 30 pg/mL or 40 pg/mL.

[0443] In some embodiments, the parameter is LDH and in some cases, development of toxicity, e.g., CRS or NT, is correlated with the LDH value that is above a threshold value. In some embodiments, the inflammatory marker is LDH and the threshold value is or is about 300 units per liter, is or is about 400 units per liter, is or is about 500 units per liter or is or is about 600 units per liter.

[0444] In some embodiments, the parameter or biomarker is LDH. In some embodiments, the biomarker is LDH and the threshold value is or is about 500 U/L or higher. In some embodiments, the parameter or biomarker is SPD. In some embodiments, the parameter is SPD,

and the threshold value is or is about 50 cm^2 or higher. In some embodiments, biomarker or parameters are SPD and LDH, and the threshold values are SPD of at or about 50 cm^2 or higher and LDH of at or about 500 U/L or higher. In some embodiments, the biomarkers or parameters are associated with increased risk of developing CRS or NT.

[0445] In some embodiments, a measurement of the parameter or marker that is above the threshold value, e.g., SPD of at or about 50 cm^2 or higher and LDH of at or about 500 U/L or higher, are associated with an approximately 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-fold or more increased risk of developing CRS or NT, such as any grade CRS or NT. In some embodiments, a measurement of the parameter or marker that is below the threshold value, e.g., SPD of lower than at or about 500 cm^2 and LDH of lower than at or about 500 U/L, are associated with an approximately 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-fold or more decreased risk of developing CRS or NT, such as any grade CRS or NT.

[0446] In some embodiments, the volumetric measure is SPD and the threshold level is or is about 30 cm^2 , is or is about 40 cm^2 , is or is about 50 cm^2 , is or is about 60 cm^2 , or is or is about 70 cm^2 . In some embodiments, the volumetric measure is SPD and the threshold level is or is about 50 cm^2 .

[0447] In some embodiments, the biomarker is LDH and the threshold level is or is about 300 units per liter (U/L), is or is about 400 U/L, is or is about 500 U/L or is or is about 600 U/L. In some embodiments, the biomarker is LDH and the threshold level is or is about 500 U/L.

[0448] In some aspects of the provided methods, a subject is determined to be at risk of developing toxicity (*e.g.* neurotoxicity, such as severe neurotoxicity or grade 3 or higher neurotoxicity, *e.g.* grade 4 or 5 neurotoxicity and/or CRS, such as severe CRS or grade 3 or higher CRS) by a comparison of the parameter (*e.g.* concentration, amount, level or activity) of the biomarker (*e.g.* analyte) or, individually, each of the biomarkers to a reference value, such as threshold level, of the corresponding parameter for the biomarker or each biomarker. In some embodiments, the comparison indicates whether the subject is or is not at risk for developing toxicity, *e.g.*, neurotoxicity such as severe neurotoxicity or grade 3 or higher neurotoxicity, *e.g.* grade 4 or 5 neurotoxicity and/or CRS, such as severe CRS or grade 3 or higher CRS, and/or indicates a degree of risk for developing said toxicity. In some embodiments, the reference value is one that is a threshold level or cut-off at which there is a good predictive value (*e.g.* accuracy, sensitivity and/or specificity) that such toxicity will occur or is likely to occur either alone or in combination with one or more biomarkers in the panel. In some cases, such reference value, *e.g.* threshold level, can be or is predetermined or known prior to performing

the method, such as from a plurality of subjects previously treated with a cell therapy and assessed for the correlation of the parameter of the biomarker or, individually, each of the biomarkers in a panel to the presence of a toxic outcome (*e.g.* the presence of neurotoxicity such as severe neurotoxicity or grade 3 or higher neurotoxicity, *e.g.* grade 4 or 5 neurotoxicity and/or CRS, such as severe CRS or grade 3 or higher CRS).

[0449] In some embodiments, a parameter of a biomarker (*e.g.* LDH, ferritin, CRP, IL-6, IL-8, IL-10, TNF- α , IFN- α 2, MCP-1 and MIP-1 β) that is higher or greater than the reference value, *e.g.* threshold level, of the corresponding parameter is associated with a positive prediction of a risk of toxicity (alone or in conjunction with assessment of the other biomarkers in the panel). In some embodiments, a parameter of a biomarker that is equal to or lower than the reference value, *e.g.* threshold level, of the corresponding parameter is associated with a negative prediction of a risk of toxicity (alone or in conjunction with assessment of the other biomarkers in the panel).

[0450] In some embodiments, the threshold level is determined based on the level, amount, concentration or other measure of the biomarker (*e.g.* analyte) in the sample positive for the biomarker. In some aspects, the threshold level is within 25%, within 20%, within 15%, within 10% or within 5% of the average level, amount or concentration or measure, and/or is within a standard deviation of the average level, amount or concentration or measure, of the biomarker or parameter in a biological sample obtained from a group of subjects prior to receiving a recombinant receptor-expressing therapeutic cell composition, wherein each of the subjects of the group went on to develop a toxicity, *e.g.* neurotoxicity such as severe neurotoxicity or grade 3 or higher neurotoxicity, *e.g.* grade 4 or 5 neurotoxicity and/or CRS, such as severe CRS or grade 3 or higher CRS, after receiving a recombinant-receptor-expressing therapeutic cell composition for treating the same disease or condition.

[0451] In some embodiments of any of the provided methods, the biomarker (*e.g.* analyte) correlates to and/or is predictive of the risk of developing severe neurotoxicity, such as severe neurotoxicity or grade 3 or higher neurotoxicity, *e.g.* grade 4 or 5 neurotoxicity and/or severe CRS or grade 3 or higher CRS. In some embodiments, the threshold level is within 25%, within 20%, within 15%, within 10% or within 5% of the average level, amount or concentration or measure, and/or is within a standard deviation of the average level, amount or concentration or measure, of the biomarker or parameter in a biological sample obtained from a group of subjects prior to receiving a recombinant receptor-expressing therapeutic cell composition, wherein each of the subjects of the group went on to develop severe neurotoxicity or grade 3 or higher

neurotoxicity, e.g. grade 4 or 5 neurotoxicity and/or severe CRS or grade 3 or higher CRS, after receiving a recombinant-receptor-expressing therapeutic cell composition for treating the same disease or condition.

[0452] In some embodiments, subjects with NHL who have high pre-treatment tumor burden (measured by the sum of product of the perpendicular diameters (SPD; $\geq 500 \text{ cm}^2$) or high serum lactate dehydrogenase (LDH; $\geq 500 \text{ U/L}$) prior to the start of lymphodepletion may also have a higher risk for developing CRS and/or neurotoxicity. In some embodiments, high pre-administration levels of inflammatory markers, such as ferritin and C-reactive protein (CRP) can be also associated with CRS. In some embodiments, peak levels of IL-6, IFN- γ , ferritin, and CRP can be associated with any grade or Grade 3 or higher neurotoxicity. In some embodiments, subjects with B-cell acute lymphoblastic leukemia (ALL) and high burden of disease may be at an elevated risk of developing CRS. In some embodiments, severe neurotoxicity can be associated with higher disease burden at the time of adoptive cell therapy. In some embodiments, protein levels in the cerebrospinal fluid (CSF) can be elevated in patients with neurotoxicity, compared with baseline measurements. In some aspects, other organ dysfunction (hepatic and renal), as well as hypoxemia, and infection, might also contribute to the encephalopathy. In some aspects, cytokine-mediated endothelial activation can be associated with coagulopathy, capillary leak, and blood-brain barrier disruption allowing transit of high concentrations of systemic cytokines into the CSF.

C. Reagents for Measuring

[0453] In some embodiments, the parameter, e.g., patient factor, biomarker, inflammatory marker and/or cytokine, is detected using one or more reagent(s) capable of detecting or that is specific for the parameter. In some embodiments, also provided are kits and articles of manufacture, for detection or assessment of the parameters and/or for modulating the therapy, e.g., cell therapy.

[0454] In some embodiments, instructions are also provided for using the reagent to assay a biological sample from a subject that is a candidate for treatment, optionally with a cell therapy, said cell therapy optionally including a dose or composition of genetically engineered cells expressing a recombinant receptor. In some embodiments of using the articles of manufacture, the level or presence of C-C Motif Chemokine Ligand 13 (CCL13), C-reactive protein (CRP), C-X-C motif chemokine 10 (CXCL10), D-dimer (fibrin degradation product), ferritin, IFN- $\alpha 2$, interleukin-2 (IL-2), IL-10, IL-15, IL-16, IL-6, IL-7, IL-8, interferon gamma (IFN- γ), lactate

dehydrogenase (LDH), macrophage inflammatory protein (MIP-1 α), MIP-1 β , Monocyte chemoattractant protein-1 (MCP-1), SAA-1, Serum Amyloid A1 (SAA-1), tumor necrosis factor alpha (TNF- α), is detected and assessed. In some embodiments of using the articles of manufacture, the level or presence of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), albumin, ferritin, β 2 microglobulin (β 2-M), or lactate dehydrogenase (LDH) is detected and assessed. Also provided are methods of detecting and assessing one or more patient attributes, factors and/or biomarkers indicative of tumor burden.

[0455] In some embodiments, measuring the value of the one or more parameters, e.g., biomarkers, comprises contacting a reagent capable of directly or indirectly detecting the analyte with the biological sample and determining the presence or absence, level, amount or concentration of the analyte in the biological sample. In some embodiments, the one or more parameters, e.g., biomarkers, is C-C Motif Chemokine Ligand 13 (CCL13), C-reactive protein (CRP), C-X-C motif chemokine 10 (CXCL10), D-dimer (fibrin degradation product), ferritin, IFN- α 2, interleukin-2 (IL-2), IL-10, IL-15, IL-16, IL-6, IL-7, IL-8, interferon gamma (IFN- γ), lactate dehydrogenase (LDH), macrophage inflammatory protein (MIP-1 α), MIP-1 β , Monocyte chemoattractant protein-1 (MCP-1), SAA-1, Serum Amyloid A1 (SAA-1), tumor necrosis factor alpha (TNF- α), is detected and assessed. In some embodiments of using the articles of manufacture, the level or presence of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), albumin, ferritin, β 2 microglobulin (β 2-M), or lactate dehydrogenase (LDH). In some embodiments, the one or more parameters, e.g., biomarkers, is or includes LDH.

[0456] In some aspects, the reagent is a binding molecule that specifically binds to the biomarker. For example, in some embodiments, the reagent is an antibody or an antigen-binding fragment thereof. In some embodiments, the reagent is or includes a substrate or binding partner of the biomarker.

[0457] In some embodiments, the presence, absence or level, amount, concentration and/or other measure of LDH is detected or determined in a sample. Various methods of detecting or determining LDH are known. For example, an assay which measures LDH conversion of lactate to pyruvate through NAD⁺ reduction to NADH can be used to detect LDH in the sample. In some embodiments, the sample is contacted with lactate in the presence of coenzyme NAD which, as a measure of LDH in the sample, results in NADH that is then oxidized in the presence of an electron transfer agent. In some embodiments, the NADH interacts with a probe or dye precursor that is detectable by measuring absorption in a visible light range. In some examples, diaphorase uses the NADH to reduce tetrazolium salt (INT) to a red formazan product

and the product is measured. Therefore, in some embodiments, the amount of colored product formed is directly proportional to the LDH activity in the sample.

[0458] In some embodiments, the patient attributes, factors and/or biomarkers is assessed using an immune assay. For example, an enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), radioimmunoassay (RIA), surface plasmon resonance (SPR), Western Blot, Lateral flow assay, immunohistochemistry, protein array or immuno-PCR (iPCR) can be used to detect the patient attributes, factors and/or biomarkers. In some embodiments, using the articles of manufacture include detecting patient attributes, factors and/or biomarkers indicative of tumor burden. In some cases, the assaying or assessing of an patient attributes, factors and/or biomarkers is using flow cytometry. In some cases, the reagent is a soluble protein that binds the patient attributes, factors and/or biomarkers. In some example, the reagent is a protein that binds C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), albumin, ferritin, $\beta 2$ microglobulin ($\beta 2$ -M), or lactate dehydrogenase (LDH).

[0459] In some embodiments, C-reactive protein (CRP) is assessed using an in vitro enzyme-linked immunosorbent assay to obtain a quantitative measurement of human CRP from a sample such as serum, plasma, or blood. In some examples, CRP is detected using a human Enzyme- Linked Immunosorbent Assay (ELISA). In some embodiments, erythrocyte sedimentation rate (ESR) is assessed by measuring the distance (in millimeters per hour) that red cells have fallen after separating from the plasma in a vertical pipette or tube. In some aspects, albumin is assessed using a colorimetric test or an in vitro enzyme-linked immunosorbent assay. In some examples, albumin is detected using a human Enzyme- Linked Immunosorbent Assay (ELISA). In some embodiments, ferritin or $\beta 2$ microglobulin is assessed using an immunoassay or detected using an ELISA. In some aspects, lactate dehydrogenase (LDH) is assessed using a colorimetric test or an in vitro enzyme-linked immunosorbent assay.

[0460] The term “antibody” herein is used in the broadest sense and includes polyclonal and monoclonal antibodies, including intact antibodies and functional (antigen-binding) antibody fragments, including fragment antigen binding (Fab) fragments, $F(ab')_2$ fragments, Fab' fragments, Fv fragments, recombinant IgG (rIgG) fragments, single chain antibody fragments, including single chain variable fragments (scFv), and single domain antibodies (e.g., sdAb, sdFv, nanobody) fragments. The term encompasses genetically engineered and/or otherwise modified forms of immunoglobulins, such as intrabodies, peptibodies, chimeric antibodies, fully human antibodies, humanized antibodies, and heteroconjugate antibodies, multispecific, e.g., bispecific, antibodies, diabodies, triabodies, and tetrabodies, tandem di-scFv, tandem tri-scFv.

Unless otherwise stated, the term “antibody” should be understood to encompass functional antibody fragments thereof. The term also encompasses intact or full-length antibodies, including antibodies of any class or sub-class, including IgG and sub-classes thereof, IgM, IgE, IgA, and IgD.

[0461] 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; single-chain antibody molecules (*e.g.* scFv); 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.

[0462] Single-domain antibodies 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.

[0463] 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, and/or that are may not be produced by enzyme digestion of a naturally-occurring intact antibody. In some aspects, the antibody fragments are scFvs.

[0464] 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.

[0465] Among the provided antibodies are human antibodies. A “human antibody” is an antibody with an amino acid sequence corresponding to that of an antibody produced by a

human or a human cell, or non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences, including human antibody libraries. The term excludes humanized forms of non-human antibodies comprising non-human antigen-binding regions, such as those in which all or substantially all CDRs are non-human.

[0466] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic animals, the endogenous immunoglobulin loci have generally been inactivated. Human antibodies also may be derived from human antibody libraries, including phage display and cell-free libraries, containing antibody-encoding sequences derived from a human repertoire.

[0467] Among the provided antibodies are monoclonal antibodies, including monoclonal antibody fragments. The term "monoclonal antibody" as used herein refers to an antibody obtained from or within a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical, except for possible variants containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different epitopes, each monoclonal antibody of a monoclonal antibody preparation is directed against a single epitope on an antigen. The term is not to be construed as requiring production of the antibody by any particular method. A monoclonal antibody may be made by a variety of techniques, including but not limited to generation from a hybridoma, recombinant DNA methods, phage-display and other antibody display methods.

[0468] Also provided are antibody immunoconjugates comprising an antibody against biomarker attached to a label, which can generate a detectable signal, indirectly or directly. These antibody immunoconjugates can be used for research or diagnostic applications. The label is preferably capable of producing, either directly or indirectly, a detectable signal. For example, the label may be radio-opaque or a radioisotope, such as ^3H , ^{14}C , ^{32}P , ^{35}S , ^{123}I , ^{125}I , ^{131}I ; a fluorescent (fluorophore) or chemiluminescent (chromophore) compound, such as fluorescein isothiocyanate, rhodamine or luciferin; an enzyme, such as alkaline phosphatase, β -galactosidase or horseradish peroxidase; an imaging agent; or a metal ion. In some embodiments, the label is a

radioactive atom for scintigraphic studies, for example ^{99}Tc or ^{123}I , or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, MRI), such as zirconium-89, iodine-123, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron. Zirconium-89 may be complexed to various metal chelating agents and conjugated to antibodies, *e.g.*, for PET imaging (WO 2011/056983).

[0469] In some embodiments, the antibody immunoconjugate is detectable indirectly. For example, a secondary antibody that is specific for the antibody against the marker expressed on a population of myeloid cells immunoconjugate and contains a detectable label can be used to detect the antibody immunoconjugate.

[0470] In some embodiments, antibodies capable of detecting or that is specific the patient attributes, factors and/or biomarkers provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various known assays. In one aspect, the antibody is tested for its antigen binding activity, *e.g.*, by known methods such as an immunoassay, ELISA, Western blotting, and/or flow cytometric assays, including cell-based binding assays.

D. Samples

[0471] In some embodiments, the, one or more patient attributes, factors and/or biomarkers is assessed from a biological sample. In some aspects, the biological sample is a bodily fluid or a tissue. In some such embodiments, the biological sample, *e.g.*, bodily fluid, is or contains whole blood, serum or plasma.

[0472] In particular embodiments, two or more samples are obtained, collected, or taken from the subject prior to administration of the therapy. In certain embodiments, the sample is a biological sample. In certain embodiments, the sample is a blood sample, plasma sample, or serum sample. In certain embodiments, the sample is a tissue sample. In some embodiments, the sample is a biopsy. In some embodiments, the sample is obtained from the subject at a screening session, such as a routine assessment or blood draw to confirm and/or identify the condition or disease in the subject.

[0473] In some embodiments, the biological sample is an apheresis or leukapheresis sample. In some embodiments, the or absence and/or a parameter of one or more biomarkers is assessed or the biological sample is obtained after administration of the cell therapy. In some embodiments, the reagents can be used prior to the administration of the cell therapy or after the administration of cell therapy, for diagnostic purposes, to identify subjects and/or to assess

treatment outcomes and/or toxicities.

[0474] In some embodiments, the biological sample is obtained from the subject prior to administration of the cell therapy (e.g., pre-infusion), e.g., obtained up to 2 days, up to 7 days, up to 14 days, up to 21 days, up to 28 days, up to 35 days or up to 40 days prior to initiation of the administration of the engineered cells.

[0475] In certain embodiments, one or more patient attributes, factors and/or biomarkers are measured, assessed, and/or determined in one or more samples obtained at two or more time points to determine a fold change in the factor indicative of disease burden. In particular embodiments, the sample is a biological sample that is taken, collected, and/or obtained from a subject. In certain embodiments, the subject has a disease or condition and/or is suspected of having a disease or condition. In some embodiments, subject has received, will receive, or is a candidate to receive a therapy. In some embodiments, the therapy is an administration of a cell therapy. In particular embodiments, the therapy is an immunotherapy. In certain embodiments, the cell therapy treats and/or is capable of treating the disease or condition. In some embodiments, the therapy is a cell therapy that contains one or more engineered cells. In some embodiments, the engineered cells express a recombinant receptor. In particular embodiments, the recombinant receptor is a CAR. In particular embodiments, the sample is taken, collected, and/or obtained from a subject who has been, who will be, or is a candidate to be administered a therapy. In particular embodiments, the sample is taken, collected, and/or obtained prior to treatment or administration with the therapy, e.g., the cell therapy.

[0476] In some embodiments, the sample does not comprise genetically engineered T cells expressing a chimeric antigen receptor (CAR) and/or is obtained from the subject prior to receiving administration of genetically engineered T cells expressing a CAR.

[0477]

[0478] In particular embodiments, the sample is taken, collected, and/or obtained from a subject who has been, who will be, or is a candidate to be administered a therapy. In particular embodiments, the sample is taken, collected, and/or obtained prior to treatment or administration with the therapy, e.g., the cell therapy. In accord with methods, kits and articles of manufacture described herein, the sample can be assessed for one or more patient attributes, factors and/or biomarkers that is associated with and/or correlate to toxicity or risk of toxicity. Exemplary patient attributes, factors and/or biomarkers associated with and/or correlated with a risk of developing toxicity and/or response that may be detected in a sample collected or obtained from a subject prior to receiving an immunotherapy include C-reactive protein (CRP), erythrocyte

sedimentation rate (ESR), albumin, ferritin, $\beta 2$ microglobulin ($\beta 2$ -M), or lactate dehydrogenase (LDH). In some embodiments, the patient attributes, factors and/or biomarkers associated with and/or correlated with a risk of developing toxicity and/or response that may be detected in a sample collected or obtained from a subject prior to or after receiving an immunotherapy include C-C Motif Chemokine Ligand 13 (CCL13), C-reactive protein (CRP), C-X-C motif chemokine 10 (CXCL10), D-dimer (fibrin degradation product), ferritin, IFN- $\alpha 2$, interleukin-2 (IL-2), IL-10, IL-15, IL-16, IL-6, IL-7, IL-8, interferon gamma (IFN- γ), lactate dehydrogenase (LDH), macrophage inflammatory protein (MIP-1 α), MIP-1 β , Monocyte chemoattractant protein-1 (MCP-1), SAA-1, Serum Amyloid A1 (SAA-1), tumor necrosis factor alpha (TNF- α). Thus, in some aspects, the provided methods relate to identifying subjects, prior to receiving an immunotherapy, such as a cell therapy (e.g. CAR-T cells), who may achieve pharmacokinetic parameters within a therapeutic window or range. In some embodiments, the provided methods relate to identifying subjects, prior to or after receiving an immunotherapy or cell therapy, for modulating the immunotherapy or cell therapy, e.g., by administration of agent to the subject capable of modulating, optionally increasing or decreasing, CAR+ T cell expansion, proliferation, and/or activity. As described elsewhere herein, the methods can be used to determine if the subject should be closely monitored following the administration of the immunotherapy, is a candidate for outpatient therapy or should receive treatment of the therapy in a hospital setting and/or is a candidate for receiving an agent capable of modulating CAR+ T cell expansion and/or proliferation and/or an intervention of preventing, treating or ameliorating a risk of a toxicity.

[0479] In some embodiments, the sample is taken, collected, and/or obtained from a subject that has or is suspected of having a condition or disease. In some embodiments, the subject has or is suspected of having a cancer or proliferative disease. In particular embodiments, the subject has a disease or condition, or is suspected of having a disease or condition, that is associated with an antigen and/or is associated with diseased cells that express the antigen. In some embodiments, the disease or condition, e.g., a cancer or proliferative disorder, is associated with $\alpha v \beta 6$ integrin (avb6 integrin), B cell maturation antigen (BCMA), B7-H3, B7-H6, carbonic anhydrase 9 (CA9, also known as CAIX or G250), a cancer-testis antigen, cancer/testis antigen 1B (CTAG, also known as NY-ESO-1 and LAGE-2), carcinoembryonic antigen (CEA), a cyclin, cyclin A2, C-C Motif Chemokine Ligand 1 (CCL-1), CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD133, CD138, CD171, chondroitin sulfate proteoglycan 4 (CSPG4), epidermal growth factor protein (EGFR), type III epidermal

growth factor receptor mutation (EGFR vIII), epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), ephrinB2, ephrine receptor A2 (EPHa2), estrogen receptor, Fc receptor like 5 (FCRL5; also known as Fc receptor homolog 5 or FCRH5), fetal acetylcholine receptor (fetal AchR), a folate binding protein (FBP), folate receptor alpha, ganglioside GD2, O-acetylated GD2 (OGD2), ganglioside GD3, glycoprotein 100 (gp100), glypican-3 (GPC3), G Protein Coupled Receptor 5D (GPRC5D), Her2/neu (receptor tyrosine kinase erb-B2), Her3 (erb-B3), Her4 (erb-B4), erbB dimers, Human high molecular weight-melanoma-associated antigen (HMW-MAA), hepatitis B surface antigen, Human leukocyte antigen A1 (HLA-A1), Human leukocyte antigen A2 (HLA-A2), IL-22 receptor alpha (IL-22R α), IL-13 receptor alpha 2 (IL-13R α 2), kinase insert domain receptor (kdr), kappa light chain, L1 cell adhesion molecule (L1-CAM), CE7 epitope of L1-CAM, Leucine Rich Repeat Containing 8 Family Member A (LRRC8A), Lewis Y, Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, MAGE-A10, mesothelin (MSLN), c-Met, murine cytomegalovirus (CMV), mucin 1 (MUC1), MUC16, natural killer group 2 member D (NKG2D) ligands, melan A (MART-1), neural cell adhesion molecule (NCAM), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME), progesterone receptor, a prostate specific antigen, prostate stem cell antigen (PSCA), prostate specific membrane antigen (PSMA), Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1), survivin, Trophoblast glycoprotein (TPBG also known as 5T4), tumor-associated glycoprotein 72 (TAG72), Tyrosinase related protein 1 (TRP1, also known as TYRP1 or gp75), Tyrosinase related protein 2 (TRP2, also known as dopachrome tautomerase, dopachrome delta-isomerase or DCT), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor receptor 2 (VEGFR2), Wilms Tumor 1 (WT-1), a pathogen-specific or pathogen-expressed antigen, or an antigen associated with a universal tag, and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV or other pathogens. Antigens targeted by the receptors in some embodiments include antigens associated with a B cell malignancy, such as any of a number of known B cell marker. In some embodiments, the antigen is or includes CD20, CD19, CD22, ROR1, CD45, CD21, CD5, CD33, Igkappa, Iglambda, CD79a, CD79b or CD30. In certain embodiments, the subject has a disease or condition, or is suspected of having a disease or condition, that is associated with CD19 and/or is associated with diseased cells that express CD19.

[0480] In some embodiments, the sample is taken, collected, and/or obtained from a subject that has or is suspected of having a cancer or proliferative disease that is a B cell malignancy or hematological malignancy. In some embodiments, the cancer or proliferative disease is a

myeloma, *e.g.*, a multiple myeloma (MM), a lymphoma or a leukemia, lymphoblastic leukemia (ALL), non-Hodgkin's lymphoma (NHL), chronic lymphocytic leukemia (CLL), a diffuse large B-cell lymphoma (DLBCL), and/or acute myeloid leukemia (AML). In some embodiments, the cancer or proliferative disorder is ALL. In some embodiments, the subject has, or is suspected of having ALL. In some embodiments, the ALL is adult ALL. In particular embodiments, the ALL is pediatric ALL.

E. Agents for Modulating Cell Expansion and Activity

[0481] In some aspects, provided are methods for modulating the expansion, proliferation and/or activity of the administered cells, *e.g.*, CAR+ T cells, based on assessment and/or determination of the parameters, *e.g.*, pharmacokinetic parameters and/or other parameters such as patient attributes and/or expression of a biomarker. In some embodiments, the method involves administering agents that modulate, such as increase or decrease, the expansion, proliferation and/or activity of the administered cells, *e.g.*, CAR+ T cells, depending on the determination of the parameters. In some embodiments, an agent is administered if the genetically engineered cells are not within the therapeutic range based on assessment of the parameters, *e.g.*, pharmacokinetic parameters, such as maximum or peak CAR+ cell concentration. In some embodiments, the agent is an agent that increases, augments or boosts the proliferation and/or expansion of the CAR+ T cells. In some embodiments, the agent is an agent that decreases, reduces, and/or dampens the proliferation and/or expansion of the CAR+ T cells.

[0482] In some embodiments, the agent can be administered sequentially, intermittently, or at the same time as or in the same composition as the therapy, such as cells for adoptive cell therapy. In some embodiments, the agent is administered before, simultaneously with, intermittently with, during, during the course of or after administration of the cells, *e.g.*, cells expressing a recombinant receptor, *e.g.* CAR. In some embodiments, such agents include agents that modulate the cell expansion and/or activity of the administered cells, *e.g.*, immune cells, such as T cells. In some embodiments, such agents include agents that reduce or decrease the expansion and/or proliferation of the cell expansion and/or activity of the administered cells, *e.g.*, immune cells, such as T cells.

[0483] In some embodiments, the agent is administered at a time that is greater than or greater than at or about 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days or 21 days after initiation of administration of the

genetically engineered cells. In some embodiments, the agent is administered at a time that is between or between about 11 to 22 days, 12 to 18 days or 14 to 16 days, each inclusive, after initiation of administration of the genetically engineered cells.

[0484] In some embodiments, the agent is administered at a time as described herein and in accord with the provided methods, and/or with the provided articles of manufacture or compositions. In some embodiments, the agent is administered at a time that is within, such as less than or no more than, 3, 4, 5, 6, 7, 8, 9 or 10 days after initiation of the immunotherapy and/or cell therapy. In some embodiments, the agent is administered within or within about 1 day, 2 days or 3 days after initiation of administration of the immunotherapy and/or cell therapy.

[0485] In some embodiments, the agent can be administered sequentially, intermittently, or at the same time as or in the same composition as the immunotherapy and/or cell therapy, e.g., cells for adoptive cell therapy, or initiation thereof. In some embodiments of any of the methods provided herein, the agent is administered prior to, simultaneously with, during and/or subsequent to initiation of administration of the cell therapy, and/or during the course of the cell therapy. In some embodiments, the agent is administered from or from about 0 to 96 hours, 0 to 72 hours, 0 to 48 hours, 0 to 24 hours, 0 to 12 hours or 0 to 6 hours or 0 to 2 hours prior to initiation of the T cell therapy; or the agent is administered no more than 96 hours, 72 hours, 48 hours, 24 hours, 12 hours, 6 hours, 2 hours or 1 hour prior to initiation of the T cell therapy. In some embodiments, the method involves administering to the subject a therapeutically effective amount of the agent at a time prior to administration of the cell therapy, e.g., adoptive cell therapy. In some embodiments, the agent is administered about 24 hours or less prior to the administration of the cells for cell therapy. In some embodiments, the agent is administered at about 24, 22, 20, 18, 16, 14, 12, 10, 8, 6, 4 or 2 hours or less prior to the administration of the cells or initiation thereof.

[0486] In some embodiments, the agent is administered simultaneously or near simultaneous with the cell therapy or initiation thereof, e.g., with about 1, 2, 3 or 4 hours of initiation of the cell therapy.

[0487] In some embodiments, the agent can be administered greater than at or about 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 18 hours, 24 hours, 36 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days or 10 days or more following administration of the cell therapy or initiation thereof. In some of such embodiments, the agent may be administered no later than 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 18 hours, 24

hours, 36 hours, 2 days, 3 days, 4 days, or 5 days or more following administration of the cell therapy or initiation thereof.

[0488] In some aspects, the agent can be administered between at or about 1 hour and at or about 5 days or at or about 4 hours and at or about 5 days following administration of cell therapy, such as between at or about 1 hour and at or about 5 days, at or about 4 hours and at or about 4 days, at or about 8 hours and at or about 3 days, at or about 1 day and at or about 3 days, at or about 2 days and at or about 3 days, or at or about 1 day and at or about 2 days following administration of cell therapy or initiation thereof. In some such cases, the agent is administered at or about 1 day, at or about 2 days, or at or about 3 days following the administration of cell therapy or initiation thereof. In some instances, the subject is treated with the agent within 3 days, within 2 days or within 1 day after administration of the cell therapy or initiation thereof. In some embodiments, the agent can be administered greater than at or about 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 18 hours, 24 hours, 36 hours, 2 days, 3 days, 4 days, or 5 days or more following administration of the cell therapy or initiation thereof. In some of such embodiments, the agent may be administered no later than at or about 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 18 hours, 24 hours, 36 hours, 2 days, 3 days, 4 days, or 5 days or more following administration of the cell therapy or initiation thereof.

[0489] In some cases, the agent or therapy or intervention, is administered alone or is administered as part of a composition or formulation, such as a pharmaceutical composition or formulation, as described herein. Thus, the agent alone or as part of a pharmaceutical composition can be administered intravenously or orally, or by any other acceptable known route of administration or as described herein.

1. Agents for Augmenting or Enhancing Cell Expansion

[0490] In some embodiments, the methods include methods involving the combined administration, *e.g.* simultaneous or sequential administration, with a drug or agent capable of augmenting, boosting or enhancing the expansion, proliferation, survival, potency and/or efficacy of the administered cells, *e.g.*, recombinant receptor expressing cells. In some embodiments, such agent is administered to achieve a peak CAR+ T cell expansion in the therapeutic range. In some embodiments, the dose of administered cells is sub-optimal and the combined administration of the agent boosts or augments expansion to achieve peak CAR+ T cells in the blood in the therapeutic range. In some embodiments, the method includes

administering a dose of cells and monitoring the peak CAR+ T cells in the blood to ensure that the therapeutic range is maintained or achieved and, if it is not, administering an agent or compound to boost or augment the therapeutic dose. In some embodiments, low or limited expansion of cells, e.g., at low pharmacokinetic parameters such as low maximum CAR+ T cell concentration (C_{\max}), tumor suppression effect may be limited.

[0491] In some embodiments, the agent is administered before, during, during the course of or after administration of the cells, e.g., cells expressing a recombinant receptor, e.g. CAR. In some embodiments, such agents include agents that specifically augment, boost or enhance the expansion, proliferation, survival, potency and/or efficacy of the engineered cells by virtue of specifically modulating the transgene, e.g., transgene encoding a recombinant receptor. In some embodiments, such agents include agents that modulate the cell expansion and/or activity of the administered cells, e.g., immune cells, such as T cells.

[0492] In some embodiments, the administered cell, e.g., cells engineered to express a recombinant receptor, are modified to augment, boost or enhance the expansion, proliferation, survival, potency and/or efficacy of the administered cells. In some embodiments, the administered cell, e.g., cells engineered to express a recombinant receptor, are modified such that the expansion, proliferation, survival, potency and/or efficacy of the engineered cells can be regulated and/or controlled, such as by administration of an agent. In some embodiments, the agent minimize the effects of inhibitory factors that suppress the proliferation, expansion and/or survival of the engineered cells *in vivo*.

[0493] In some embodiments, the additional agent is a small molecule, a peptide, a polypeptide, an antibody or antigen-binding fragment thereof, an antibody mimetic, an aptamer or a nucleic acid molecule (e.g. siRNA), a lipid, a polysaccharide or any combination thereof. In some embodiments, the additional agent is an inhibitor or an activator of a particular factor, molecule, receptor, function and/or enzyme. In some embodiments, the additional agent is an agonist or an antagonist of a particular factor, molecule, receptor, function and/or enzyme. In some embodiments, the additional agent is an analog or a derivative of one or more factors and/or metabolites. In some embodiments, the additional agent is a protein or polypeptide. In some embodiments, the additional agent is a cell, e.g., an engineered cell, such as an additional dose of the same engineered cell that was administered and/or a different engineered cell.

[0494] In some embodiments, the agent is capable of transgene-specific expansion. In some embodiments, exemplary methods or agents for transgene-specific expansion include endogenous antigen exposure, vaccination, anti-idiotypic antibodies or antigen-binding fragment

thereof and/or regulatable recombinant receptor. For example, in some embodiments, methods for transgene-specific expansion include vaccination methods. In some embodiments, the agent is a peptide vaccine or a cell-based vaccine, *e.g.* cells engineered to express a particular antigen recognized by the recombinant receptor (see, *e.g.*, WO 2016/069647, WO 2011/066048, US 2016/0304624, US Pat. No. 9,476,028 and Hailemichael and Overwijk, *Int J Biochem Cell Biol.* (2014) 53: 46–50). In some embodiments, the methods for transgene-specific expansion include administering anti-idiotypic antibodies. Anti-idiotypic antibodies, including antigen-binding fragments thereof, specifically recognizes, is specifically targeted to, and/or specifically binds to an idiotope of an antibody or an antigen binding fragment thereof, *e.g.*, the antigen-binding domain of a recombinant receptor such as a chimeric antigen receptor (CAR). An idiotope is any single antigenic determinant or epitope within the variable portion of an antibody. In some embodiments, the anti-idiotypic antibodies or antigen-binding fragments thereof are agonists and/or exhibit specific activity to stimulate cells expressing a particular antibody including conjugates or recombinant receptors containing the same or an antigen-binding fragment thereof (see, *e.g.*, U.S. Pat. Publication Nos. US 2016/0096902; US 2016/0068601; US 2014/0322183; US 2015/0175711; US 2015/283178; U.S. Pat. No. 9,102,760; Jena *et al.* *PloS one* (2013) 8(3):e57838; Long *et al.*, *Nature Medicine* (2015) 21(6):581-590; Lee *et al.*, *The Lancet* (2015) 385(9967):517-528; Zhao *et al.*, *PloS One* (2014) 9(5):e96697; Leung *et al.*, *MAbs.* (2015) 7(1):66-76).

[0495] In some embodiments, the methods include modulating the expansion of the engineered cells, for example, by inhibiting negative regulator of proliferation, expansion and/or activation of administered cells, *e.g.*, engineered immune cells. In particular environment in the body of the subject administered cells expressing the recombinant receptor, can encounter an environment that represses or suppresses the growth, proliferation, expansion and/or survival of the cells, *e.g.* immunosuppressive environment. For example, immunosuppressive environments can contain immunosuppressive cytokines, regulatory modulators and co-inhibitory receptors. In some embodiments, an additional agent can be used to modulate the expansion of the administered cells, *e.g.*, overcome suppressive environments.

[0496] In some embodiments, the additional agent includes an immunomodulatory agent, immune checkpoint inhibitor, modulators of metabolic pathways, adenosine pathway or adenosine receptor antagonist or agonist and modulators of signaling pathways, *e.g.*, kinase inhibitors.

[0497] In some embodiments, the additional agent is an immunomodulatory agent, such as

an immune checkpoint inhibitor. In some examples, the additional agent increases, enhances or augments the expansion and/or proliferation of the administered cells and thereby increases, enhances or augments the immune response by blocking an immune checkpoint protein (i.e., immune checkpoint inhibitor). In some embodiments, the additional agent is an agent that enhances the activity of the engineered cell, *e.g.*, a recombinant receptor-expressing cell, is a molecule that inhibits an immune inhibitory molecule or an immune checkpoint molecule. Examples of immune inhibitory molecules include PD-1, PD-L1, CTLA4, TEVI3, CEACAM (*e.g.*, CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4 and TGF β R. In some embodiments, the immune checkpoint inhibitor can be an antibody directed against an immune checkpoint protein, such as an antibody directed against cytotoxic T-lymphocyte antigen 4 (CTLA4 or CD152), programmed cell death protein 1 (PD-1), or programmed cell death protein 1 ligand 1 (PD-L1) (see, *e.g.*, Pardoll, Nat Rev Cancer. 2012 Mar. 22; 12(4):252-264).

[0498] Immune checkpoint inhibitors include any agent that blocks or inhibits in a statistically significant manner, the inhibitory pathways of the immune system. Such inhibitors may include small molecule inhibitors or may include antibodies, or antigen binding fragments thereof, that bind to and block or inhibit immune checkpoint receptors, ligands and/or receptor-ligand interaction. In some embodiments, modulation, enhancement and/or stimulation of particular receptors can overcome immune checkpoint pathway components. Illustrative immune checkpoint molecules that may be targeted for blocking, inhibition, modulation, enhancement and/or stimulation include, but are not limited to, PD-1 (CD279), PD-L1 (CD274, B7-H1), PDL2 (CD273, B7-DC), CTLA-4, LAG-3 (CD223), TIM-3, 4-1BB (CD137), 4-1BBL (CD137L), GITR (TNFRSF18, AITR), CD40, OX40 (CD134, TNFRSF4), CXCR2, tumor associated antigens (TAA), B7-H3, B7-H4, BTLA, HVEM, GAL9, B7H3, B7H4, VISTA, KIR, 2B4 (belongs to the CD2 family of molecules and is expressed on all NK, $\gamma\delta$, and memory CD8+ ($\alpha\beta$) T cells), CD160 (also referred to as BY55), CGEN-15049, CEACAM (*e.g.*, CEACAM-1, CEACAM-3 and/or CEACAM-5), TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and a transforming growth factor receptor (TGFR; *e.g.*, TGFR beta). Immune checkpoint inhibitors include antibodies, or antigen binding fragments thereof, or other binding proteins, that bind to and block or inhibit and/or enhance or stimulate the activity of one or more of any of the said molecules.

[0499] Exemplary immune checkpoint inhibitors include Tremelimumab (CTLA-4 blocking

antibody, also known as ticilimumab, CP-675,206), anti-OX40, PD-L1 monoclonal antibody (Anti-B7-H1; MEDI4736), MK-3475 (PD-1 blocker), nivolumab (anti-PD-1 antibody), CT-011 (anti-PD-1 antibody), BY55 monoclonal antibody, AMP224 (anti-PD-L1 antibody), BMS-936559 (anti-PD-L1 antibody), MPLDL3280A (anti-PD-L1 antibody), MSB0010718C (anti-PD-L1 antibody) and ipilimumab (anti-CTLA-4 antibody, also known as Yervoy®, MDX-010 and MDX-101). Exemplary of immunomodulatory antibodies include, but are not limited to, Daclizumab (Zenapax), Bevacizumab (Avastin®), Basiliximab, Ipilimumab, Nivolumab, pembrolizumab, MPDL3280A, Pidilizumab (CT-011), MK-3475, BMS-936559, MPDL3280A (Atezolizumab), tremelimumab, IMP321, BMS-986016, LAG525, urelumab, PF-05082566, TRX518, MK-4166, dacetuzumab (SGN-40), lucatumumab (HCD122), SEA-CD40, CP-870, CP-893, MEDI6469, MEDI6383, MOXR0916, AMP-224, MSB0010718C (Avelumab), MEDI4736, PDR001, rHiGM12B7, Ulocuplumab, BKT140, Varlilumab (CDX-1127), ARGX-110, MGA271, lirilumab (BMS-986015, IPH2101), IPH2201, ARGX-115, Emactuzumab, CC-90002 and MNRP1685A or an antibody-binding fragment thereof. Other exemplary immunomodulators include, e.g., afutuzumab (available from Roche®); pegfilgrastim (Neulasta®); lenalidomide (CC-5013, Revlimid®); thalidomide (Thalomid®), actimid (CC4047); and IRX-2 (mixture of human cytokines including interleukin 1, interleukin 2, and interferon .gamma., CAS 951209-71-5, available from IRX Therapeutics).

[0500] In some embodiments, the agent includes a molecule that decreases the regulatory T cell (Treg) population. Methods that decrease the number of (e.g., deplete) Treg cells are known in the art and include, e.g., CD25 depletion, cyclophosphamide administration, and modulating Glucocorticoid-induced TNFR family related gene (GITR) function. GITR is a member of the TNFR superfamily that is upregulated on activated T cells, which enhances the immune system. Reducing the number of Treg cells in a subject prior to apheresis or prior to administration of engineered cells, e.g., CAR-expressing cells, can reduce the number of unwanted immune cells (e.g., Tregs) in the tumor microenvironment and reduces the subject's risk of relapse. In some embodiments, the agent includes a molecule targeting GITR and/or modulating GITR functions, such as a GITR agonist and/or a GITR antibody that depletes regulatory T cells (Tregs). In some embodiments, the agent includes cyclophosphamide. In some embodiments, the GITR binding molecule and/or molecule modulating GITR function (e.g., GITR agonist and/or Treg depleting GITR antibodies) is administered prior to the engineered cells, e.g., CAR-expressing cells. For example, in some embodiments, the GITR agonist can be administered prior to apheresis of the cells. In some embodiments, cyclophosphamide is administered to the subject prior to

administration (e.g., infusion or re-infusion) of the engineered cells, e.g., CAR-expressing cells or prior to apheresis of the cells. In some embodiments, cyclophosphamide and an anti-GITR antibody are administered to the subject prior to administration (e.g., infusion or re-infusion) of the engineered cells, e.g., CAR-expressing cells or prior to apheresis of the cells.

[0501] In some embodiments, the agent is a GITR agonist. Exemplary GITR agonists include, e.g., GITR fusion proteins and anti-GITR antibodies (e.g., bivalent anti-GITR antibodies) such as, e.g., a GITR fusion protein described in U.S. Patent No. 6,111,090, European Patent No. 090505B 1, U.S. Patent No. 8,586,023, PCT Publication Nos.: WO 2010/003118 and 2011/090754, or an anti-GITR antibody described, e.g., in U.S. Patent No. 7,025,962, European Patent No. 1947183B 1, U.S. Patent No. 7,812,135, U.S. Patent No. 8,388,967, U.S. Patent No. 8,591,886, European Patent No. EP 1866339, PCT Publication No. WO 2011/028683, PCT Publication No. WO 2013/039954, PCT Publication No. WO2005/007190, PCT Publication No. WO 2007/133822, PCT Publication No. WO2005/055808, PCT Publication No. WO 99/40196, PCT Publication No. WO 2001/03720, PCT Publication No. WO99/20758, PCT Publication No. WO2006/083289, PCT Publication No. WO 2005/115451, U.S. Patent No. 7,618,632, and PCT Publication No. WO 2011/051726. An exemplary anti-GITR antibody is TRX518.

[0502] In some embodiments, the agent is a structural or functional analog or derivative of thalidomide and/or an inhibitor of E3 ubiquitin ligase. In some embodiments, the immunomodulatory agent binds to cereblon (CRBN). In some embodiments, the immunomodulatory agent binds to the CRBN E3 ubiquitin-ligase complex. In some embodiments, the immunomodulatory agent binds to CRBN and the CRBN E3 ubiquitin-ligase complex. In some embodiments, the immunomodulatory agent up-regulates the protein or gene expression of CRBN. In some aspects, CRBN is the substrate adaptor for the CRL4^{CRBN} E3 ubiquitin ligase, and modulates the specificity of the enzyme. In some embodiments, binding to CRBN or the CRBN E3 ubiquitin ligase complex inhibits E3 ubiquitin ligase activity. In some embodiments, the immunomodulatory agent induces the ubiquitination of IKZF1 (Ikaros) and IKZF3 (Aiolos) and/or induces degradation of IKZF1 (Ikaros) and IKZF3 (Aiolos). In some embodiments, the immunomodulatory agent induces the ubiquitination of casein kinase 1A1 (CK1 α) by the CRL4^{CRBN} E3 ubiquitin ligase. In some embodiments, the ubiquitination of CK1 α results in CK1 α degradation.

[0503] In some embodiments, the agent is an inhibitor of the Ikaros (IKZF1) transcription factor. In some embodiments, the agent enhances ubiquitination of Ikaros. In some

embodiments, the agent enhances the degradation of Ikaros. In some embodiments, the agent down-regulates the protein or gene expression of Ikaros. In some embodiments, administration of the agent causes a decrease in Ikaros protein levels.

[0504] In some embodiments, the agent is an inhibitor of the Aiolos (IKZF3) transcription factor. In some embodiments, the agent enhances ubiquitination of Aiolos. In some embodiments, the agent enhances the degradation of Aiolos. In some embodiments, the agent down-regulates the protein or gene expression of Aiolos. In some embodiments, administration of the agent causes a decrease in Aiolos protein levels.

[0505] In some embodiments, the agent is thalidomide (2-(2,6-dioxopiperidin-3-yl)-1H-isoindole-1,3(2H)-dione) or an analog or derivative of thalidomide. In certain embodiments, a thalidomide derivative includes structural variants of thalidomide that have a similar biological activity. Exemplary thalidomide derivatives include, but are not limited to lenalidomide (REVLIMMUNOMODULATORY COMPOUND™; Celgene Corporation), pomalidomide (also known as ACTIMMUNOMODULATORY COMPOUND™ or POMALYST™ (Celgene Corporation)), CC-1088, CDC-501, and CDC-801, and the compounds disclosed in U.S. Pat. Nos. 5,712,291; 7,320,991; and 8,716,315; U.S. Appl. No. 2016/0313300; and PCT Pub. Nos. WO 2002/068414 and WO 2008/154252.

[0506] In some embodiments, the agent is 1-oxo- and 1,3 dioxo-2-(2,6-dioxopiperidin-3-yl)isoindolines substituted with amino in the benzo ring as described in U.S. Pat. No. 5,635,517 which is incorporated herein by reference.

[0507] In some embodiments, the agent is a compound that belongs to a class of isoindole-immunomodulatory compounds disclosed in U.S. Pat. No. 7,091,353, U.S. Patent Publication No. 2003/0045552, and International Application No. PCT/US01/50401 (International Publication No. WO02/059106), each of which are incorporated herein by reference. For example, in some embodiments, the agent is [2-(2,6-dioxo-piperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-ylmethyl]-amide; (2-(2,6-dioxo-piperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-ylmethyl)-carbamic acid tert-butyl ester; 4-(aminomethyl)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione; N-(2-(2,6-dioxo-piperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-ylmethyl)-acetamide; N-{(2-(2,6-dioxo(3-piperidyl))-1,3-dioxoisindolin-4-yl)methyl}cyclopropyl-carboxamide; 2-chloro-N-{(2-(2,6-dioxo(3-piperidyl))-1,3-dioxoisindolin-4-yl)methyl}acetamide; N-(2-(2,6-dioxo(3-piperidyl))-1,3-dioxoisindolin-4-yl)-3-pyridylcarboxamide; 3-{1-oxo-4-(benzylamino)isoindolin-2-yl}piperidine-2,6-dione; 2-(2,6-dioxo(3-piperidyl))-4-(benzylamino)isoindoline-1,3-dione; N-{(2-(2,6-dioxo(3-piperidyl))-

1,3-dioxoisindolin-4-yl)methyl}propanamide; N-{(2-(2,6-dioxo(3-piperidyl))-1,3-dioxoisindolin-4-yl)methyl}-3-pyridylcarboxamide; N-{(2-(2,6-dioxo(3-piperidyl))-1,3-dioxoisindolin-4-yl)methyl}heptanamide; N-{(2-(2,6-dioxo(3-piperidyl))-1,3-dioxoisindolin-4-yl)methyl}-2-furylcarboxamide; {N-(2-(2,6-dioxo(3-piperidyl))-1,3-dioxoisindolin-4-yl)carbamoyl}methyl acetate; N-(2-(2,6-dioxo(3-piperidyl))-1,3-dioxoisindolin-4-yl)pentanamide; N-(2-(2,6-dioxo(3-piperidyl))-1,3-dioxoisindolin-4-yl)-2-thienylcarboxamide; N-{[2-(2,6-dioxo(3-piperidyl))-1,3-dioxoisindolin-4-yl]methyl}(butylamino)carboxamide; N-{[2-(2,6-dioxo(3-piperidyl))-1,3-dioxoisindolin-4-yl]methyl}(octylamino)carboxamide; or N-{[2-(2,6-dioxo(3-piperidyl))-1,3-dioxoisindolin-4-yl]methyl}(benzylamino)carboxamide.

[0508] In some embodiments, the agent is lenalidomide, pomalidomide, avadomide, a stereoisomer of lenalidomide, pomalidomide, avadomide or a pharmaceutically acceptable salt, solvate, hydrate, co-crystal, clathrate, or polymorph thereof. In some embodiments, the immunomodulatory compound is lenalidomide, a stereoisomer of lenalidomide or a pharmaceutically acceptable salt, solvate, hydrate, co-crystal, clathrate, or polymorph thereof. In some embodiments, the immunomodulatory compound is lenalidomide, or ((RS)-3-(4-Amino-1-oxo-1,3-dihydro-2H-isoindol-2-yl)piperidine-2,6-dione).

[0509] In some embodiments, the methods include contacting the cells expressing the recombinant receptor with an agent that inhibits inhibitory cell surface receptors, *e.g.*, transforming growth factor beta receptor (TGFβR). In some embodiments, administered cells, *e.g.*, recombinant receptor expressing cells, can be engineered to resist the effects of immunosuppressive cytokines that can inhibit their effector functions (see, *e.g.*, Foster *et al.*, J Immunother. (2008) 31:500–505; Bollard *et al.*, Molecular Therapy. (2012) 20:S22; Bendle *et al.*, J. Immunol. (2013) 191(6):3232-3239). In some embodiments, the additional agent is an anti-TGFβ antibody or an anti-TGFβR antibody (see, *e.g.*, WO 2011/109789).

[0510] In some embodiments, the additional agent modulates the metabolism, signaling and/or transport of immunosuppressive factors, *e.g.*, adenosine. In some embodiments, the additional agent is an inhibitor of extracellular adenosine or adenosine receptor, or an agent that causes a reduction or a decrease of extracellular adenosine levels, such as an agent that prevents the formation of, degrades, renders inactive, and/or decreases extracellular adenosine. In some embodiments, the additional agent is an adenosine receptor antagonist such as the A2a, A2b and/or A3 receptor. In some embodiments, the antagonist is a peptide, or a peptidomimetic, that binds the adenosine receptor but does not trigger a G1 protein dependent intracellular pathway. Exemplary adenosine receptor antagonists are described in U.S. Pat. Nos. 5,565,566; 5,545,627,

5,981,524; 5,861,405; 6,066,642; 6,326,390; 5,670,501; 6,117,998; 6,232,297; 5,786,360; 5,424,297; 6,313,131, 5,504,090; and 6,322,771; and Jacobson and Gao, *Nat Rev Drug Discov.* (2006) 5(3): 247–264.

[0511] In some embodiments, the agent is an A2 receptor (A2R) antagonist, such as an A2a antagonist. Exemplary A2R antagonists include KW6002 (istradefyline), SCH58261, caffeine, paraxanthine, 3,7-dimethyl-1-propargylxanthine (DMPX), 8-(m-chlorostyryl) caffeine (CSC), MSX-2, MSX-3, MSX-4, CGS-15943, ZM-241385, SCH-442416, preladenant, vipadenant (BII014), V2006, ST-1535, SYN-115, PSB-1115, ZM241365, FSPTP, and an inhibitory nucleic acid targeting A2R expression, e.g., siRNA or shRNA, or any antibodies or antigen-binding fragment thereof that targets an A2R. In some embodiments, the agent is an A2R antagonist described in, e.g., Ohta et al., *Proc Natl Acad Sci U S A* (2006) 103:13132-13137; Jin et al., *Cancer Res.* (2010) 70(6):2245-2255; Leone et al., *Computational and Structural Biotechnology Journal* (2015) 13:265-272; Beavis et al., *Proc Natl Acad Sci U S A* (2013) 110:14711–14716; and Pinna, A., *Expert Opin Investig Drugs* (2009) 18:1619-1631; Sitkovsky et al., *Cancer Immunol Res* (2014) 2(7):598-605; US 8,080,554; US 8,716,301; US 20140056922; WO2008/147482; US 8,883,500; US 20140377240; WO02/055083; US 7,141,575; US 7,405,219; US 8,883,500; US 8,450,329 and US 8,987,279).

[0512] In some embodiments, the methods include administering additional agents that are immunostimulatory. In some embodiments, the additional agent can generally promote the proliferation, expansion, survival, potency and/or efficacy of immune cells. In some embodiments, the additional agent can specifically promote administered cells, e.g., recombinant receptor-expressing cells. In some embodiments, the additional agent is a cytokine. In some embodiments, the additional agent is a ligand.

[0513] In some embodiments, the additional agent is an immunostimulatory ligand, e.g., CD40L. In some embodiments, the additional agent is a cytokine, e.g., IL-2, IL-3, IL-6, IL-11, IL-7, IL-12, IL-15, IL-21, granulocyte macrophage colony stimulating factor (GM-CSF), alpha, beta or gamma interferon (IFN) and erythropoietin (EPO). In some embodiments, the agent is a cytokine. In some embodiments, the immunomodulatory agent is a cytokine or is an agent that induces increased expression of a cytokine in the tumor microenvironment. Cytokines have important functions related to T cell expansion, differentiation, survival, and homeostasis. Cytokines that can be administered to the subject receiving the cells and/or compositions provided herein include one or more of IL-2, IL-4, IL-7, IL-9, IL-15, IL-18, and IL-21. In some embodiments, the cytokine administered is IL-7, IL-15, or IL-21, or a combination thereof. In

some embodiments, administration of the cytokine to the subject that has sub-optimal response to the administration of the engineered cells, e.g., CAR-expressing cells improves potency and/or efficacy and/or anti-tumor activity of the administered cells, e.g., CAR-expressing cells.

[0514] In some embodiments, the agent is an inhibitor of hypoxia inducible factor 1 alpha (HIF-1 α) signaling. Exemplary inhibitors of HIF-1 α include digoxin, acriflavine, sirtuin-7 and ganetespib.

[0515] In some embodiments, the agent includes a protein tyrosine phosphatase inhibitor, e.g., a protein tyrosine phosphatase inhibitor described herein. In some embodiments, the protein tyrosine phosphatase inhibitor is an SHP-1 inhibitor, e.g., an SHP-1 inhibitor described herein, such as, e.g., sodium stibogluconate. In some embodiments, the protein tyrosine phosphatase inhibitor is an SHP-2 inhibitor, e.g., an SHP-2 inhibitor described herein.

[0516] In some aspects, the method results in at least a 2-fold, at least a 4-fold, at least a 10-fold, or at least a 20-fold increase in copies of nucleic acid encoding the recombinant receptor, e.g., CAR, per microgram of DNA, e.g., in the serum, plasma, blood or tissue, e.g., tumor sample, of the subject. (from old section, move to method of modulation section)

[0517] In some aspects, the method results in high *in vivo* proliferation of the administered cells, for example, as measured by flow cytometry. In some aspects, high peak proportions of the cells are detected. For example, in some embodiments, at a peak or maximum level or concentration following the administration of the T cells, e.g., CAR-expressing T cells, in the blood or disease-site of the subject or white blood cell fraction thereof, e.g., PBMC fraction or T cell fraction, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90% of the cells express the recombinant receptor, e.g., the CAR.

[0518] In some embodiments, the method results in a maximum concentration, in the blood or serum or other bodily fluid or organ or tissue of the subject, of at least 100, 500, 1000, 1500, 2000, 5000, 10,000 or 15,000 copies of or nucleic acid encoding the receptor, e.g., the CAR, per microgram of DNA, or at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, or 0.9 receptor-expressing, e.g., CAR-expressing cells per total number of peripheral blood mononuclear cells (PBMCs), total number of mononuclear cells, total number of T cells, or total number of microliters of the blood or serum or other bodily fluid or organ or tissue of the subject. In some embodiments, the cells expressing the receptor are detected as at least 10, 20, 30, 40, 50, or 60% of total PBMCs in the blood of the subject, and/or at such a level for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24, 36, 48, or 52 weeks following the T cells, e.g., CAR-expressing T cells or for 1, 2, 3, 4, or 5, or

more years following such administration.

2. Agents for Reducing Cell Expansion

[0519] In some embodiments, the provided methods and articles of manufacture can be used in connection with, or involve or include, one or more agents or treatments capable of modulating, e.g., increasing or decreasing, CAR+ T cell expansion, proliferation, and/or activity. In some embodiments, the agent is capable of reducing, decreasing, and/or dampening CAR+ T cell expansion and/or proliferation. In some embodiments, expansion and/or proliferation of CAR+ T cells above a certain threshold value, or high expression of certain biomarkers, such as inflammatory markers, can be associated with a reduced response and/or reduced durable response. In some embodiments, if the administered cells in the subject are determined to have very high or excessive expansion, or if the subject is determined to express biomarkers associated with very high expansion or excessive expansion, the subject may be determined not likely to achieve response and/or durable response. In some embodiments, very high expansion or excessive expansion is also associated with high tumor burden and inflammatory cytokine production. In some embodiments, an agent that can reduce, decrease and/or dampen CAR+ T cell expansion and/or proliferation can be administered to such subjects.

[0520] In some contexts, optimal efficacy of an administered cell therapy, e.g., CAR+ T cell therapy, can depend on the ability of the administered cells to become activated, expand, to exert various effector functions, to persist, including long-term, to differentiate, transition or engage in reprogramming into certain phenotypic states (such as long-lived memory, less-differentiated, and effector states), to avoid or reduce immunosuppressive conditions in the local microenvironment of a disease, to provide effective and robust recall responses following clearance and re-exposure to target ligand or antigen, and avoid or reduce exhaustion, anergy, peripheral tolerance, terminal differentiation, and/or differentiation into a suppressive state. In some aspects, excessive or very high expansion or proliferation of the administered T cells may result in exhaustion, anergy, peripheral tolerance, terminal differentiation, and/or differentiation into a suppressive state. In some aspects, an agent that can reduce, decrease and/or dampen CAR+ T cell expansion and/or proliferation can prevent or reduce such exhaustion or differentiation.

[0521] In some embodiments, the administration of the agent is capable of reducing, decreasing, and/or dampening CAR+ T cell expansion and/or proliferation, such as a steroid, can result in reduced expansion of the administered CAR+ T cells. In some embodiments,

administration of the agent can result in changes in parameters, e.g., reduced volumetric measures, e.g., SPD, or expression of inflammatory markers, e.g., LDH.

[0522] In some embodiments, the agent capable of reducing, decreasing, and/or dampening CAR+ T cell expansion and/or proliferation is a steroid, is an antagonist or inhibitor of a cytokine receptor, such as IL-6 receptor, CD122 receptor (IL-2Rbeta receptor), or CCR2, or is an inhibitor of a cytokine, such as IL-6, MCP-1, IL-10, IFN- γ , IL-8, or IL-18. In some embodiments, the agent is an agonist of a cytokine receptor and/or cytokine, such as TGF- β . In some embodiments, the agent, e.g., agonist, antagonist or inhibitor, is an antibody or antigen-binding fragment, a small molecule, a protein or peptide, or a nucleic acid.

[0523] In some embodiments, the agent capable of reducing, decreasing, and/or dampening CAR+ T cell expansion and/or proliferation is a steroid, e.g., corticosteroid. Corticosteroids typically include glucocorticoids and mineralocorticoids.

[0524] Any corticosteroid, e.g., glucocorticoid, can be used in the methods provided herein. In some embodiments, glucocorticoids include synthetic and non-synthetic glucocorticoids. Exemplary glucocorticoids include, but are not limited to: alclomethasones, algestones, beclomethasones (e.g. beclomethasone dipropionate), betamethasones (e.g. betamethasone 17-valerate, betamethasone sodium acetate, betamethasone sodium phosphate, betamethasone valerate), budesonides, clobetasols (e.g. clobetasol propionate), clobetasones, clocortolones (e.g. clocortolone pivalate), cloprednols, corticosterones, cortisones and hydrocortisones (e.g. hydrocortisone acetate), cortivazols, deflazacorts, desonides, desoximethasones, dexamethasones (e.g. dexamethasone 21-phosphate, dexamethasone acetate, dexamethasone sodium phosphate), diflorasones (e.g. diflorasone diacetate), diflucortolones, difluprednates, enoxolones, fluazacorts, flucloronides, fludrocortisones (e.g., fludrocortisone acetate), flumethasones (e.g. flumethasone pivalate), flunisolides, fluocinolones (e.g. fluocinolone acetonide), fluocinonides, fluocortins, fluocortolones, fluorometholones (e.g. fluorometholone acetate), fluperolones (e.g., fluperolone acetate), fluprednidenes, fluprednisolones, flurandrenolides, fluticasones (e.g. fluticasone propionate), formocortals, halcinonides, halobetasols, halometasones, halopredones, hydrocortamates, hydrocortisones (e.g. hydrocortisone 21-butyrate, hydrocortisone aceponate, hydrocortisone acetate, hydrocortisone buteprate, hydrocortisone butyrate, hydrocortisone cypionate, hydrocortisone hemisuccinate, hydrocortisone probutate, hydrocortisone sodium phosphate, hydrocortisone sodium succinate, hydrocortisone valerate), loteprednol etabonate, mazipredones, medrysones, meprednisones, methylprednisolones (methylprednisolone aceponate, methylprednisolone acetate,

methylprednisolone hemisuccinate, methylprednisolone sodium succinate), mometasones (e.g., mometasone furoate), paramethasones (e.g., paramethasone acetate), prednicarbates, prednisolones (e.g. prednisolone 25-diethylaminoacetate, prednisolone sodium phosphate, prednisolone 21-hemisuccinate, prednisolone acetate; prednisolone farnesylate, prednisolone hemisuccinate, prednisolone-21 (beta-D-glucuronide), prednisolone metasulphobenzoate, prednisolone steaglate, prednisolone tebutate, prednisolone tetrahydrophthalate), prednisones, prednivals, prednylidenes, rimexolones, tixocortols, triamcinolones (e.g. triamcinolone acetonide, triamcinolone benetonide, triamcinolone hexacetonide, triamcinolone acetonide 21-palmitate, triamcinolone diacetate). These glucocorticoids and the salts thereof are discussed in detail, for example, in Remington's Pharmaceutical Sciences, A. Osol, ed., Mack Pub. Co., Easton, Pa. (16th ed. 1980).

[0525] In some embodiments, the steroid is administered prior to, concurrently with and/or after administration of an immunotherapy and/or cell therapy, such as cell therapy with an engineered cell composition as described herein. In some embodiments, the steroid is administered after administration of the immunotherapy and/or cell therapy, or a first administration or dose thereof, or after the initiation of any of the foregoing. In some embodiments, the steroid is administered within at or about 12, 18, 24, 36 or 48 hours, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more, or 2, 3, 4, 5, 6, 7, 8, 9, 10 weeks or more, after administration of the immunotherapy and/or cell therapy, or a first administration or dose thereof, or after the initiation of any of the foregoing. In some embodiments, the steroid is administered within at or about 12, 24, 36 or 48 hours, or within at or about 2, 3, or 4 days after administration of the immunotherapy and/or cell therapy, or a first administration or dose thereof, or after the initiation of any of the foregoing.

[0526] In some embodiments, the steroid, e.g., corticosteroid, is administered in multiple doses over a period of time. In some aspects, the steroid, e.g., corticosteroid, can be administered over a period of more than at or about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more, or more than at or about 2, 3, 4, 5, 6, 7, 8, 9, 10 weeks or more, or within at or about a range defined by any of the foregoing. In some aspects, the steroid, e.g., corticosteroid, can be administered over a period of more than at or about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40 days or more, or within at or about a range defined by any of the foregoing. In some embodiments, the steroid, e.g., corticosteroid, can be administered in multiple or repeat doses over a total duration of about 6, 12, 18, 24 hours or more, or 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13,

14 days or more, or 2, 3, 4, 5, 6, 7, 8, 9, 10 weeks or more, or within at or about a range defined by any of the foregoing. In some embodiments, the steroid, e.g., corticosteroid, can be administered one per day, twice per day, or three times or more per day. In some embodiments, the steroid, e.g., corticosteroid, can be administered at least or at least about every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18, 24, 36, 48 hours, or every 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days, or every 2, 3, 4, 5, 6, 7, 8, 9 or 10 weeks or more, or within at or about a range defined by any of the foregoing. In some aspects, the steroid, e.g., glucocorticoid, can be administered in multiple or repeated doses, over a period of more than at or about one day, such as over two days, over 3 days, or over 4 or more days. In some embodiments, the steroid, e.g., corticosteroid or glucocorticoid, can be administered for a total duration of 6, 12, 18, 24 hours or 2, 3, 4, 5, 6, 7, 8, 9 or 10 days or more, or within at or about a range defined by any of the foregoing. In some embodiments, the corticosteroid can be administered one per day, twice per day, or three times or four times or more per day. In some embodiments, the corticosteroid can be administered at least or at least about every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18, 24, 36, 48 hours or more, or within at or about a range defined by any of the foregoing.

[0527] In some embodiments, the steroid is dexamethasone, and the dexamethasone is administered in multiple doses over a period of time. In some aspects, the dexamethasone can be administered over a period of more than at or about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more, or more than at or about 2, 3, 4, 5, 6, 7, 8, 9, 10 weeks or more, or within at or about a range defined by any of the foregoing. In some embodiments, the dexamethasone can be administered in multiple or repeat doses over a total duration of about 6, 12, 18, 24 hours or more, or 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more, or 2, 3, 4, 5, 6, 7, 8, 9, 10 weeks or more, or within at or about a range defined by any of the foregoing. In some embodiments, the dexamethasone can be administered one per day, twice per day, or three times or more per day. In some embodiments, the dexamethasone is administered daily, for up to 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40 days or more, or within at or about a range defined by any of the foregoing.

[0528] In some embodiments, the steroid, e.g., dexamethasone, is first administered after initiation of the an immunotherapy and/or cell therapy, and subsequently administered in multiple doses over a period of time. In some embodiments, the initial administration of the steroid, e.g., dexamethasone, is within at or about 12, 18, 24, 36 or 48 hours, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more, or 2, 3, 4, 5, 6, 7, 8, 9, 10 weeks or more, after administration of the immunotherapy and/or cell therapy, or a first administration or dose thereof, or after the

initiation of any of the foregoing. In some embodiments, the steroid, e.g., dexamethasone, is first administered within at or about 12, 24, 36 or 48 hours, or within at or about 2, 3, or 4 days after administration of the immunotherapy and/or cell therapy, or a first administration or dose thereof, or after the initiation of any of the foregoing. The administration of the steroid, e.g., dexamethasone, is subsequently administered over a period of more than at or about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more, or more than at or about 2, 3, 4, 5, 6, 7, 8, 9, 10 weeks or more, or within at or about a range defined by any of the foregoing. For example, the steroid, e.g., dexamethasone, can be administered beginning at within at or about 12, 24, 36 or 48 hours, or within at or about 2, 3, or 4 days after the initial administration of the cell therapy, and administered in multiple or repeat doses until about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more, or 2, 3, 4, 5, 6, 7, 8, 9, 10 weeks or more after the initial administration of the cell therapy. In some embodiments, the steroid, e.g., dexamethasone, is can be administered one per day, twice per day, or three times or more per day, during the period of administration. In some embodiments, the steroid, e.g., dexamethasone, can be administered, beginning at day 1, 2, 3, 4, or 5 after the initial administration of the cell therapy, and ending at days 6, 7, 8, 9, 10, 11, 12, 13, 14 days or week 2, 3 or 4 after the initial administration of the cell therapy.

[0529] In some examples, the glucocorticoid is selected from among cortisones, dexamethasones, hydrocortisones, methylprednisolones, prednisolones and prednisones. In a particular example, the glucocorticoid is dexamethasone.

[0530] In some embodiments, the agent is a corticosteroid and is administered in an amount that is therapeutically effective to reduce, decrease and/or dampen CAR⁺ T cell expansion and/or proliferation. In some embodiments, indicators of improvement or successful treatment include determination of pharmacokinetic parameters, e.g., any described herein, such as peak CAR⁺ T cell concentration and/or AUC.

[0531] In some aspects, the corticosteroid is provided in a therapeutically effective dose. Therapeutically effective concentration can be determined empirically by testing in known in vitro or in vivo (e.g. animal model) systems. In addition, animal models can be employed to help identify optimal dosage ranges. The precise dosage, which can be determined empirically, can depend on the particular therapeutic preparation, the regime and dosing schedule, the route of administration and the seriousness of the disease.

[0532] The corticosteroid can be administered in any amount that is effective reduce, decrease and/or dampen CAR⁺ T cell expansion and/or proliferation. The corticosteroid, e.g., glucocorticoid, can be administered, for example, at an amount between at or about 0.1 and at or

about 100 mg, per dose, at or about 0.1 and at or about 80 mg, at or about 0.1 and at or about 60 mg, at or about 0.1 and at or about 40 mg, at or about 0.1 and at or about 30 mg, at or about 0.1 and at or about 20 mg, at or about 0.1 and at or about 15 mg, at or about 0.1 and at or about 10 mg, at or about 0.1 and at or about 5 mg, at or about 0.2 and at or about 40 mg, at or about 0.2 and at or about 30 mg, at or about 0.2 and at or about 20 mg, at or about 0.2 and at or about 15 mg, at or about 0.2 and at or about 10 mg, at or about 0.2 and at or about 5 mg, at or about 0.4 and at or about 40 mg, at or about 0.4 and at or about 30 mg, at or about 0.4 and at or about 20 mg, at or about 0.4 and at or about 15 mg, at or about 0.4 and at or about 10 mg, at or about 0.4 and at or about 5 mg, at or about 0.4 and at or about 4 mg, at or about 1 and at or about 20 mg, at or about 1 and at or about 15 mg or 1 and at or about 10 mg to a 70 kg adult human subject.

Typically, the corticosteroid, such as a glucocorticoid is administered at an amount between at or about 0.4 and at or about 20 mg, for example, at or about 0.4 mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.75 mg, 0.8 mg, 0.9 mg, 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg or 20 mg per dose (or equivalents thereof), to an average adult human subject. In some embodiments, the corticosteroid, such as a glucocorticoid is administered at an amount between at or about 10 and at or about 80 mg, for example, at or about 10 mg, 15 mg, 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, 60 mg, 65 mg, 70 mg, 75 mg or 80 mg, per dose (or equivalents thereof), to an average adult human subject.

[0533] In some embodiments, the corticosteroid, e.g., glucocorticoid, can be administered, at or about for example, at or about at an amount between at or about 0.1 and at or about at or about 100 mg per day, at or about 0.1 and at or about 80 mg per day, at or about 0.1 and at or about 60 mg per day, at or about 0.1 and at or about 40 mg per day, at or about 0.1 and at or about 30 mg per day, at or about 0.1 and at or about 20 mg per day, at or about 0.1 and at or about 15 mg per day, at or about 0.1 and at or about 10 mg per day, at or about 0.1 and at or about 5 mg per day, at or about 0.2 and at or about 80 mg per day, at or about 0.2 and at or about 60 mg per day, at or about 0.2 and at or about 40 mg per day, at or about 0.2 and at or about 30 mg per day, at or about 0.2 and at or about 20 mg per day, at or about 0.2 and at or about 15 mg per day, at or about 0.2 and at or about 10 mg per day, at or about 0.2 and at or about 5 mg per day, at or about 0.4 and at or about 40 mg per day, at or about 0.4 and at or about 30 mg per day, at or about 0.4 and at or about 20 mg per day, at or about 0.4 and at or about 15 mg per day, at or about 0.4 and at or about 10 mg per day, at or about 0.4 and at or about 5 mg per day, at or about 0.4 and at or about 4 mg per day, at or about 1 and at or about 20 mg per day, at or about 1 and

at or about 15 mg per day or at or about 1 and at or about 10 mg per day (or equivalents thereof), to an adult human subject. In some embodiments, the corticosteroid, such as a glucocorticoid is administered at an amount between at or about 0.4 and at or about 20 mg per day, for example, at or about 0.4 mg per day, 0.5 mg per day, 0.6 mg per day, 0.7 mg per day, 0.75 mg per day, 0.8 mg per day, 0.9 mg per day, 1 mg per day, 2 mg per day, 3 mg per day, 4 mg per day, 5 mg per day, 6 mg per day, 7 mg per day, 8 mg per day, 9 mg per day, 10 mg per day, 11 mg per day, 12 mg per day, 13 mg per day, 14 mg per day, 15 mg per day, 16 mg per day, 17 mg per day, 18 mg per day, 19 mg per day or 20 mg per day (or equivalents thereof), to an average adult human subject. In some embodiments, the corticosteroid, such as a glucocorticoid is administered at an amount between at or about 10 and at or about 80 mg per day, for example, at or about 10 mg per day, 15 mg per day, 20 mg per day, 25 mg per day, 30 mg per day, 35 mg per day, 40 mg per day, 45 mg per day, 50 mg per day, 55 mg per day, 60 mg per day, 65 mg per day, 70 mg per day, 75 mg per day or 80 mg per day (or equivalents thereof), to an average adult human subject.

[0534] In some embodiments, the corticosteroid is dexamethasone. Exemplary doses of dexamethasone that can be administered include an amount between at or about 0.1 and at or about 100 mg, per dose, 0.1 and 80 mg, 0.1 and 60 mg, 0.1 and 40 mg, 0.1 and 30 mg, 0.1 and 20 mg, 0.1 and 15 mg, 0.1 and 10 mg, 0.1 and 5 mg, 0.2 and 40 mg, 0.2 and 30 mg, 0.2 and 20 mg, 0.2 and 15 mg, 0.2 and 10 mg, 0.2 and 5 mg, 0.4 and 40 mg, 0.4 and 30 mg, 0.4 and 20 mg, 0.4 and 15 mg, 0.4 and 10 mg, 0.4 and 5 mg, 0.4 and 4 mg, 1 and 20 mg, 1 and 15 mg or 1 and 10 mg, to a 70 kg adult human subject. In some embodiments, the dexamethasone is administered at an amount between at or about 0.4 and at or about 20 mg, for example, at or about 0.4 mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.75 mg, 0.8 mg, 0.9 mg, 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg or 20 mg per dose, to an average adult human subject. In some embodiments, the dexamethasone is administered at an amount between at or about 10 and at or about 40 mg, for example, at or about 10 mg, 15 mg, 20 mg, 25 mg, 30 mg, 35 mg or 40 mg per dose, to an average adult human subject.

[0535] In some embodiments, the dexamethasone can be administered, for example, at an amount between at or about 0.1 and at or about 100 mg per day, 0.1 and 80 mg per day, 0.1 and 60 mg per day, 0.1 and 40 mg per day, 0.1 and 30 mg per day, 0.1 and 20 mg per day, 0.1 and 15 mg per day, 0.1 and 10 mg per day, 0.1 and 5 mg per day, 0.2 and 80 mg per day, 0.2 and 60 mg per day, 0.2 and 40 mg per day, 0.2 and 30 mg per day, 0.2 and 20 mg per day, 0.2 and 15 mg per day, 0.2 and 10 mg per day, 0.2 and 5 mg per day, 0.4 and 40 mg per day, 0.4 and 30 mg per

day, 0.4 and 20 mg per day, 0.4 and 15 mg per day, 0.4 and 10 mg per day, 0.4 and 5 mg per day, 0.4 and 4 mg per day, 1 and 20 mg per day, 1 and 15 mg per day or 1 and 10 mg per day, to an adult human subject. In some embodiments, the dexamethasone is administered at an amount between at or about 0.4 and at or about 20 mg per day, for example, at or about 0.4 mg per day, 0.5 mg per day, 0.6 mg per day, 0.7 mg per day, 0.75 mg per day, 0.8 mg per day, 0.9 mg per day, 1 mg per day, 2 mg per day, 3 mg per day, 4 mg per day, 5 mg per day, 6 mg per day, 7 mg per day, 8 mg per day, 9 mg per day, 10 mg per day, 11 mg per day, 12 mg per day, 13 mg per day, 14 mg per day, 15 mg per day, 16 mg per day, 17 mg per day, 18 mg per day, 19 mg per day or 20 mg per day, to an average adult human subject. In some embodiments, the dexamethasone is administered at a high dose, such as in an amount between at or about 10 and at or about 40 mg per day, for example, at or about 10 mg per day, 15 mg per day, 20 mg per day, 25 mg per day, 30 mg per day, 35 mg per day or 40 mg per day, to an average adult human subject. In some embodiments, a high-dose of dexamethasone can be administered. Exemplary high-dose dexamethasone include a dosage amount of at or about 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, 60 mg, 65 mg, 70 mg, 75 mg or 80 mg dexamethasone or equivalent thereof, or a range defined by any of the foregoing, each inclusive, to an average adult human subject. Exemplary high-dose dexamethasone include a dose of 20 mg per day, 25 mg per day, 30 mg per day, 35 mg per day, 40 mg per day, 45 mg per day, 50 mg per day, 55 mg per day, 60 mg per day, 65 mg per day, 70 mg per day, 75 mg per day or 80 mg per day, or a range defined by any of the foregoing, each inclusive, to an average adult human subject.

[0536] In some embodiments, the corticosteroid can be administered, for example, at a dosage of at or about 0.001 mg/kg (of the subject), 0.002 mg/kg, 0.003 mg/kg, 0.004 mg/kg, 0.005 mg/kg, 0.006 mg/kg, 0.007 mg/kg, 0.008 mg/kg, 0.009 mg/kg, 0.01 mg/kg, 0.015 mg/kg, 0.02 mg/kg, 0.025 mg/kg, 0.03 mg/kg, 0.035 mg/kg, 0.04 mg/kg, 0.045 mg/kg, 0.05 mg/kg, 0.055 mg/kg, 0.06 mg/kg, 0.065 mg/kg, 0.07 mg/kg, 0.075 mg/kg, 0.08 mg/kg, 0.085 mg/kg, 0.09 mg/kg, 0.095 mg/kg, 0.1 mg/kg, 0.15 mg/kg, 0.2 mg/kg, 0.25 mg/kg, 0.30 mg/kg, 0.35 mg/kg, 0.40 mg/kg, 0.45 mg/kg, 0.50 mg/kg, 0.55 mg/kg, 0.60 mg/kg, 0.65 mg/kg, 0.70 mg/kg, 0.75 mg/kg, 0.80 mg/kg, 0.85 mg/kg, 0.90 mg/kg, 0.95 mg/kg, 1 mg/kg, 1.05 mg/kg, 1.1 mg/kg, 1.15 mg/kg, 1.20 mg/kg, 1.25 mg/kg, 1.3 mg/kg, 1.35 mg/kg or 1.4 mg/kg, to an average adult human subject, typically weighing about 70 kg to 75 kg.

[0537] The corticosteroid, or glucocorticoid, for example dexamethasone, can be administered orally (tablets, liquid or liquid concentrate), PO, intravenously (IV), intramuscularly or by any other known route or route described herein (e.g., with respect to

pharmaceutical formulations). In some aspects, the corticosteroid is administered as a bolus, and in other aspects it may be administered over a period of time. In some aspects, the corticosteroid is administered as a bolus, and in other aspects it may be administered over a period of time, e.g., over 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 120, 180, 240, 360, 480 or 720 minutes or more, or a range defined by any two of the foregoing values.

[0538] In some aspects, the glucocorticoid can be administered over a period of more than one day, such as over two days, over 3 days, or over 4 or more days. In some embodiments, the corticosteroid can be administered one per day, twice per day, or three times or more per day. For example, the corticosteroid, e.g., dexamethasone, may in some examples be administered at 10 mg (or equivalent) IV twice a day for three days.

[0539] In some embodiments, the steroid, e.g., corticosteroid or glucocorticoid, can be administered at a given dose per day, e.g., a specific dose per day. In some embodiments, exemplary dose per day includes at or about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2, 3, 4, 5, 6, 7, 8, 9 or 10 mg/kg per day, or a range defined by any two of the foregoing values and equivalents thereof. In some embodiments, the steroid, e.g., corticosteroid or glucocorticoid, can be administered at or about 0.25, 0.5, 0.75, 1, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0, 13.0, 14.0, 15.0, 16.0, 17.0, 18.0, 19.0, 20.0, 25.0, 50.0 or 100.0 mg/kg/day, or a range defined by any two of the foregoing values and equivalents thereof. In some embodiments, exemplary dose per day includes 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150 or 200 mg per day, or a range defined by any two of the foregoing values and equivalents thereof. In some embodiments, the steroid, e.g., corticosteroid or glucocorticoid, can be administered at or about 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150 or 200 mg/day, or a range defined by any two of the foregoing values and equivalents thereof.

[0540] In some embodiments, a steroid, such as a corticosteroid, e.g., dexamethasone, may in some examples be administered at between or between about 5 mg and about 40 mg, such as about 10 mg and about 20 mg (or equivalent) IV or about 20 mg and about 40 mg (or equivalent) IV, at a frequency of once, twice, three times or four times a day, for a duration of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40 days. In some embodiments, a steroid, such as a corticosteroid, e.g., dexamethasone, may in some examples be administered at between or between about 5 mg and about 40 mg, such as about 10 mg and about 20 mg (or equivalent) IV or about 20 mg and about 40 mg (or equivalent) IV, at a frequency of once, twice, three times or four times a day, for a duration of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20,

21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40 days. The corticosteroid, e.g., dexamethasone, may in some examples be administered at or about 40 mg (or equivalent) IV four times a day, at or about 40 mg (or equivalent) IV three times a day, at or about 40 mg (or equivalent) IV twice a day, or about 40 mg (or equivalent) IV once a day, 20 mg (or equivalent) IV four times a day, at or about 20 mg (or equivalent) IV three times a day, at or about 20 mg (or equivalent) IV twice a day, or about 20 mg (or equivalent) IV once a day, or about 10 mg (or equivalent) IV four times a day, at or about 10 mg (or equivalent) IV three times a day, at or about 10 mg (or equivalent) IV twice a day, or at or about 10 mg (or equivalent) IV once a day.

[0541] In some embodiments, a steroid, such as a glucocorticoid e.g., methylprednisolone, may in some examples be administered at between at or about 0.5 mg/kg and at or about 5 mg/kg, such as at or about 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg or 5 mg/kg (or equivalent). In some embodiments, the glucocorticoid e.g., methylprednisolone, is administered once, twice, three times or four times a day for 2, 3, 4 or 5 days. In some embodiments, the glucocorticoid e.g., methylprednisolone, is administered in multiple doses, including a loading dose and a follow-up dose. In some embodiments, the glucocorticoid e.g., methylprednisolone, is administered at a loading dose of between about 1 and about 3 mg/kg, such as 2 mg/kg (or equivalent), followed by a follow-up dose of between at or about 1 and at or about 3 mg/kg, such as 2 mg/kg (or equivalent), that is divided between 1, 2, 3, 4 or 5 times over a day.

[0542] In some embodiments, the dosage of corticosteroid, e.g., glucocorticoid, is administered in successively lower dosages per treatment. Hence, in some such treatment regimes, the dose of corticosteroid is tapered, for example, gradually reduced over time. For example, the corticosteroid may be administered at an initial dose (or equivalent dose, such as with reference to dexamethasone) of 4 mg, and upon each successive administration the dose may be lowered, such that the dose is 3 mg for the next administration, 2 mg for the next administration, and 1 mg for the next administration. In some embodiments, the corticosteroid, e.g., dexamethasone, may be administered at an initial dose (or equivalent dose, such as with reference to dexamethasone) of 4 mg per day, and upon some of the successive administration the dose may be lowered. In some embodiments, exemplary tapered doses can include 3 mg for one of the next administrations, 2 mg for some of the subsequent administrations, and 1 mg for some of the subsequent administration. In some embodiments, the corticosteroid, e.g., dexamethasone, may be administered at an initial dose (or equivalent dose, such as with reference to dexamethasone) of at or about 40 mg per day, and upon some of the successive

administration the dose may be lowered. In some embodiments, exemplary tapered doses can include at or about 30 mg for one of the next administrations, at or about 20 mg for some of the subsequent administrations, and 10 mg for some of the subsequent administration.

[0543] Generally, the dose of corticosteroid administered is dependent upon the specific corticosteroid, as a difference in potency exists between different corticosteroids. It is typically understood that drugs vary in potency, and that doses can therefore vary, in order to obtain equivalent effects. **Table 6** shows equivalence in terms of potency for various glucocorticoids and routes of administration. Equivalent potency in clinical dosing is well known. Information relating to equivalent steroid dosing (in a non-chronotherapeutic manner) may be found in the British National Formulary (BNF) 37, March 1999.

Table 6: Glucocorticoid administration	
Glucocorticoid (Route)	Equivalency Potency
Hydrocortisone (IV or PO)	20
Prednisone	5
Prednisolone (IV or PO)	5
Methylprednisolone sodium succinate (IV)	4
Dexamethasone (IV or PO)	0.5-0.75

[0544] Thus, in some embodiments, the steroid is administered in an equivalent dosage amount of from at or about 1.0 mg to at or about 20 mg dexamethasone per day, such as at or about 1.0 mg to at or about 15 mg dexamethasone per day, at or about 1.0 mg to at or about 10 mg dexamethasone per day, at or about 2.0 mg to at or about 8 mg dexamethasone per day, or at or about 2.0 mg to at or about 6.0 mg dexamethasone per day, each inclusive. In some cases, the steroid is administered in an equivalent dose of at or about 4 mg or at or about 8 mg dexamethasone per day.

[0545] In some embodiments, the steroid is administered if fever persists after treatment with tocilizumab. For example, in some embodiments, dexamethasone is administered orally or intravenously at a dosage of 5-10 mg up to every 6-12 hours with continued fevers. In some embodiments, tocilizumab is administered concurrently with or subsequent to oxygen supplementation.

[0546] In some embodiments, the agent capable of reducing, decreasing, and/or dampening CAR+ T cell expansion and/or proliferation is an inhibitor of a microglial cell activity. In some embodiments, the administration of the inhibitor modulates the activity of microglia. In some embodiments, the inhibitor is an antagonist that inhibits the activity of a signaling pathway in microglia. In some embodiments, the microglia inhibitor affects microglial homeostasis,

survival, and/or proliferation. In some embodiments, the inhibitor targets the CSF1R signaling pathway. In some embodiments, the inhibitor is an inhibitor of CSF1R. In some embodiments, the inhibitor is a small molecule. In some cases, the inhibitor is an antibody.

[0547] In some aspects, administration of the inhibitor results in one or more effects selected from an alteration in microglial homeostasis and viability, a decrease or blockade of microglial cell proliferation, a reduction or elimination of microglial cells, a reduction in microglial activation, a reduction in nitric oxide production from microglia, a reduction in nitric oxide synthase activity in microglia, or protection of motor neurons affected by microglial activation. In some embodiments, the agent alters the level of a serum or blood biomarker of CSF1R inhibition, or a decrease in the level of urinary collagen type 1 cross-linked N-telopeptide (NTX) compared to at a time just prior to initiation of the administration of the inhibitor. In some embodiments, the administration of the agent transiently inhibits the activity of microglia activity and/or wherein the inhibition of microglia activity is not permanent. In some embodiments, the administration of the agent transiently inhibits the activity of CSF1R and/or wherein the inhibition of CSF1R activity is not permanent.

[0548] In some embodiments, the agent capable of reducing, decreasing, and/or dampening CAR⁺ T cell expansion and/or proliferation is selected from an anti-inflammatory agent, an inhibitor of NADPH oxidase (NOX2), a calcium channel blocker, a sodium channel blocker, inhibits GM-CSF, inhibits CSF1R, specifically binds CSF-1, specifically binds IL-34, inhibits the activation of nuclear factor kappa B (NF- κ B), activates a CB₂ receptor and/or is a CB₂ agonist, a phosphodiesterase inhibitor, inhibits microRNA-155 (miR-155), upregulates microRNA-124 (miR-124), inhibits nitric oxide production in microglia, inhibits nitric oxide synthase, or activates the transcription factor NRF2 (also called nuclear factor (erythroid-derived 2)-like 2, or NFE2L2).

[0549] In some embodiments, the agent capable of reducing, decreasing, and/or dampening CAR⁺ T cell expansion and/or proliferation is one that targets a cytokine, e.g., is an antagonist or inhibitor of a cytokine, such as transforming growth factor beta (TGF-beta), interleukin 6 (IL-6), interleukin 10 (IL-10), IL-2, MIP1 β (CCL4), TNF alpha, IL-1, interferon gamma (IFN-gamma), or monocyte chemoattractant protein-1 (MCP-1). In some embodiments, the agent capable of reducing, decreasing, and/or dampening CAR⁺ T cell expansion and/or proliferation is one that targets (e.g. inhibits or is an antagonist of) a cytokine receptor, such as IL-6 receptor (IL-6R), IL-2 receptor (IL-2R/CD25), MCP-1 (CCL2) receptor (CCR2 or CCR4), a TGF-beta receptor (TGF-beta I, II, or III), IFN-gamma receptor (IFNGR), MIP1 β receptor (e.g., CCR5),

TNF alpha receptor (e.g., TNFR1), IL-1 receptor (IL1-R α /IL-1R β), or IL-10 receptor (IL-10R).

[0550] The amount of a selected agent capable of reducing, decreasing, and/or dampening CAR+ T cell expansion and/or proliferation can be determined by standard clinical techniques. Exemplary adverse events include, but are not limited to, an increase in alanine aminotransferase, an increase in aspartate aminotransferase, chills, febrile neutropenia, headache, hypotension, left ventricular dysfunction, encephalopathy, hydrocephalus, seizure, and/or tremor.

[0551] In some embodiments, the agent is administered in a dosage amount of from or from about 30 mg to at or about 5000 mg, such as at or about 50 mg to at or about 1000 mg, at or about 50 mg to at or about 500 mg, at or about 50 mg to at or about 200 mg, at or about 50 mg to at or about 100 mg, at or about 100 mg to at or about 1000 mg, at or about 100 mg to at or about 500 mg, at or about 100 mg to at or about 200 mg, at or about 200 mg to at or about 1000 mg, at or about 200 mg to at or about 500 mg or at or about 500 mg to at or about 1000 mg.

[0552] In some embodiments, the agent is administered from or from about 0.5 mg/kg to at or about 100 mg/kg, such as from at or about 1 mg/kg to at or about at or about 50 mg/kg, at or about 1 mg/kg to at or about 25 mg/kg, at or about 1 mg/kg to at or about 10 mg/kg, at or about 1 mg/kg to at or about 5 mg/kg, at or about 5 mg/kg to at or about 100 mg/kg, at or about 5 mg/kg to at or about 50 mg/kg, at or about 5 mg/kg to at or about 25 mg/kg, at or about 5 mg/kg to at or about 10 mg/kg, at or about 10 mg/kg to at or about 100 mg/kg, at or about 10 mg/kg to at or about 50 mg/kg, at or about 10 mg/kg to at or about 25 mg/kg, at or about 25 mg/kg to at or about 100 mg/kg, at or about 25 mg/kg to at or about 50 mg/kg to at or about 50 mg/kg to at or about 100 mg/kg. In some embodiments, the agent is administered in a dosage amount of from or from about 1 mg/kg to at or about 10 mg/kg, at or about 2 mg/kg to at or about 8 mg/kg, at or about 2 mg/kg to at or about 6 mg/kg, at or about 2 mg/kg to at or about 4 mg/kg or 6 mg/kg to at or about 8 mg/kg, at or about each inclusive. In some aspects, the agent is administered in a dosage amount of at least or at least about or about 1 mg/kg, 2 mg/kg, 4 mg/kg, 6 mg/kg, 8 mg/kg, 10 mg/kg or more. In some embodiments, the agent is administered at a dose of 4 mg/kg or 8 mg/kg. In some embodiments, the agent is administered in a dosage amount of from at or about 1 mg/kg to at or about 20 mg/kg, at or about 2 mg/kg to at or about 19 mg/kg, at or about 4 mg/kg to at or about 16 mg/kg, at or about 6 mg/kg to at or about 14 mg/kg or at or about 8 mg/kg to at or about 12 mg/kg, each inclusive. In some aspects, the agent is administered in a dosage amount of at least or at least about or about 1 mg/kg, 2 mg/kg, 4 mg/kg, 6 mg/kg, 8 mg/kg, 10 mg/kg, 12 mg/kg, 14 mg/kg, 16 mg/kg, 18 mg/kg, 20 mg/kg or more. In some

embodiments, the agent is administered at a dosage amount of between about 8 mg/kg and at or about 12 mg/kg, such as at or about 8 mg/kg, 9 mg/kg, 10 mg/kg, 11 mg/kg or 12 mg/kg.

[0553] In some embodiments, the agent is administered in a total dose per day of from at or about 30 mg/day to at or about at or about 5000 mg/day, such as at or about 50 mg/day to at or about 1000 mg/day, at or about 50 mg/day to at or about 500 mg/day, at or about 50 mg/day to at or about 200 mg/day, at or about 50 mg/day to at or about 100 mg/day, at or about 100 mg/day to at or about 1000 mg/day, at or about 100 mg/day to at or about 500 mg/day, at or about 100 mg/day to at or about 200 mg/day, at or about 200 mg/day to at or about 1000 mg/day, at or about 200 mg/day to at or about 500 mg/day or 500 mg/day to at or about 1000 mg/day.

[0554] In some embodiments, the agent is administered in a total dose per day of from at or about 1 mg/kg/day to at or about at or about 20 mg/kg/day, at or about 2 mg/kg/day to at or about 19 mg/kg/day, at or about 4 mg/kg/day to at or about 16 mg/kg/day, at or about 6 mg/kg/day to at or about 14 mg/kg/day or at or about 8 mg/kg/day to at or about 12 mg/kg/day, each inclusive. In some aspects, the agent is administered in a dosage amount of at least or at least about or about 1 mg/kg/day, 2 mg/kg/day, 4 mg/kg/day, 6 mg/kg/day, 8 mg/kg/day, 10 mg/kg/day, 12 mg/kg/day, 14 mg/kg/day, 16 mg/kg/day, 18 mg/kg/day, 20 mg/kg/day or more. In some embodiments, the agent is administered at a dose of between at or about 8 mg/kg/day and at or about 12 mg/kg/day, such as at or about 8 mg/kg/day, 9 mg/kg/day, 10 mg/kg/day, 11 mg/kg/day or 12 mg/kg/day.

[0555] In some embodiments, the agent is administered 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, subconjunctival 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.

[0556] In some embodiments, the agent is administered via intravenous delivery. In some embodiments, the agent is administered via intravenous delivery over a period of time, e.g., over 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 120, 180, 240, 360, 480 or 720 minutes or more, or a range defined by any two of the foregoing values.

[0557] In some embodiments, the amount of the agent is administered about or

approximately twice daily, daily, every other day, three times a week, weekly, every other week or once a month.

[0558] In some embodiments, the agent is administered in multiple or repeated doses, e.g., more than one dose. In some embodiments, the agent is administered in repeated doses until a desired expansion is observed or is likely to be observed and/or suppression of toxicity or symptoms associated with toxicity occurs and/or the risk for developing the toxicity has passed. In some embodiments, agent is administered in a total of 2, 3, 4, 5, 6, 7, 8, 9, 10 or more doses.

[0559] In some embodiments, the agent is administered in multiple doses over a period of time. In some aspects, the agent can be administered over a period of more than at or about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more, or more than 2, 3, 4, 5, 6, 7, 8, 9, 10 weeks or more. In some embodiments, the agent can be administered in multiple or repeat doses over a total duration of about 6, 12, 18, 24 hours or more, or 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more, or 2, 3, 4, 5, 6, 7, 8, 9, 10 weeks or more. In some embodiments, the agent can be administered one per day, twice per day, or three times or more per day. In some embodiments, the agent can be administered at least or at least about every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18, 24, 36, 48 hours, or every 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days, or every 2, 3, 4, 5, 6, 7, 8, 9 or 10 weeks.

[0560] In some embodiments, the agent is administered as part of a composition or formulation, such as a pharmaceutical composition or formulation as described below. Thus, in some cases, the composition comprising the agent is administered as described below. In other aspects, the agent is administered alone and may be administered by any known acceptable route of administration or by one described herein, such as with respect to compositions and pharmaceutical formulations.

[0561] In some embodiments, the agent is a small molecule, peptide, protein, antibody or antigen-binding fragment thereof, an antibody mimetic, an aptamer, or a nucleic acid molecule. In some embodiments, the method involves administration of an inhibitor of microglia activity. In some embodiments, the agent is an antagonist that inhibits the activity of a signaling pathway in microglia. In some embodiments, the agent affects microglial homeostasis, survival, and/or proliferation.

[0562] In some embodiments, the agent capable of reducing, decreasing, and/or dampening CAR⁺ T cell expansion and/or proliferation, is an antibody or antigen binding fragment. In some embodiments, the agent is tocilizumab, siltuximab, sarilumab, olokizumab (CDP6038), elsilimomab, ALD518/BMS-945429, sirukumab (CNTO 136), CPSI-2634, ARGX-109, FE301,

or FM101.

[0563] In some embodiments, the agent is an antagonist or inhibitor of IL-6 or the IL-6 receptor (IL-6R). In some aspects, the agent is an antibody that neutralizes IL-6 activity, such as an antibody or antigen-binding fragment that binds to IL-6 or IL-6R. For example, in some embodiments, the agent is or comprises tocilizumab (atlizumab) or sarilumab, anti-IL-6R antibodies. In some embodiments, the agent is an anti-IL-6R antibody described in U.S. Patent No: 8,562,991. In some cases, the agent that targets IL-6 is an anti-IL-6 antibody, such as siltuximab, elsilimomab, ALD518/BMS-945429, sirukumab (CNTO 136), CPSI-2634, ARGX-109, FE301, FM101, or olokizumab (CDP6038). In some aspects, the agent may neutralize IL-6 activity by inhibiting the ligand-receptor interactions. The feasibility of this general type of approach has been demonstrated with a natural occurring receptor antagonist for interleukin-1. *See* Harmurn, C. H. et al., *Nature* (1990) 343:336-340. In some aspects, the IL-6/IL-6R antagonist or inhibitor is an IL-6 mutein, such as one described in U.S. Patent No. 5591827. In some embodiments, the agent that is an antagonist or inhibitor of IL-6/IL-6R is a small molecule, a protein or peptide, or a nucleic acid.

[0564] In some embodiments, the agent is tocilizumab. In some embodiments, tocilizumab is administered as an early intervention in accord with the provided methods, and/or with the provided articles of manufacture or compositions, at a dosage of from or from about 1 mg/kg to at or about 12 mg/kg, such as at or about 4 mg/kg, 8 mg/kg, or 10 mg/kg. In some embodiments, tocilizumab is administered by intravenous infusion. In some embodiments, tocilizumab is administered for a persistent fever of greater than 39°C lasting 10 hours that is unresponsive to acetaminophen. In some embodiments, a second administration of tocilizumab is provided if symptoms recur after 48 hours of the initial dose. In some embodiments, tocilizumab is administered in accord with the provided methods at a dosage of from or from about 1 mg/kg to about 20 mg/kg, such as from at or about 8 mg/kg to at or about 12 mg/kg. In some embodiments, tocilizumab is administered by intravenous infusion. In some embodiments, tocilizumab is administered by intravenous infusion over approximately 1 hour, at a dose or dosage amount of approximately 4-12 mg/kg, e.g., at or about 8 mg/kg. In some embodiments, tocilizumab is administered as multiple or repeated doses, e.g., at least or at least about every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18, 24, 36, 48 hours, or every 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days, or every 2, 3, 4, 5, 6, 7, 8, 9 or 10 weeks or more. In some embodiments, one or more dose of tocilizumab is administered. In some embodiments, and tocilizumab is administered every 8, 10, 12, 14, 16, 18, 24 or 36 or more hours, or 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or more

days, or 3, 4, 5, 6, 7, 8, 10, 11, 12 or more weeks.

[0565] In some embodiments, the agent is an agent that binds to IL-6, e.g., an anti-IL-6 antibody or antigen-binding fragment thereof, such as siltuximab, clazakizumab, elsilimomab, ALD518/BMS-945429, sirukumab (CNTO 136), CPSI-2634, ARGX-109, FE301, FM101, or olokizumab (CDP6038) or antigen-binding fragment thereof. In some aspects, the agent may neutralize IL-6 activity by inhibiting the ligand-receptor interactions. The feasibility of this general type of approach has been demonstrated with a natural occurring receptor antagonist for interleukin-1. *See* Harmurn, C. H. et al., *Nature* (1990) 343:336-340. In some aspects, the IL-6/IL-6R antagonist or inhibitor is an IL-6 mutein or a modified IL-6 protein or portion thereof, such as one described in U.S. Patent No. 5591827. In some embodiments, the agent that is an antagonist or inhibitor of IL-6/IL-6R is a small molecule, a protein or peptide, or a nucleic acid. In some embodiments, agent is siltuximab.

[0566] In some embodiments, siltuximab is administered in accord with the provided methods at a dosage of from or from about 1 mg/kg to about 20 mg/kg, such as from at or about 8 mg/kg to at or about 12 mg/kg. In some embodiments, siltuximab is administered by intravenous infusion. In some embodiments, siltuximab is administered by intravenous infusion over approximately 1 hour, at a dose of approximately 11 mg/kg. In some embodiments, siltuximab is administered as multiple or repeated doses, e.g., at least or at least about every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18, 24, 36, 48 hours, or every 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days, or every 2, 3, 4, 5, 6, 7, 8, 9 or 10 weeks, or a range defined by any two of the foregoing values. In some embodiments, one or more dose of the siltuximab is administered. In some embodiments, and the siltuximab is administered every 8, 10, 12, 14, 16, 18, 24 or 36 or more hours, or 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or more days, or 3, 4, 5, 6, 7, 8, 10, 11, 12 or more weeks or a range defined by any two of the foregoing values. In some embodiments, the agent is an agonist or stimulator of TGF- β or a TGF- β receptor (e.g., TGF- β receptor I, II, or III). In some aspects, the agent is an antibody that increases TGF- β activity, such as an antibody or antigen-binding fragment that binds to TGF- β or one of its receptors. In some embodiments, the agent that is an agonist or stimulator of TGF- β and/or its receptor is a small molecule, a protein or peptide, or a nucleic acid.

[0567] In some embodiments, the agent is an antagonist or inhibitor of MCP-1 (CCL2) or a MCP-1 receptor (e.g., MCP-1 receptor CCR2 or CCR4). In some aspects, the agent is an antibody that neutralizes MCP-1 activity, such as an antibody or antigen-binding fragment that binds to MCP-1 or one of its receptors (CCR2 or CCR4). In some embodiments, the MCP-1

antagonist or inhibitor is any described in Gong et al. J Exp Med. 1997 Jul 7; 186(1): 131–137 or Shahrara et al. J Immunol 2008; 180:3447-3456. In some embodiments, the agent that is an antagonist or inhibitor of MCP-1 and/or its receptor (CCR2 or CCR4) is a small molecule, a protein or peptide, or a nucleic acid.

[0568] In some embodiments, the agent is an antagonist or inhibitor of IFN- γ or an IFN- γ receptor (IFNGR). In some aspects, the agent is an antibody that neutralizes IFN- γ activity, such as an antibody or antigen-binding fragment that binds to IFN- γ or its receptor (IFNGR). In some aspects, the IFN-gamma neutralizing antibody is any described in Dobber et al. Cell Immunol. 1995 Feb;160(2):185-92 or Ozmen et al. J Immunol. 1993 Apr 1;150(7):2698-705. In some embodiments, the agent that is an antagonist or inhibitor of IFN- γ /IFNGR is a small molecule, a protein or peptide, or a nucleic acid.

[0569] In some embodiments, the agent is an antagonist or inhibitor of IL-10 or the IL-10 receptor (IL-10R). In some aspects, the agent is an antibody that neutralizes IL-10 activity, such as an antibody or antigen-binding fragment that binds to IL-10 or IL-10R. In some aspects, the IL-10 neutralizing antibody is any described in Dobber et al. Cell Immunol. 1995 Feb;160(2):185-92 or Hunter et al. J Immunol. 2005 Jun 1;174(11):7368-75. In some embodiments, the agent that is an antagonist or inhibitor of IL-10/IL-10R is a small molecule, a protein or peptide, or a nucleic acid.

[0570] In some embodiments, the agent is an antagonist or inhibitor of IL-1 or the IL-1 receptor (IL-1R). In some aspects, the agent is an IL-1 receptor antagonist, which is a modified form of IL-1R, such as anakinra (see, e.g., Fleischmann et al., (2006) Annals of the rheumatic diseases. 65(8):1006–12). In some aspects, the agent is an antibody that neutralizes IL-1 activity, such as an antibody or antigen-binding fragment that binds to IL-1 or IL-1R, such as canakinumab (see also EP 2277543). In some embodiments, the agent that is an antagonist or inhibitor of IL-1/IL-1R is a small molecule, a protein or peptide, or a nucleic acid.

[0571] In some embodiments, the agent is an antagonist or inhibitor of a tumor necrosis factor (TNF) or a tumor necrosis factor receptor (TNFR). In some aspects, the agent is an antibody that blocks TNF activity, such as an antibody or antigen-binding fragment that binds to a TNF, such as TNF α , or its receptor (TNFR, e.g., TNFRp55 or TNFRp75). In some aspects, the agent is selected from among infliximab, adalimumab, certolizumab pegol, golimumab and etanercept. In some embodiments, the agent that is an antagonist or inhibitor of TNF/TNFR is a small molecule, a protein or peptide, or a nucleic acid.

[0572] In some embodiments, the agent is an antagonist or inhibitor of signaling through the

Janus kinase (JAK) and two Signal Transducer and Activator of Transcription (STAT) signaling cascade. JAK/STAT proteins are common components of cytokine and cytokine receptor signaling. In some embodiments, the agent that is an antagonist or inhibitor of JAK/STAT, such as ruxolitinib (see, e.g., Mesa et al. (2012) *Nature Reviews Drug Discovery*. 11(2):103-104), tofacitinib (also known as Xeljanz, Jakvinus tasocitinib and CP-690550), Baricitinib (also known as LY-3009104, INCB-28050), Filgotinib (G-146034, GLPG-0634), Gandotinib (LY-2784544), Lestaurtinib (CEP-701), Momelotinib (GS-0387, CYT-387), Pacritinib (SB1518), and Upadacitinib (ABT-494). In some embodiments, the agent is a small molecule, a protein or peptide, or a nucleic acid.

[0573] In some embodiments, the agent is a kinase inhibitor. Kinase inhibitors, such as a CDK4 kinase inhibitor, a BTK kinase inhibitor, a MNK kinase inhibitor, or a DGK kinase inhibitor, can regulate the constitutively active survival pathways that exist in tumor cells and/or modulate the function of immune cells. In some embodiments, the kinase inhibitor is a Bruton's tyrosine kinase (BTK) inhibitor, e.g., ibrutinib. In some embodiments, the kinase inhibitor is a phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) inhibitor. In some embodiments, the kinase inhibitor is a CDK4 inhibitor, e.g., a CDK4/6 inhibitor. In some embodiments, the kinase inhibitor is an mTOR inhibitor, such as, e.g., rapamycin, a rapamycin analog, OSI-027. The mTOR inhibitor can be, e.g., an mTORC1 inhibitor and/or an mTORC2 inhibitor, e.g., an mTORC1 inhibitor and/or mTORC2 inhibitor. In some embodiments, the kinase inhibitor is an MNK inhibitor, or a dual PI3K/mTOR inhibitor. In some embodiments, other exemplary kinase inhibitors include the AKT inhibitor perifosine, the mTOR inhibitor temsirolimus, the Src kinase inhibitors dasatinib and fostamatinib, the JAK2 inhibitors pacritinib and ruxolitinib, the PKC β inhibitors enzastaurin and bryostatin, and the AAK inhibitor alisertib.

[0574] In some embodiments, the kinase inhibitor is a BTK inhibitor selected from ibrutinib (PCI- 32765); GDC-0834; RN-486; CGI-560; CGI-1764; HM-71224; CC-292; ONO-4059; CNX-774; and LFM-A13. In some embodiments, the BTK inhibitor does not reduce or inhibit the kinase activity of interleukin-2-inducible kinase (ITK), and is selected from GDC-0834; RN-486; CGI-560; CGI-1764; HM-71224; CC-292; ONO-4059; CNX-774; and LFM-A13.

[0575] In some embodiments, the kinase inhibitor is a BTK inhibitor, e.g., ibrutinib (1-[(3R)-3-[4-Amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl]piperidin-1-yl]prop-2-en-1-one; also known as PCI-32765). In some embodiments, the kinase inhibitor is a BTK inhibitor, e.g., ibrutinib (PCI-32765). In some embodiments, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more cycles of ibrutinib are administered. In some embodiments, the BTK inhibitor is a BTK

inhibitor described in International Application WO 2015/079417.

[0576] In some embodiments, the kinase inhibitor is a PI3K inhibitor. PI3K is central to the PI3K/Akt/mTOR pathway involved in cell cycle regulation and lymphoma survival. Exemplary PI3K inhibitor includes idelalisib (PI3K δ inhibitor). In some embodiments, the agent is idelalisib and rituximab.

[0577] In some embodiments, the agent is an inhibitor of mammalian target of rapamycin (mTOR). In some embodiments, the kinase inhibitor is an mTOR inhibitor selected from temsirolimus; ridaforolimus (also known as AP23573 and MK8669); everolimus (RAD001); rapamycin (AY22989); simapimod; AZD8055; PF04691502; SF1126; and XL765. In some embodiments, the agent is an inhibitor of mitogen-activated protein kinase (MAPK), such as vemurafenib, dabrafenib, and trametinib.

[0578] In some embodiments, a device, such as absorbent resin technology with blood or plasma filtration, can be used to reduce cytokine levels. In some embodiments, the device used to reduce cytokine levels is a physical cytokine absorber, such as an extracorporeal cytokine absorber. In some embodiments, a physical cytokine absorber can be used to eliminate cytokines from the bloodstream in an *ex vivo*, extracorporeal manner. In some embodiments, the agent is a porous polymer. In some embodiments, the agent is CytoSorb (see, e.g., Basu et al. Indian J Crit Care Med. (2014) 18(12): 822–824).

V. METHODS FOR TREATING OR AMELIORATING SYMPTOMS OF TOXICITY

[0579] In some embodiments, provided are methods for treating, ameliorating or reducing the toxicity associated with cell therapy. In some embodiments, also provided are treatment regimens, e.g., including assessment, dosing and/or timing of administration of agents that can ameliorate or treat signs or symptoms of toxicity. In some embodiments, the methods include administration of one or more agents or treatments for treating, preventing, delaying, or attenuating the development of a toxicity. In some examples, the agent or other treatment capable of treating, preventing, delaying, or attenuating the development of a toxicity is administered prior to and/or concurrently with administration of a therapeutic cell composition comprising the genetically engineered cells.

[0580] In some embodiments, the agent is a steroid. In some embodiments, the agent is an agent capable of binding an interleukin-6 receptor (IL-6R). In some embodiments, the agent is an agent capable of binding an interleukin-6 (IL-6). In some embodiments, the agent can be

administered according to any dose, frequency, route of delivery and/or timing of administration described in Section IV.E above. In some embodiments, the agent is or comprises any agents that are capable of modulating the expansion and/or activity of cell therapy, such as any described in Section IV.E above. In some embodiments, one or more of the agents, such as any described herein, e.g., for use in modulating cell expansion or activity and/or treating, ameliorating or reducing the toxicity, can be administered in combination. In some embodiments, one or more doses, such as multiple doses, of any of the agents can be administered according to the methods provided herein. In some embodiments, the dose, frequency, route of delivery and/or timing of administration of one or more of the doses of the agent include any of those described herein.

[0581] In some embodiments, the agent is administered in combination with one or more further agent(s) for modulating cell expansion or activity and/or treating, ameliorating or reducing the toxicity, such as a steroid and/or an anti-IL-6 receptor (IL-6R) antibody. In some embodiments, the methods include administering to a subject one or more additional or further agent(s), intervention(s) and/or treatment(s) capable of modulating cell expansion or activity and/or treating, preventing, delaying, or attenuating the development of a toxicity. In some embodiments, a multiple dose or a repeated dose of the one or more further agent is administered.

[0582] In some embodiments, the agent is administered simultaneously or near simultaneously with the one or more further agents, such as within up to 1, 2, 3, 4, 5, 6, 7 or 8 hours of administration of the agent. In some embodiments, the agent is administered before and/or after administering the one or more further agents. In some embodiments, the agent is administered as a first-line therapy to modulate the expansion or persistence of cells and/or to treat, prevent, reduce and/or ameliorate symptoms of a toxicity of a cell therapy. In some embodiments, the one or more further agents are administered as a second-, third- or fourth-line or subsequent therapy. In some embodiments, a combination of the agent and the further agent(s) is administered as a first-line therapy. In some embodiments, the one or more further agents are administered as a first-line therapy. In some embodiments, the agent is administered as a second-, third- or fourth-line or subsequent therapy. In some embodiments, the agent and/or one or more further agents, can be administered in multiple doses. In some embodiments, each of the agent and/or one or more further agents, can be administered in multiple or repeat doses depending on the grade, progression and/or manifestations of symptoms of the toxicity or potential toxicity, e.g., CRS or neurotoxicity, and/or based on the assessment of biomarkers, e.g.

any described herein and/or according to exemplary methods and/or procedures described herein.

[0583] In some embodiments, the agent is administered with or without concurrent administration of a further agent, as a first-line therapy for treating, ameliorating or reducing the toxicity. In some embodiments, a further agent, is administered with or without the agent and/or a different further agent, e.g., a steroid, as a first-line therapy for treating, ameliorating or reducing the toxicity. In some embodiments, a first dose of the agent is administered within at or about 24, 36, 48, 72, 96 or 120 hours of administration of the immunotherapy and/or cell therapy. In some embodiments, an additional dose of the agent is administered within at or about 6, 12, 18, 24, 36, 48, 72, 96 or 120 hours of the first or initial dose, or prior doses of the agent. In some embodiments, one or more doses of one or more further agent(s), can be administered simultaneously with and/or subsequently to the first dose of the agent. In some embodiments, one or more doses of the one or more further agent(s) can be administered within at or about 6, 12, 18, 24, 36, 48, 72, 96 or 120 hours of the first or initial dose, or prior doses of the agent or within at or about 6, 12, 18, 24, 36, 48, 72, 96 or 120 hours of the first or initial dose, or prior doses of the further agent. In some embodiments, if the signs and/or symptoms of the toxicity do not resolve or improve, a different further agent, e.g., a different steroid, can be administered as second-, third- or fourth-line or subsequent therapy.

[0584] In some embodiments, a further agent, e.g., anti-IL-6R antibody and/or a steroid, with or without concurrent administration of the agent and/or a different further agent, is administered as a first-line therapy for treating, ameliorating or reducing the toxicity. In some embodiments, an additional dose of the further agent, is administered within at or about 6, 12, 18, 24, 36, 48, 72, 96 or 120 hours of the first or initial dose, or prior doses of the further agent. In some embodiments, one or more doses of the agent or a different further agent, can be administered within at or about 6, 12, 18, 24, 36, 48, 72, 96 or 120 hours of the first or initial dose, or prior doses of the further agent, e.g., anti-IL-6R antibody and/or a steroid, or within at or about 6, 12, 18, 24, 36, 48, 72, 96 or 120 hours of the first or initial dose, or prior doses of the agent.

[0585] In some embodiments, if the signs and/or symptoms of the toxicity do not resolve or improve, the agent can be administered as second-, third- or fourth-line or subsequent therapy.

[0586] In some embodiments, one or more doses of the agent and/or further agent(s) is administered prior to administration of the immunotherapy or cell therapy or initiation thereof. In some embodiments, one or more doses of the agent and/or further agent(s) is administered

after administration of the immunotherapy or cell therapy or initiation thereof. In some embodiments, one or more doses of the agent and/or further agent(s) is administered simultaneously with the administration of the immunotherapy or cell therapy or initiation thereof. In some embodiments, the additional doses of one or both of the agent and the further agent are administered after administration of the immunotherapy or cell therapy. In some cases, the one or more further agent(s), is administered alone or is administered as part of a composition or formulation, such as a pharmaceutical composition or formulation, as described herein.

[0587] Also provided are methods for ameliorating a toxicity, e.g., cytokine release syndrome (CRS), comprising administering, to a subject exhibiting one or more physical signs or symptom of a toxicity, one or more agent(s) capable of reducing and/or ameliorating the one or more physical signs or symptoms associated with the toxicity, said subject having been administered a dose of genetically engineered cells comprising T cells expressing a recombinant receptor. In some aspects, the one or more agent(s) is administered in a treatment regimen.

[0588] In some aspects, after administration of the dose of genetically engineered cells comprising T cells expressing a recombinant receptor, the subject is monitored for manifestation of one or more physical signs or symptom of a toxicity, e.g., CRS. In some aspects, after administration of the dose of genetically engineered cells comprising T cells expressing a recombinant receptor, the subject is monitored for development of a fever. In some embodiments, the fever is a fever of or greater than 38°C or 100.4°F. In some embodiments, the subject is monitored for one or more physical signs or symptoms indicative of grade 1, grade 2, grade 3 or grade 4 or higher CRS, e.g., signs or symptoms described herein. In some embodiments, a subject is monitored for signs or symptoms of CRS during and/or after administration of the immunotherapy or cell therapy. In some embodiments, a subject is monitored if they develop a fever of or greater than 38°C or 100.4°F more than or equal to 72 hours post administration of the immunotherapy or cell therapy. In such embodiments, the subject being monitored may be moved on to first or subsequent lines of therapy if they exhibit clinical progression of CRS and/or rapidly deteriorate after developing a fever of or greater than 38°C or 100.4°F. In some embodiments, the treatment regimen comprises administering one or more agent(s) if: at or greater than 72 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits a fever, and exhibits one or more physical signs or symptoms associated with the toxicity, e.g., cytokine release syndrome (CRS), exhibits clinical progression of CRS, and/or exhibits a rapid progression of the physical signs or

symptoms associated with the toxicity; or within 48 or 72 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits a fever and/or one or more physical signs or symptoms associated with grade 2 or higher CRS. In some embodiments, the one or more agent(s) is administered as a first-line therapy or first-line treatment for ameliorating the toxicity.

[0589] In some embodiments, the treatment regimen comprises administering one or more agent(s) if, within 24, 48 or 72 hours after administration of any one or more agent(s) above, e.g., first-line therapy, the subject does not exhibit an improvement of the fever and/or the one or more physical signs or symptoms associated with the toxicity and/or exhibits a rapid progression of the physical signs or symptoms associated with the toxicity, which one or more agent(s) optionally are different from any one or more agent(s) administered above and/or is administered at the same or higher dose and/or frequency as any one or more agent(s) administered above, e.g., first-line therapy. In some embodiments, the one or more agent(s) is administered as a second-line therapy or second-line treatment for ameliorating the toxicity.

[0590] In some embodiments, the treatment regimen comprises administering one or more agent(s) if, within 24, 48 or 72 hours after administration of any one or more agent(s) above, e.g., second-line therapy, the subject does not exhibit an improvement of the fever and/or the one or more physical signs or symptoms associated with the toxicity and/or exhibits a rapid progression of the physical signs or symptoms associated with the toxicity, which one or more agent(s) optionally are different from any one or more agent(s) administered above and/or is administered at the same or higher dose and/or frequency as any one or more agent(s) administered above, e.g., first- or second-line therapy. In some embodiments, the one or more agent(s) is administered as a third-line therapy or third-line treatment for ameliorating the toxicity.

[0591] In some embodiments, the treatment regimen comprises administering one or more agent(s) if, after administration of any one or more agent(s) above, e.g., third-line therapy, the subject does not exhibit an improvement of the fever and/or the one or more physical signs or symptoms associated with the toxicity, which one or more agent(s) optionally are different from any one or more agent(s) administered above and/or is administered at the same or higher dose and/or frequency as any one or more agent(s) administered above, e.g., first-, second- or third-line therapy. In some embodiments, the one or more agent(s) is administered as a fourth-line therapy or fourth-line treatment for ameliorating the toxicity.

[0592] In some embodiments, the one or more agent(s) is selected from an agent capable of

binding an interleukin-6 receptor (IL-6R) or one or more steroid, optionally one or more doses of the one or more steroid.

[0593] Also provided are methods for ameliorating a toxicity, e.g., optionally neurotoxicity (NT), comprising administering, to a subject exhibiting one or more physical signs or symptom of a toxicity, one or more agent(s) capable of reducing and/or ameliorating the one or more physical signs or symptoms associated with the toxicity, said subject having been administered a dose of genetically engineered cells comprising T cells expressing a recombinant receptor, In some aspects, the one or more agent(s) is administered in a treatment regimen.

[0594] In some aspects, after administration of the dose of genetically engineered cells comprising T cells expressing a recombinant receptor, the subject is monitored for manifestation of one or more physical signs or symptom of a toxicity, e.g., NT. In some embodiments, the subject is monitored for one or more physical signs or symptoms indicative of grade 1, grade 2, grade 3 or grade 4 or higher NT, e.g., signs or symptoms described herein.

[0595] In some embodiments, the treatment regimen comprises administering one or more agent(s) if: at or greater than 72 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits one or more physical signs or symptoms associated with the toxicity, optionally neurotoxicity (NT); or within at or about 48 or 72 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits one or more physical signs or symptoms associated with the toxicity. In some embodiments, the one or more agent(s) is administered as a first-line therapy or first-line treatment for ameliorating the toxicity.

[0596] In some embodiments, the treatment regimen comprises administering one or more agent(s) if, within at or about 24, 48 or 72 hours after administration of any one or more agent(s) above, e.g., first-line therapy, the subject does not exhibit an improvement of the one or more physical signs or symptoms associated with the toxicity and/or exhibits a progression of the physical signs or symptoms associated with the toxicity, which one or more agent(s) optionally are different from any one or more agent(s) administered above and/or is administered at the same or higher dose and/or frequency as any one or more agent(s) administered above, e.g., first-line therapy. In some embodiments, the one or more agent(s) is administered as a second-line therapy or second-line treatment for ameliorating the toxicity.

[0597] In some embodiments, the treatment regimen comprises administering one or more agent(s) if, within at or about 24, 48 or 72 hours after administration of any one or more agent(s) above, the subject does not exhibit an improvement of the one or more physical signs or

symptoms associated with the toxicity and/or exhibits a rapid progression of the physical signs or symptoms associated with the toxicity, which one or more agent(s) optionally are different from any one or more agent(s) administered in above and/or is administered at the same or higher dose and/or frequency as any one or more agent(s) administered above, e.g., first- or second-line therapy. In some embodiments, the one or more agent(s) is administered as a third-line therapy or third-line treatment for ameliorating the toxicity.

[0598] In some embodiments, the one or more agent(s) is one or more steroid, optionally one or more doses of the one or more steroid.

[0599] In some embodiments, the agent used for first-line therapy is a steroid. In some embodiments, the agent used for first-line therapy is an agent capable of binding an IL-6 receptor, e.g., an anti-IL-6R antibody. In some embodiments, the agent used for first-line therapy is a combination of a steroid and an anti-IL-6R antibody.

[0600] In some embodiments, the agent used for second-line therapy is a steroid. In some embodiments, the agent used for second-line therapy is an agent capable of binding an IL-6 receptor, e.g., an anti-IL-6R antibody. In some embodiments, the agent used for second-line therapy is a combination of a steroid and an anti-IL-6R antibody. In some embodiments, the agent used for second-line therapy is different from the agent used for first-line therapy. In some embodiments, one or more further agents are used for second-line therapy. In some embodiments, the agent used for second-line therapy is the same as the agent used for first-line therapy. In some embodiments, the agent(s) for second-line therapy is administered at the same or higher dose and/or frequency as the first-line therapy.

[0601] In some embodiments, the agent used for third-line therapy is a steroid. In some embodiments, the agent used for third-line therapy is an agent capable of binding an IL-6 receptor, e.g., an anti-IL-6R antibody. In some embodiments, the agent used for third-line therapy is a combination of a steroid and an anti-IL-6R antibody. In some embodiments, the agent used for third-line therapy is different from the agent used for first- or second-line therapy. In some embodiments, one or more further agents are used for third-line therapy. In some embodiments, the agent used for third-line therapy is the same as the agent used for first- or second-line therapy. In some embodiments, the agent(s) for third-line therapy is administered at the same or higher dose and/or frequency as the first- or second-line therapy.

[0602] In some embodiments, the agent used for fourth-line therapy or subsequent therapy is a steroid. In some embodiments, the agent used for fourth-line therapy or subsequent therapy is an agent capable of binding an IL-6 receptor, e.g., an anti-IL-6R antibody. In some

embodiments, the agent used for fourth-line therapy or subsequent therapy is a combination of a steroid and an anti-IL-6R antibody. In some embodiments, the agent used for fourth-line therapy or subsequent therapy is different from the agent used for first-, second- or third-line therapy. In some embodiments, one or more further agents are used for fourth-line therapy or subsequent therapy. In some embodiments, the agent(s) for fourth-line therapy or subsequent therapy is administered at the same or higher dose and/or frequency as the first-, second- or third-line therapy.

[0603] In some embodiments, any one or more of the agent(s) described in Section IV.E.2, or a combination thereof, such as one or more steroid and/or an anti-IL-6R antibody and/or an anti-IL-6 antibody, can be administered as the one or more agent(s) capable of reducing and/or ameliorating the one or more physical signs or symptoms associated with the toxicity. In some embodiments, the dose and/or frequency of administration is the same as described can be the dose and/or frequency for each of the agents described in Section IV.E.2.

[0604] In some embodiments, the agent is a steroid, e.g., dexamethasone or methylprednisolone. In some embodiments, the further agent is an anti-IL-6R antibody, e.g., a tocilizumab. In some embodiments, the agent is an anti-IL-6R antibody, e.g., a tocilizumab. In some embodiments, the further agent is a steroid, e.g., dexamethasone or methylprednisolone.

[0605] In some embodiments, the steroid is dexamethasone or methylprednisolone. In some embodiments, the steroid is dexamethasone. In some embodiments, the steroid is dexamethasone, and the dexamethasone is administered after the administration of the anti-IL-6 antibody. In some embodiments, the steroid is dexamethasone, and the dexamethasone is administered prior to the administration of the anti-IL-6 antibody.

[0606] In some embodiments, the agent is administered to a subject after initiation of administration of the immunotherapy and/or cell therapy. In some embodiments, the agent is administered prior to or after the subject exhibits signs or symptoms of toxicity, e.g., cytokine release syndrome (CRS) or neurotoxicity (NT). In some embodiments, the agent is also an agent that can treat or ameliorate a toxicity.

[0607] In some embodiments, the agent can be administered based on or according certain procedures or guidelines, e.g., based on assessment and monitoring of outcomes, such as toxicity and/or response outcomes, and/or monitoring of parameters or biomarkers, e.g., pharmacokinetic parameters, patient attributes or factors and/or expression of biomarkers, such as any described herein. In some embodiments, the agent is administered according to exemplary procedure or guidelines for treatment or amelioration of toxicity, such as those described in **Table 7** below.

Table 7. Exemplary Guidelines for Administering Agents for Modulating Cell Therapy		
CRS ^a	Tocilizumab	Dexamethasone
Any Grade		
Admit to hospital for observation, infectious work-up; antibiotics per institutional guidelines; symptomatic support; recommend seizure prophylaxis (e.g., Levetiracetam)		
Grade 1	None	None
≥ 96 hours after CAR+ T cell administration		
Grade 1	8 mg/kg every 24 hours x 1-2 doses	10 mg every 12-24 hours ≥ 3 days
≤ 96 hours after CAR+ T cell administration		
Grade 2	8 mg/kg every 24 hours x 1-2 doses	10 mg every 12-24 hours ≥ 3 days
Grade 3	8 mg/kg every 24 hours x 1-2 doses	10-20 mg every 12 hours ≥ 3 days
Grade 4	8 mg/kg every 24 hours x 1-2 doses	20 mg every 6 hours ≥ 3 days
Neurotoxicity ^b		
Grade 1		
Mild Symptoms	Admit to hospital for observation: <ul style="list-style-type: none">• Rule out other causes of neurologic symptoms• Start anti-seizure medicines (e.g., levetiracetam) for seizure prophylaxis	
Grade 2		
Moderate symptoms and/or limiting age-appropriate instrumental ADL	<ul style="list-style-type: none">• Consider dexamethasone 10 mg IV every 12-24 hours• Continue dexamethasone use until the event is ≤ Grade 1, then taper over 3 days, if necessary	
Grade 3		
Severe or Medically significant and/or limiting self care ADL	<ul style="list-style-type: none">• Administer dexamethasone 10 mg IV every 12 hours• Continue dexamethasone use until the event is ≤ Grade 1, then taper over at least 3 days	
Grade 4		
Life-threatening symptoms	<ul style="list-style-type: none">• Administer dexamethasone 10-20 mg IV every 6-12 hours• Continue dexamethasone use until the event is ≤ Grade 1, then taper over at least 3 days	
Any grade CRS or NT: In cases with very early onset (< 72 hours) and/or rapid progression, aggressive intervention with high dose steroids (20 mg every 6-12 hours or methylprednisolone) is recommended		

^a Grading per Lee et al, Blood. 2014;124(2):188-95.

^b CTCAE v4.03.

[0608] Other non-limiting examples of administering the agent or therapy or intervention, are described in **Table 8**. In some embodiments, the intervention includes tocilizumab or other agent as described, which can be at a time in which there is a sustained or persistent fever of greater than or about 38 °C or greater than or greater than about 39 °C in the subject. In some embodiments, the fever is sustained in the subject for more than 10 hours, more than 12 hours, more than 16 hours, or more than 24 hours before intervention.

Table 8. Exemplary Guidelines for Administering Agents for Modulating Cell Therapy	
Symptoms or Signs	Suggested Intervention
Fever of $\geq 38.3^{\circ}\text{C}$	Acetaminophen (12.5 mg/kg) PO/IV up to every four hours
Persistent fever of $\geq 39^{\circ}\text{C}$ for 10 hours that is unresponsive to acetaminophen	Tocilizumab (8-12 mg/kg) IV
Persistent fever of $\geq 39^{\circ}\text{C}$ after tocilizumab	Dexamethasone 5-10 mg IV/PO up to every 6-12 hours with continued fevers
Recurrence of symptoms 48 hours after initial dose of tocilizumab	Tocilizumab (8-12 mg/kg) IV
Hypotension	Fluid bolus, target hematocrit $>24\%$
Persistent/recurrent hypotension after initial fluid bolus (within 6 hours)	Tocilizumab (8-12 mg/kg) IV
Use of low dose pressors for hypotension for longer than 12 hours	Dexamethasone 5-10 mg IV/PO up to every 6 hours with continued use of pressors
Initiation of higher dose pressors or addition of a second pressor for hypotension	Dexamethasone 5-10 mg IV/PO up to every 6 hours with continued use of pressors
Initiation of oxygen supplementation	Tocilizumab (8-12 mg/kg) IV
Increasing respiratory support with concern for impending intubation	Dexamethasone 5-10 mg IV/PO up to every 6 hours with continued use of pressors
Recurrence/Persistence of symptoms for which tocilizumab was given ≥ 48 hours after initial dose was administered	Tocilizumab (8-12 mg/kg) IV

[0609] Other non-limiting examples of administering the agent or therapy or intervention, are described in **Table 9** below.

Table 9. Toxicity Management Algorithms		
	Management	
CRS^a	Tocilizumab	Dexamethasone
Grade 1 Onset < 72 hours after CAR+ T cell administration	8 mg/kg every 24 hours	Optionally 10 mg every 24 hours
Grade 2 Onset < 72 hours after CAR+ T cell administration	8 mg/kg every 12-24 hours	10 mg every 12-24 hours
Grade 2 Onset ≥ 72 hours after CAR+ T cell administration	8 mg/kg every 12-24 hours	Optionally 10 mg every 24 hours
Grade 3	8 mg/kg every 12 hours	10 mg every 12 hours
Grade 4	8 mg/kg every 6 hours	10 mg every 6 hours

^a Grading per Lee et al, Blood. 2014;124(2):188-95.

[0610] Other non-limiting examples of administering the agent or therapy or intervention, are described in **Table 10** (CRS) and **Table 11** (NT) below.

Table 10. Exemplary Guidelines for Administering Agents for Modulating Cell Therapy for
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Cytokine Release Syndrome (CRS)	
After CAR+ T cell administration	Monitoring: <ul style="list-style-type: none"> • Monitor for CRS symptoms (fever, hemodynamic instability, hypoxia) with neurologic evaluations • Follow serum CRP, ferritin, and coagulation parameters • Consider hospitalization for close monitoring
If onset of fever $\geq 38^{\circ}\text{C}$ / $100.4^{\circ}\text{F} \geq 72$ hours post CAR+ T cell administration:	Monitoring: <ul style="list-style-type: none"> • Check absolute neutrophil count (ANC), evaluate fever, rule out infection (surveillance cultures) • Admit for/continue close monitoring of cardiac and organ function, including routine neurologic exams • Follow serum C-reactive protein (CRP), ferritin and coagulation parameters (international normalized ratio (INR), partial thromboplastin time (PTT), fibrinogen) • Symptomatic support (e.g. antipyretics, analgesics), antibiotics as per institutional guidelines (febrile neutropenia)
If rapid onset of CRS signs or symptoms (defined as fever $\geq 38.5^{\circ}\text{C}$ / 101.3°F seen < 72 hours post-CAR+ T cell administration or any signs or symptoms defining CRS Grade ≥ 2); or If clinical progression of CRS or rapid deterioration when monitoring after onset of fever, initiate 1st line treatment	First line treatment: <u>Grade 1:</u> <ul style="list-style-type: none"> • If slow onset ($\geq 72\text{h}$), treat symptomatically • If rapid onset ($< 72\text{h}$), consider tocilizumab 8 mg/kg IV \pm dexamethasone 10 mg q24h <u>Grade 2:</u> <ul style="list-style-type: none"> • If slow onset ($\geq 72\text{h}$), give tocilizumab 8 mg/kg IV \pm dexamethasone 10 mg IV q12-24h if needed • If rapid onset ($< 72\text{h}$), give tocilizumab 8mg/kg IV and dexamethasone 10 mg IV q12-24h <u>Grade 3:</u> <ul style="list-style-type: none"> • Give tocilizumab 8 mg/kg IV and dexamethasone 10 mg IV q12h <u>Grade 4:</u> <ul style="list-style-type: none"> • Give tocilizumab 8 mg/kg IV and dexamethasone 20 mg IV q6
If no improvement with 1st line treatment within 24 hours or rapid progression of CRS, initiate 2nd line treatment	Second line Treatment: <ul style="list-style-type: none"> • Give 2nd dose of tocilizumab 8 mg/kg IV and dexamethasone 20 mg IV q6-12h • Consider other causes for clinical deterioration (e.g. sepsis, adrenal insufficiency)
If no improvement with 2nd line treatment within 24 hours or rapid progression of CRS, initiate 3rd line treatment	Third line treatment: <ul style="list-style-type: none"> • Methylprednisolone 2 mg/kg followed by 2 mg/kg divided 4 times per day (taper within 7 days) • Consider other anti-IL-6 agents
If ongoing CRS despite prior therapies initiate 4th line treatment	Fourth line treatment: <ul style="list-style-type: none"> • Consider anti-T cell therapies such as cyclophosphamide (1.5 mg/m^2) or others
Other considerations: <ul style="list-style-type: none"> • Once dexamethasone is initiated, give for a minimum of 3 doses or until resolution of CRS and any associated neurological symptoms • Grade 1: consider seizure prophylaxis (e.g. levetiracetam) • Grade 2: frequent inpatient monitoring until fever and symptom resolution, 	

include neurologic evaluations and symptomatic support (supplemental oxygen, IV fluids with aggressive electrolyte replacement, antipyretics, low-dose vasopressor support); initiate seizure prophylaxis (e.g. levetiracetam) and consider electroencephalogram (EEG) monitoring if concurrent neurotoxicity (NT); also see **Table 11** below for NT management guidelines

- Grade ≥ 3 : ICU-level monitoring and symptomatic, hemodynamic, and respiratory support, include neurologic exams; initiate seizure prophylaxis (e.g. levetiracetam) and consider EEG monitoring if concurrent NT; also see **Table 11** below for NT management guidelines

Table 11. Exemplary Guidelines for Administering Agents for Modulating Cell Therapy for Neurotoxicity (NT)

After CAR+ T cell administration	Monitoring: <ul style="list-style-type: none"> • Monitor for NT symptoms (aphasia, confusion, altered mental status) • Consider seizure prophylaxis (e.g., levetiracetam) for subjects at high risk of NT
Early onset NT (event onset < 72 hours):	First line treatment: <ul style="list-style-type: none"> • Start seizure prophylaxis • <u>Grade 1</u>: consider dexamethasone 10 mg q8-12h • <u>Grade 2</u>: dexamethasone 10 mg q8-12h • <u>Grade 3</u>: dexamethasone 20 mg q6-8h • <u>Grade 4</u>: dexamethasone 20 mg q6h
Late Onset NT (event onset \geq 72 hours)	First line treatment: <ul style="list-style-type: none"> • Start seizure prophylaxis • <u>Grade 1</u>: Observe • <u>Grade 2</u>: Consider dexamethasone 10 mg q12-24h • <u>Grade 3</u>: Give dexamethasone 10-20 mg q8-12h; use lower doses/longer interval for aphasia or confusion and higher doses/longer interval for events leading to depressed level of consciousness. High-dose corticosteroids are not recommended for isolated Grade 3 headaches • <u>Grade 4</u>: Give dexamethasone 10-20 mg q6-8h; use higher dose/shorter interval for events requiring respiratory support or seizures
If no improvement with 1st line treatment within 24 hours or worsening of NT, initiate 2nd line treatment	Second line treatment: <ul style="list-style-type: none"> • Increase dose and/or frequency of dexamethasone • Consider methylprednisolone (2 mg/kg loading dose followed by 2 mg/ kg divided 4 times per day (taper within 7 days)) if life-threatening complications arise (require respiratory support or if seizures)
If no improvement with 2nd line treatment within 24 hours or symptoms progress rapidly initiate 3rd line treatment	Third line treatment: <ul style="list-style-type: none"> • Further increase dose and/or frequency of dexamethasone • Give methylprednisolone if at maximum doses of dexamethasone
If cerebral edema occurs	Cerebral edema: <ul style="list-style-type: none"> • Give high-dose methylprednisolone (1-2 g, repeat q24 if needed). Taper as clinically indicated. Consider hyperventilation and hyperosmolar therapy
Other considerations:	

- Hospitalize for monitoring if subject is an outpatient upon start of event; initiate neurologic consultation
- If concurrent with CRS, treat CRS per CRS management guidelines (e.g., as indicated in **Table 10** above) in addition to NT recommendations; use the most aggressive interventions recommended between the two guidelines
- Consider other causes of neurologic symptoms (e.g., infection, metabolic syndrome, disease progression, medications)
- Steroids could be continued for a minimum of 48 hours; consider longer course with potential taper for a total of 5 to 7 days for higher grade or persistent/recurrent symptoms
- Imaging (magnetic resonance imaging (MRI) or computed tomography (CT) scan), electroencephalogram (EEG) and lumbar puncture (LP) should be done and imaging repeated if no clinical improvement; continuous monitoring by EEG should be considered
- For subjects who have seizures or seizure-like activity, antiepileptic drugs are recommended; antiepileptic drug combinations may be required for multiple or refractory seizure activity
- intensive care unit (ICU) monitoring may be required; mechanical ventilation for airway protection may be indicated

[0611] In some embodiments, biomarkers such as CRP, ferritin, and serum cytokine levels (e.g., those described in Section IV.B. herein) can be associated with higher risk for developing CRS, but management of CRS symptoms is always considered for treating or management of CRS, in some cases, based on close observation of the subjects.

[0612] In some embodiments, the agent or therapy or intervention is a fluid bolus or the use of absorbent resin technology with blood or plasma filtration. In some cases, the intervention includes dialysis, plasmapheresis, or similar technologies. In some embodiments, vassopressors or acetaminophen can be employed.

[0613] In some aspects, any of the signs, symptoms, factors or parameters associated with a toxicity, such as CRS or neurotoxicity, including any described herein, for example in Section II.A, can be assessed or monitored, in some cases, in a hospital setting or an outpatient setting. In some cases, symptomatic support for CRS can be provided, including administration of antipyretics, analgesics and/or antibiotics. In some aspects, seizure prophylaxis (e.g., Levetiracetam) can be administered for subjects who have a high risk of developing neurotoxicity.

VI. ENGINEERED CELLS

[0614] In some embodiments, the provided methods are associated with the administration of a cell therapy, such as for the treatment of diseases or conditions including various tumors. In some embodiments, the T cell therapy for use in accord with the provided methods includes administering engineered cells expressing recombinant receptors designed to recognize and/or

specifically bind to molecules associated with the disease or condition and result in a response, such as an immune response against such molecules upon binding to such molecules. The receptors may include chimeric receptors, *e.g.*, chimeric antigen receptors (CARs), and other transgenic antigen receptors including transgenic T cell receptors (TCRs) or chimeric autoantibody receptors (CAARs).

[0615] In some embodiments, the cells contain or are engineered to contain an engineered receptor, *e.g.*, an engineered antigen receptor, such as a chimeric antigen receptor (CAR), or a T cell receptor (TCR). Also provided are populations of such cells, compositions containing such cells and/or enriched for such cells, such as in which cells of a certain type such as T cells or CD8⁺ or CD4⁺ cells are enriched or selected. Among the compositions are pharmaceutical compositions and formulations for administration, such as for adoptive cell therapy. Also provided are therapeutic methods for administering the cells and compositions to subjects, *e.g.*, patients.

[0616] Thus, 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.Recombinant Receptors

[0617] The cells generally express recombinant receptors, such as antigen receptors including functional non-TCR antigen receptors, *e.g.*, chimeric antigen receptors (CARs), and other antigen-binding receptors such as transgenic T cell receptors (TCRs). Also among the receptors are other chimeric receptors, such as chimeric autoantibody receptors (CAARs).

1. Chimeric Antigen Receptors (CARs)

[0618] In some embodiments, the recombinant receptor includes a chimeric antigen receptor (CAR). In some embodiments, the CAR is specific for a particular antigen (or marker or ligand), such as an antigen expressed on the surface of a particular cell type. In some embodiments, the antigen is a polypeptide. In some embodiments, it is a carbohydrate or other molecule. In some embodiments, the antigen is selectively expressed or overexpressed on cells of the disease or condition, *e.g.*, the tumor or pathogenic cells, as compared to normal or non-

targeted cells or tissues. In other embodiments, the antigen is expressed on normal cells and/or is expressed on the engineered cells.

[0619] In particular embodiments, the recombinant receptor, such as a chimeric receptor, contains an intracellular signaling region, which includes a cytoplasmic signaling domain (also interchangeably called an intracellular signaling domain), such as a cytoplasmic (intracellular) region capable of inducing a primary activation signal in a T cell, for example, a cytoplasmic signaling domain of a T cell receptor (TCR) component (e.g. a cytoplasmic signaling domain of a zeta chain of a CD3-zeta (CD3 ζ) chain or a functional variant or signaling portion thereof) and/or that comprises an immunoreceptor tyrosine-based activation motif (ITAM).

[0620] In some embodiments, the chimeric receptor further contains an extracellular binding domain that specifically binds to an antigen (or a ligand). In some embodiments, the chimeric receptor is a CAR that contains an extracellular antigen-recognition domain that specifically binds to an antigen. In some embodiments, the antigen (or a ligand), is a protein expressed on the surface of cells. In some embodiments, the CAR is a TCR-like CAR and the antigen is a processed peptide antigen, such as a peptide antigen of an intracellular protein, which, like a TCR, is recognized on the cell surface in the context of a major histocompatibility complex (MHC) molecule.

[0621] 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.*, 3(4): 388–398 (2013); Davila et al. *PLoS ONE* 8(4): e61338 (2013); Turtle et al., *Curr. Opin. Immunol.*, 24(5): 633-39 (2012); Wu et al., *Cancer*, 18(2): 160-75 (2012). 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., *Nature Reviews Clinical Oncology*, 10, 267-276 (2013); Wang et al., *J. Immunother.* 35(9): 689-701 (2012); and Brentjens et al., *Sci Transl Med.*, 5(177) (2013). 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. 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.

[0622] In some embodiments, the antigen targeted by the receptor is a polypeptide. In some embodiments, it is a carbohydrate or other molecule. In some embodiments, the antigen is selectively expressed or overexpressed on cells of the disease or condition, *e.g.*, the tumor or pathogenic cells, as compared to normal or non-targeted cells or tissues. In other embodiments, the antigen is expressed on normal cells and/or is expressed on the engineered cells.

[0623] In some embodiments, the CAR is constructed with a specificity for a particular antigen (or marker or ligand), such as an antigen expressed in a particular cell type to be targeted by adoptive therapy, *e.g.*, a cancer marker, and/or an antigen intended to induce a dampening response, such as an antigen expressed on a normal or non-diseased cell type. Thus, the CAR typically includes in its extracellular portion one or more antigen binding molecules, such as one or more antigen-binding fragment, domain, or portion, or one or more antibody variable domains, and/or antibody molecules. 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).

[0624] In some embodiments, the antibody or antigen-binding portion thereof is expressed on cells as part of a recombinant receptor, such as an antigen receptor. Among the antigen receptors are functional non-TCR antigen receptors, such as chimeric antigen receptors (CARs). Generally, a CAR containing an antibody or antigen-binding fragment that exhibits TCR-like specificity directed against peptide-MHC complexes also may be referred to as a TCR-like CAR. In some embodiments, the extracellular antigen binding domain specific for an MHC-peptide complex of a TCR-like CAR is linked to one or more intracellular signaling components, in some aspects via linkers and/or transmembrane domain(s). In some embodiments, such molecules can typically mimic or approximate a signal through a natural antigen receptor, such as a TCR, and, optionally, a signal through such a receptor in combination with a costimulatory receptor.

[0625] In some embodiments, the recombinant receptor, such as a chimeric receptor (*e.g.* CAR), includes a ligand-binding domain that binds, such as specifically binds, to an antigen (or

a ligand). Among the antigens targeted by the chimeric receptors are those expressed in the context of a disease, condition, or cell type to be targeted via the adoptive cell therapy. Among the diseases and conditions are proliferative, neoplastic, and malignant diseases and disorders, including cancers and tumors, including hematologic cancers, cancers of the immune system, such as lymphomas, leukemias, and/or myelomas, such as B, T, and myeloid leukemias, lymphomas, and multiple myelomas.

[0626] In some embodiments, the antigen (or a ligand) is a polypeptide. In some embodiments, it is a carbohydrate or other molecule. In some embodiments, the antigen (or a ligand) is selectively expressed or overexpressed on cells of the disease or condition, *e.g.*, the tumor or pathogenic cells, as compared to normal or non-targeted cells or tissues.

[0627] In some embodiments, the CAR contains an antibody or an antigen-binding fragment (*e.g.* scFv) that specifically recognizes an antigen, such as an intact antigen, expressed on the surface of a cell.

[0628] Antigens targeted by the receptors in some embodiments are or include $\alpha\text{v}\beta 6$ integrin ($\alpha\text{v}\beta 6$ integrin), B cell maturation antigen (BCMA), B7-H3, B7-H6, carbonic anhydrase 9 (CA9, also known as CAIX or G250), a cancer-testis antigen, cancer/testis antigen 1B (CTAG, also known as NY-ESO-1 and LAGE-2), carcinoembryonic antigen (CEA), a cyclin, cyclin A2, C-C Motif Chemokine Ligand 1 (CCL-1), CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD133, CD138, CD171, chondroitin sulfate proteoglycan 4 (CSPG4), epidermal growth factor protein (EGFR), type III epidermal growth factor receptor mutation (EGFR vIII), epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), ephrinB2, ephrine receptor A2 (EPHa2), estrogen receptor, Fc receptor like 5 (FCRL5; also known as Fc receptor homolog 5 or FCRH5), fetal acetylcholine receptor (fetal AchR), a folate binding protein (FBP), folate receptor alpha, ganglioside GD2, O-acetylated GD2 (OGD2), ganglioside GD3, glycoprotein 100 (gp100), glypican-3 (GPC3), G Protein Coupled Receptor 5D (GPRC5D), Her2/neu (receptor tyrosine kinase erb-B2), Her3 (erb-B3), Her4 (erb-B4), erbB dimers, Human high molecular weight-melanoma-associated antigen (HMW-MAA), hepatitis B surface antigen, Human leukocyte antigen A1 (HLA-A1), Human leukocyte antigen A2 (HLA-A2), IL-22 receptor alpha (IL-22R α), IL-13 receptor alpha 2 (IL-13R α 2), kinase insert domain receptor (kdr), kappa light chain, L1 cell adhesion molecule (L1-CAM), CE7 epitope of L1-CAM, Leucine Rich Repeat Containing 8 Family Member A (LRRC8A), Lewis Y, Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, MAGE-A10, mesothelin (MSLN), c-Met, murine cytomegalovirus (CMV), mucin 1 (MUC1), MUC16, natural killer group 2 member

D (NKG2D) ligands, melan A (MART-1), neural cell adhesion molecule (NCAM), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME), progesterone receptor, a prostate specific antigen, prostate stem cell antigen (PSCA), prostate specific membrane antigen (PSMA), Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1), survivin, Trophoblast glycoprotein (TPBG also known as 5T4), tumor-associated glycoprotein 72 (TAG72), Tyrosinase related protein 1 (TRP1, also known as TYRP1 or gp75), Tyrosinase related protein 2 (TRP2, also known as dopachrome tautomerase, dopachrome delta-isomerase or DCT), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor receptor 2 (VEGFR2), Wilms Tumor 1 (WT-1), a pathogen-specific or pathogen-expressed antigen, or an antigen associated with a universal tag, and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV or other pathogens. Antigens targeted by the receptors in some embodiments include antigens associated with a B cell malignancy, such as any of a number of known B cell marker. In some embodiments, the antigen is or includes CD20, CD19, CD22, ROR1, CD45, CD21, CD5, CD33, Igkappa, Iglambda, CD79a, CD79b or CD30.

[0629] Antigens targeted by the receptors in some embodiments are or include orphan tyrosine kinase receptor ROR1, Her2, L1-CAM, CD19, CD20, CD22, mesothelin, CEA, and hepatitis B surface antigen, anti-folate receptor, CD23, CD24, CD30, CD33, CD38, CD44, EGFR, EGP-2, EGP-4, EPHA2, ErbB2, 3, or 4, FBP, fetal acetylcholine e receptor, GD2, GD3, HMW-MAA, IL-22R-alpha, IL-13R-alpha2, kdr, kappa light chain, Lewis Y, L1-cell adhesion molecule, MAGE-A1, mesothelin, MUC1, MUC16, PSCA, NKG2D Ligands, NY-ESO-1, MART-1, gp100, oncofetal antigen, ROR1, TAG72, VEGF-R2, carcinoembryonic antigen (CEA), prostate specific antigen, PSMA, Her2/neu, estrogen receptor, progesterone receptor, ephrinB2, CD123, c-Met, GD-2, and MAGE A3, CE7, Wilms Tumor 1 (WT-1), a cyclin, such as cyclin A1 (CCNA1), and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV or other pathogens. In some embodiments, the CAR binds a pathogen-specific or pathogen-expressed antigen. In some embodiments, the CAR is specific for viral antigens (such as HIV, HCV, HBV, etc.), bacterial antigens, and/or parasitic antigens.

[0630] In some embodiments, the CAR contains a TCR-like antibody, such as an antibody or an antigen-binding fragment (*e.g.* scFv) that specifically recognizes an intracellular antigen, such as a tumor-associated antigen, presented on the cell surface as a MHC-peptide complex. In some embodiments, an antibody or antigen-binding portion thereof that recognizes an MHC-peptide complex can be expressed on cells as part of a recombinant receptor, such as an antigen

receptor. Among the antigen receptors are functional non-TCR antigen receptors, such as chimeric antigen receptors (CARs). Generally, a CAR containing an antibody or antigen-binding fragment that exhibits TCR-like specificity directed against peptide-MHC complexes also may be referred to as a TCR-like CAR.

[0631] Reference to “Major histocompatibility complex” (MHC) refers to a protein, generally a glycoprotein, that contains a polymorphic peptide binding site or binding groove that can, in some cases, complex with peptide antigens of polypeptides, including peptide antigens processed by the cell machinery. In some cases, MHC molecules can be displayed or expressed on the cell surface, including as a complex with peptide, *i.e.* MHC-peptide complex, for presentation of an antigen in a conformation recognizable by an antigen receptor on T cells, such as a TCRs or TCR-like antibody. Generally, MHC class I molecules are heterodimers having a membrane spanning α chain, in some cases with three α domains, and a non-covalently associated $\beta 2$ microglobulin. Generally, MHC class II molecules are composed of two transmembrane glycoproteins, α and β , both of which typically span the membrane. An MHC molecule can include an effective portion of an MHC that contains an antigen binding site or sites for binding a peptide and the sequences necessary for recognition by the appropriate antigen receptor. In some embodiments, MHC class I molecules deliver peptides originating in the cytosol to the cell surface, where a MHC-peptide complex is recognized by T cells, such as generally CD8⁺ T cells, but in some cases CD4⁺ T cells. In some embodiments, MHC class II molecules deliver peptides originating in the vesicular system to the cell surface, where they are typically recognized by CD4⁺ T cells. Generally, MHC molecules are encoded by a group of linked loci, which are collectively termed H-2 in the mouse and human leukocyte antigen (HLA) in humans. Hence, typically human MHC can also be referred to as human leukocyte antigen (HLA).

[0632] The term “MHC-peptide complex” or “peptide-MHC complex” or variations thereof, refers to a complex or association of a peptide antigen and an MHC molecule, such as, generally, by non-covalent interactions of the peptide in the binding groove or cleft of the MHC molecule. In some embodiments, the MHC-peptide complex is present or displayed on the surface of cells. In some embodiments, the MHC-peptide complex can be specifically recognized by an antigen receptor, such as a TCR, TCR-like CAR or antigen-binding portions thereof.

[0633] In some embodiments, a peptide, such as a peptide antigen or epitope, of a polypeptide can associate with an MHC molecule, such as for recognition by an antigen

receptor. Generally, the peptide is derived from or based on a fragment of a longer biological molecule, such as a polypeptide or protein. In some embodiments, the peptide typically is about 8 to about 24 amino acids in length. In some embodiments, a peptide has a length of from or from about 9 to 22 amino acids for recognition in the MHC Class II complex. In some embodiments, a peptide has a length of from or from about 8 to 13 amino acids for recognition in the MHC Class I complex. In some embodiments, upon recognition of the peptide in the context of an MHC molecule, such as MHC-peptide complex, the antigen receptor, such as TCR or TCR-like CAR, produces or triggers an activation signal to the T cell that induces a T cell response, such as T cell proliferation, cytokine production, a cytotoxic T cell response or other response.

[0634] In some embodiments, a TCR-like antibody or antigen-binding portion, are known or can be produced by known methods (see e.g. US Published Application Nos. US 2002/0150914; US 2003/0223994; US 2004/0191260; US 2006/0034850; US 2007/00992530; US20090226474; US20090304679; and International PCT Publication No. WO 03/068201).

[0635] In some embodiments, an antibody or antigen-binding portion thereof that specifically binds to a MHC-peptide complex, can be produced by immunizing a host with an effective amount of an immunogen containing a specific MHC-peptide complex. In some cases, the peptide of the MHC-peptide complex is an epitope of antigen capable of binding to the MHC, such as a tumor antigen, for example a universal tumor antigen, myeloma antigen or other antigen as described below. In some embodiments, an effective amount of the immunogen is then administered to a host for eliciting an immune response, wherein the immunogen retains a three-dimensional form thereof for a period of time sufficient to elicit an immune response against the three-dimensional presentation of the peptide in the binding groove of the MHC molecule. Serum collected from the host is then assayed to determine if desired antibodies that recognize a three-dimensional presentation of the peptide in the binding groove of the MHC molecule is being produced. In some embodiments, the produced antibodies can be assessed to confirm that the antibody can differentiate the MHC-peptide complex from the MHC molecule alone, the peptide of interest alone, and a complex of MHC and irrelevant peptide. The desired antibodies can then be isolated.

[0636] In some embodiments, an antibody or antigen-binding portion thereof that specifically binds to an MHC-peptide complex can be produced by employing antibody library display methods, such as phage antibody libraries. In some embodiments, phage display libraries of mutant Fab, scFv or other antibody forms can be generated, for example, in which members

of the library are mutated at one or more residues of a CDR or CDRs. See *e.g.* US published application No. US20020150914, US2014/0294841; and Cohen CJ. *et al.* (2003) *J Mol. Recogn.* 16:324-332.

[0637] The term “antibody” herein is used in the broadest sense and includes polyclonal and monoclonal antibodies, including intact antibodies and functional (antigen-binding) antibody fragments, including fragment antigen binding (Fab) fragments, F(ab')₂ fragments, Fab' fragments, Fv fragments, recombinant IgG (rIgG) fragments, variable heavy chain (V_H) regions capable of specifically binding the antigen, single chain antibody fragments, including single chain variable fragments (scFv), and single domain antibodies (*e.g.*, sdAb, sdFv, nanobody) fragments. The term encompasses genetically engineered and/or otherwise modified forms of immunoglobulins, such as intrabodies, peptibodies, chimeric antibodies, fully human antibodies, humanized antibodies, and heteroconjugate antibodies, multispecific, *e.g.*, bispecific, antibodies, diabodies, triabodies, and tetrabodies, tandem di-scFv, tandem tri-scFv. Unless otherwise stated, the term “antibody” should be understood to encompass functional antibody fragments thereof. The term also encompasses intact or full-length antibodies, including antibodies of any class or sub-class, including IgG and sub-classes thereof, IgM, IgE, IgA, and IgD.

[0638] In some embodiments, the antigen-binding proteins, antibodies and antigen binding fragments thereof specifically recognize an antigen of a full-length antibody. In some embodiments, the heavy and light chains of an antibody can be full-length or can be an antigen-binding portion (a Fab, F(ab')₂, Fv or a single chain Fv fragment (scFv)). In other embodiments, the antibody heavy chain constant region is chosen from, *e.g.*, IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD, and IgE, particularly chosen from, *e.g.*, IgG1, IgG2, IgG3, and IgG4, more particularly, IgG1 (*e.g.*, human IgG1). In another embodiment, the antibody light chain constant region is chosen from, *e.g.*, kappa or lambda, particularly kappa.

[0639] 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.

[0640] 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).

[0641] 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).

[0642] 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.

[0643] **Table 12**, 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 12. Boundaries of CDRs according to various numbering schemes.				
CDR	Kabat	Chothia	AbM	Contact
CDR-L1	L24--L34	L24--L34	L24--L34	L30--L36
CDR-L2	L50--L56	L50--L56	L50--L56	L46--L55
CDR-L3	L89--L97	L89--L97	L89--L97	L89--L96
CDR-H1 (Kabat Numbering ¹)	H31--H35B	H26--H32..34	H26--H35B	H30--H35B
CDR-H1 (Chothia Numbering ²)	H31--H35	H26--H32	H26--H35	H30--H35
CDR-H2	H50--H65	H52--H56	H50--H58	H47--H58
CDR-H3	H95--H102	H95--H102	H95--H102	H93--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

[0644] 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.

[0645] 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.

[0646] 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 domains 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).

[0647] Single-domain antibodies 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 a cancer marker or cell surface antigen of a cell or disease to be targeted, such as a tumor cell or a cancer cell, such as any of the target antigens described herein or known.

[0648] 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, and/or that are may not be produced by enzyme digestion of a naturally-occurring intact antibody. In some embodiments, the antibody fragments are scFvs.

[0649] 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.

[0650] Thus, in some embodiments, the chimeric antigen receptor, including TCR-like CARs, includes an extracellular portion containing an antibody or antibody fragment. In some embodiments, the antibody or fragment includes an scFv. In some aspects, the chimeric antigen receptor includes an extracellular portion containing the antibody or fragment and an intracellular signaling region. In some embodiments, the intracellular signaling region comprises an intracellular signaling domain. In some embodiments, the intracellular signaling domain is or comprises a primary signaling domain, a signaling domain that is capable of inducing a primary activation signal in a T cell, a signaling domain of a T cell receptor (TCR) component, and/or a signaling domain comprising an immunoreceptor tyrosine-based activation motif (ITAM).

[0651] In some embodiments, the recombinant receptor such as the CAR, including the antibody portion of the recombinant receptor, *e.g.*, CAR, further includes at least a portion of an immunoglobulin constant region, 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 such as the CAR, including the antibody portion thereof, further includes a spacer, which 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. Exemplary spacers, *e.g.*, hinge regions, include those described in international patent application publication number WO2014031687. In some examples, the spacer is or is 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 C_H2 and C_H3 domains, or IgG4 hinge linked to the C_H3 domain. Exemplary spacers include, but are not limited to, those described in Hudecek et al. *Clin. Cancer Res.*, 19:3153 (2013), international patent application publication number WO2014031687, U.S. Patent No. 8,822,647 or published app. No. US2014/0271635.

[0652] In some embodiments, the constant region or portion is of a human IgG, such as IgG4 or IgG1. In some embodiments, the spacer has the sequence ESKYGPPCPPCP (set forth in SEQ ID NO: 1), and is encoded by the sequence set forth in SEQ ID NO: 2. In some embodiments, the spacer has the sequence set forth in SEQ ID NO: 3. In some embodiments, the spacer has the sequence set forth in SEQ ID NO: 4. 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. In some embodiments, the spacer has the sequence set forth in SEQ ID NOS: 26-34. 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: 26-34.

[0653] The antigen recognition 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. Thus, in some embodiments, the antigen-binding component (e.g., antibody) is linked to one or more transmembrane and intracellular signaling domains or regions. In some embodiments, the transmembrane domain is fused to the extracellular domain. 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.

[0654] 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, 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).

[0655] Among the intracellular signaling domains or regions 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 or region of the CAR.

[0656] The receptor, e.g., the CAR, generally includes at least one intracellular signaling component or components. 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., CAR, further includes a portion of one or more additional molecules such as Fc receptor γ , CD8, 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 CD8, CD4, CD25 or CD16.

[0657] In some embodiments, upon ligation of the CAR or other chimeric receptor, the cytoplasmic domain or intracellular signaling domains or regions of the receptor 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 domain or 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 domain or domains or regions 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 receptors 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.

[0658] 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.

[0659] 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.

[0660] 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 TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD8, CD22, CD79a, CD79b, and CD66d. In some embodiments, cytoplasmic signaling molecule(s) in the CAR contain(s) a cytoplasmic signaling domain or region, portion thereof, or sequence derived from CD3 zeta.

[0661] In some embodiments, the CAR includes a signaling domain or region and/or transmembrane portion of a costimulatory receptor, such as CD28, 4-1BB, OX40, DAP10, and ICOS. In some aspects, the same CAR includes both the activating and costimulatory components.

[0662] 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) (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.

[0663] In some embodiments, the cells expressing the recombinant receptor further include inhibitory CARs (iCARs, see Fedorov *et al.*, *Sci. Transl. Medicine*, 5(215) (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.

[0664] In some embodiments, the two receptors induce, respectively, an activating and an inhibitory signal to the cell, such that ligation of one of the receptor 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.

[0665] 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.

[0666] In certain embodiments, the intracellular signaling domain comprises a CD28 transmembrane and signaling domain linked to a CD3 (e.g., CD3-zeta) intracellular domain. In some embodiments, the intracellular signaling domain comprises a chimeric CD28 and CD137 (4-1BB, TNFRSF9) co-stimulatory domains, linked to a CD3 zeta intracellular domain.

[0667] 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.

[0668] In some embodiments, the CAR or other antigen receptor further includes a marker, such as a cell surface marker, which may be used to confirm transduction or engineering of the cell to express the receptor, such as a truncated version of a cell surface receptor, such as truncated EGFR (tEGFR). In some aspects, the marker includes all or part (e.g., truncated form) of CD34, a NGFR, or epidermal growth factor receptor (e.g., tEGFR).

[0669] 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.

[0670]

[0671] 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). 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.

[0672] 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.

[0673] 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. 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 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: 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 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: 6 or 17.

[0674] 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.

[0675] 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.

[0676] 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 is one that includes multiple costimulatory domains of different costimulatory receptors.

[0677] In some embodiments, the chimeric antigen receptor includes an extracellular portion containing an antibody or antibody fragment. In some aspects, the chimeric antigen receptor includes an extracellular portion containing the antibody or fragment and an intracellular signaling domain. In some embodiments, the antibody or fragment includes an scFv 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 (CD3 ζ) chain. In some embodiments,

the chimeric antigen receptor includes a transmembrane domain linking the extracellular domain and the intracellular signaling domain. In some aspects, the transmembrane domain contains a transmembrane portion of CD28. In some embodiments, the chimeric antigen receptor contains an intracellular domain of a T cell costimulatory molecule. The extracellular domain and transmembrane domain can be linked directly or indirectly. In some embodiments, the extracellular domain and transmembrane are linked by a spacer, such as any described herein. In some embodiments, the receptor contains extracellular portion of the molecule from which the transmembrane domain is derived, such as a CD28 extracellular portion. In some embodiments, the chimeric antigen receptor contains an intracellular domain derived from a T cell costimulatory molecule or a functional variant thereof, such as between the transmembrane domain and intracellular signaling domain. In some aspects, the T cell costimulatory molecule is CD28 or 4-1BB.

[0678] In some embodiments, the antigen or antigen binding domain is CD19. 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 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.

[0679] In some embodiments, the scFv is derived from FMC63. 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 NOS: 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 set forth in SEQ ID NO: 37 or 34. 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.

[0680] 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. In some embodiments, the scFv comprises a 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 variable heavy and variable light chains are connected by a linker. In some embodiments, the linker is set forth in SEQ ID NO:56. 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:57 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:57. 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.

[0681] In some embodiments the scFv is derived from SJ25C1. 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.

[0682] In some embodiments, the scFv 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. In some embodiments, the scFv comprises a variable heavy chain region set forth in SEQ ID NO:50 and a variable light chain region set forth in SEQ ID NO:51. In some embodiments, the variable heavy and variable light chain 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.

[0683] In some embodiments, the antigen is CD20. In some embodiments, the scFv contains a V_H and a V_L derived from an antibody or an antibody fragment specific to CD20. In some embodiments, the antibody or antibody fragment that binds CD20 is an antibody that is or

is derived from Rituximab, such as is Rituximab scFv.

[0684] In some embodiments, the antigen is CD22. In some embodiments, the scFv contains a V_H and a V_L derived from an antibody or an antibody fragment specific to CD22. In some embodiments, the antibody or antibody fragment that binds CD22 is an antibody that is or is derived from m971, such as is m971 scFv.

[0685] In some embodiments, the antigen or antigen binding domain is BCMA. In some embodiments, the scFv contains a V_H and a V_L derived from an antibody or an antibody fragment specific to BCMA. In some embodiments, the antibody or antibody fragment that binds BCMA is or contains a V_H and a V_L from an antibody or antibody fragment set forth in International Patent Applications, Publication Number WO 2016/090327 and WO 2016/090320.

[0686] In some embodiments, the antigen or antigen binding domain is GPRC5D. In some embodiments, the scFv contains a V_H and a V_L derived from an antibody or an antibody fragment specific to GPRC5D. In some embodiments, the antibody or antibody fragment that binds GPRC5D is or contains a V_H and a V_L from an antibody or antibody fragment set forth in International Patent Applications, Publication Number WO 2016/090329 and WO 2016/090312.

[0687] For example, 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. 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.

[0688] In some embodiments, the transmembrane domain of the recombinant receptor, e.g., the CAR, is or includes a transmembrane domain of human CD28 (e.g. Accession No. P01747.1) or variant thereof, such as 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 at or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto, or such as a 27-amino acid transmembrane domain of a human CD28.

[0689] In some embodiments, the chimeric antigen receptor contains an intracellular domain of a T cell costimulatory molecule. In some aspects, the T cell costimulatory molecule is CD28 or 4-1BB.

[0690] In some embodiments, the intracellular signaling domain, region or component(s) of the recombinant receptor, e.g. the CAR, contains an intracellular costimulatory signaling domain of human CD28 or a functional variant or portion thereof, such as a domain with an LL to GG substitution at positions 186-187 of a native CD28 protein. For example, in some embodiments, the intracellular signaling domain or region 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 domain or region comprises an intracellular costimulatory signaling domain or region of 4-1BB (e.g., 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 or such as a 42-amino acid cytoplasmic domain of a human 4-1BB.

[0691] In some embodiments, the intracellular signaling domain or region of the recombinant receptor, e.g. the CAR, comprises a human CD3 chain, optionally a zeta stimulatory signaling domain or region or functional variant thereof, such as an 112 AA cytoplasmic domain or region of isoform 3 of human CD3 ζ (Accession No.: P20963.2) or a CD3 zeta signaling domain or region as described in U.S. Patent No.: 7,446,190 or U.S. Patent No. 8,911,993. For example, in some embodiments, the intracellular signaling domain or region comprises the sequence of amino acids as 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.

[0692] In some aspects, 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. In other embodiments, the spacer is or contains an Ig hinge, e.g., an IgG4-derived hinge, optionally 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 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.

[0693] For example, in some embodiments, the CAR includes an antibody such as an antibody fragment, including scFvs, 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. In some embodiments, the CAR includes an antibody or fragment, such as scFv, 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.

[0694] 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 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: 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 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: 7 or 16.

[0695] 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.

2. T Cell receptors (TCRs)

[0696] In some embodiments, engineered cells, such as T cells, are provided that express a T cell receptor (TCR) or antigen-binding portion thereof that recognizes an peptide epitope or T cell epitope of a target polypeptide, such as an antigen of a tumor, viral or autoimmune protein.

[0697] In some embodiments, a “T cell receptor” or “TCR” is a molecule that contains a variable α and β chains (also known as TCR α and TCR β , respectively) or a variable γ and δ chains (also known as TCR α and TCR β , respectively), or antigen-binding portions thereof, and which is capable of specifically binding to a peptide bound to an MHC molecule. In some embodiments, the TCR is in the $\alpha\beta$ form. Typically, TCRs that exist in $\alpha\beta$ and $\gamma\delta$ forms are generally structurally similar, but T cells expressing them may have distinct anatomical locations or functions. A TCR can be found on the surface of a cell or in soluble form. Generally, a TCR is found on the surface of T cells (or T lymphocytes) where it is generally responsible for recognizing antigens bound to major histocompatibility complex (MHC) molecules.

[0698] Unless otherwise stated, the term “TCR” should be understood to encompass full TCRs as well as antigen-binding portions or antigen-binding fragments thereof. In some embodiments, the TCR is an intact or full-length TCR, including TCRs in the $\alpha\beta$ form or $\gamma\delta$ form. In some embodiments, the TCR is an antigen-binding portion that is less than a full-length TCR but that binds to a specific peptide bound in an MHC molecule, such as binds to an MHC-peptide complex. In some cases, an antigen-binding portion or fragment of a TCR can contain only a portion of the structural domains of a full-length or intact TCR, but yet is able to bind the peptide epitope, such as MHC-peptide complex, to which the full TCR binds. In some cases, an antigen-binding portion contains the variable domains of a TCR, such as variable α chain and variable β chain of a TCR, sufficient to form a binding site for binding to a specific MHC-peptide complex. Generally, the variable chains of a TCR contain complementarity determining regions involved in recognition of the peptide, MHC and/or MHC-peptide complex.

[0699] In some embodiments, the variable domains of the TCR contain hypervariable loops, or complementarity determining regions (CDRs), which generally are the primary contributors to antigen recognition and binding capabilities and specificity. In some embodiments, a CDR of a TCR or combination thereof forms all or substantially all of the antigen-binding site of a given

TCR molecule. The various CDRs within a variable region of a TCR chain generally are separated by framework regions (FRs), which generally display less variability among TCR molecules as compared to the CDRs (see, e.g., Jores et al., Proc. Nat'l Acad. Sci. U.S.A. 87:9138, 1990; Chothia et al., EMBO J. 7:3745, 1988; see also Lefranc et al., Dev. Comp. Immunol. 27:55, 2003). In some embodiments, CDR3 is the main CDR responsible for antigen binding or specificity, or is the most important among the three CDRs on a given TCR variable region for antigen recognition, and/or for interaction with the processed peptide portion of the peptide-MHC complex. In some contexts, the CDR1 of the alpha chain can interact with the N-terminal part of certain antigenic peptides. In some contexts, CDR1 of the beta chain can interact with the C-terminal part of the peptide. In some contexts, CDR2 contributes most strongly to or is the primary CDR responsible for the interaction with or recognition of the MHC portion of the MHC-peptide complex. In some embodiments, the variable region of the β -chain can contain a further hypervariable region (CDR4 or HVR4), which generally is involved in superantigen binding and not antigen recognition (Kotb (1995) Clinical Microbiology Reviews, 8:411-426).

[0700] In some embodiments, a TCR also can contain a constant domain, a transmembrane domain and/or a short cytoplasmic tail (see, e.g., Janeway et al., *Immunobiology: The Immune System in Health and Disease*, 3rd Ed., Current Biology Publications, p. 4:33, 1997). In some aspects, each chain of the TCR can possess one N-terminal immunoglobulin variable domain, one immunoglobulin constant domain, a transmembrane region, and a short cytoplasmic tail at the C-terminal end. In some embodiments, a TCR is associated with invariant proteins of the CD3 complex involved in mediating signal transduction.

[0701] In some embodiments, a TCR chain contains one or more constant domain. For example, the extracellular portion of a given TCR chain (e.g., α -chain or β -chain) can contain two immunoglobulin-like domains, such as a variable domain (e.g., V α or V β ; typically amino acids 1 to 116 based on Kabat numbering Kabat et al., "Sequences of Proteins of Immunological Interest, US Dept. Health and Human Services, Public Health Service National Institutes of Health, 1991, 5th ed.) and a constant domain (e.g., α -chain constant domain or C α , typically positions 117 to 259 of the chain based on Kabat numbering or β chain constant domain or C β , typically positions 117 to 295 of the chain based on Kabat) adjacent to the cell membrane. For example, in some cases, the extracellular portion of the TCR formed by the two chains contains two membrane-proximal constant domains, and two membrane-distal variable domains, which variable domains each contain CDRs. The constant domain of the TCR may contain short

connecting sequences in which a cysteine residue forms a disulfide bond, thereby linking the two chains of the TCR. In some embodiments, a TCR may have an additional cysteine residue in each of the α and β chains, such that the TCR contains two disulfide bonds in the constant domains.

[0702] In some embodiments, the TCR chains contain a transmembrane domain. In some embodiments, the transmembrane domain is positively charged. In some cases, the TCR chain contains a cytoplasmic tail. In some cases, the structure allows the TCR to associate with other molecules like CD3 and subunits thereof. For example, a TCR containing constant domains with a transmembrane region may anchor the protein in the cell membrane and associate with invariant subunits of the CD3 signaling apparatus or complex. The intracellular tails of CD3 signaling subunits (e.g. CD3 γ , CD3 δ , CD3 ϵ and CD3 ζ chains) contain one or more immunoreceptor tyrosine-based activation motif or ITAM that are involved in the signaling capacity of the TCR complex.

[0703] In some embodiments, the TCR may be a heterodimer of two chains α and β (or optionally γ and δ) or it may be a single chain TCR construct. In some embodiments, the TCR is a heterodimer containing two separate chains (α and β chains or γ and δ chains) that are linked, such as by a disulfide bond or disulfide bonds.

[0704] In some embodiments, the TCR can be generated from a known TCR sequence(s), such as sequences of V α , β chains, for which a substantially full-length coding sequence is readily available. Methods for obtaining full-length TCR sequences, including V chain sequences, from cell sources are well known. In some embodiments, nucleic acids encoding the TCR can be obtained from a variety of sources, such as by polymerase chain reaction (PCR) amplification of TCR-encoding nucleic acids within or isolated from a given cell or cells, or synthesis of publicly available TCR DNA sequences.

[0705] In some embodiments, the TCR is obtained from a biological source, such as from cells such as from a T cell (e.g. cytotoxic T cell), T-cell hybridomas or other publicly available source. In some embodiments, the T-cells can be obtained from in vivo isolated cells. In some embodiments, the TCR is a thymically selected TCR. In some embodiments, the TCR is a neoepitope-restricted TCR. In some embodiments, the T-cells can be a cultured T-cell hybridoma or clone. In some embodiments, the TCR or antigen-binding portion thereof can be synthetically generated from knowledge of the sequence of the TCR.

[0706] In some embodiments, the TCR is generated from a TCR identified or selected from screening a library of candidate TCRs against a target polypeptide antigen, or target T cell

epitope thereof. TCR libraries can be generated by amplification of the repertoire of V α and V β from T cells isolated from a subject, including cells present in PBMCs, spleen or other lymphoid organ. In some cases, T cells can be amplified from tumor-infiltrating lymphocytes (TILs). In some embodiments, TCR libraries can be generated from CD4+ or CD8+ cells. In some embodiments, the TCRs can be amplified from a T cell source of a normal or healthy subject, i.e. normal TCR libraries. In some embodiments, the TCRs can be amplified from a T cell source of a diseased subject, i.e. diseased TCR libraries. In some embodiments, degenerate primers are used to amplify the gene repertoire of V α and V β , such as by RT-PCR in samples, such as T cells, obtained from humans. In some embodiments, scTv libraries can be assembled from naïve V α and V β libraries in which the amplified products are cloned or assembled to be separated by a linker. Depending on the source of the subject and cells, the libraries can be HLA allele-specific. Alternatively, in some embodiments, TCR libraries can be generated by mutagenesis or diversification of a parent or scaffold TCR molecule. In some aspects, the TCRs are subjected to directed evolution, such as by mutagenesis, e.g., of the α or β chain. In some aspects, particular residues within CDRs of the TCR are altered. In some embodiments, selected TCRs can be modified by affinity maturation. In some embodiments, antigen-specific T cells may be selected, such as by screening to assess CTL activity against the peptide. In some aspects, TCRs, e.g. present on the antigen-specific T cells, may be selected, such as by binding activity, e.g., particular affinity or avidity for the antigen.

[0707] In some embodiments, the genetically engineered antigen receptors include recombinant T cell receptors (TCRs) and/or TCRs cloned from naturally occurring T cells. In some embodiments, a high-affinity T cell clone for a target antigen (e.g., a cancer antigen) is identified, isolated from a patient, and introduced into the cells. In some embodiments, the TCR clone for a target antigen has been generated in transgenic mice engineered with human immune system genes (e.g., the human leukocyte antigen system, or HLA). See, e.g., tumor antigens (see, e.g., Parkhurst et al. (2009) Clin Cancer Res. 15:169–180 and Cohen et al. (2005) J Immunol. 175:5799–5808. In some embodiments, phage display is used to isolate TCRs against a target antigen (see, e.g., Varela-Rohena et al. (2008) Nat Med. 14:1390–1395 and Li (2005) Nat Biotechnol. 23:349–354.

[0708] In some embodiments, the TCR or antigen-binding portion thereof is one that has been modified or engineered. In some embodiments, directed evolution methods are used to generate TCRs with altered properties, such as with higher affinity for a specific MHC-peptide complex. In some embodiments, directed evolution is achieved by display methods including,

but not limited to, yeast display (Holler et al. (2003) *Nat Immunol*, 4, 55-62; Holler et al. (2000) *Proc Natl Acad Sci U S A*, 97, 5387-92), phage display (Li et al. (2005) *Nat Biotechnol*, 23, 349-54), or T cell display (Chervin et al. (2008) *J Immunol Methods*, 339, 175-84). In some embodiments, display approaches involve engineering, or modifying, a known, parent or reference TCR. For example, in some cases, a wild-type TCR can be used as a template for producing mutagenized TCRs in which in one or more residues of the CDRs are mutated, and mutants with an desired altered property, such as higher affinity for a desired target antigen, are selected.

[0709] In some embodiments, peptides of a target polypeptide for use in producing or generating a TCR of interest are known or can be readily identified by a skilled artisan. In some embodiments, peptides suitable for use in generating TCRs or antigen-binding portions can be determined based on the presence of an HLA-restricted motif in a target polypeptide of interest, such as a target polypeptide described below. In some embodiments, peptides are identified using computer prediction models known to those of skill in the art. In some embodiments, for predicting MHC class I binding sites, such models include, but are not limited to, ProPred1 (Singh and Raghava (2001) *Bioinformatics* 17(12):1236-1237, and SYFPEITHI (see Schuler et al. (2007) *Immunoinformatics Methods in Molecular Biology*, 409(1): 75-93 2007). In some embodiments, the MHC-restricted epitope is HLA-A0201, which is expressed in approximately 39-46% of all Caucasians and therefore, represents a suitable choice of MHC antigen for use preparing a TCR or other MHC-peptide binding molecule.

[0710] HLA-A0201-binding motifs and the cleavage sites for proteasomes and immune-proteasomes using computer prediction models are known to those of skill in the art. For predicting MHC class I binding sites, such models include, but are not limited to, ProPred1 (described in more detail in Singh and Raghava, ProPred: prediction of HLA-DR binding sites. *BIOINFORMATICS* 17(12):1236-1237 2001), and SYFPEITHI (see Schuler et al. SYFPEITHI, Database for Searching and T-Cell Epitope Prediction. in *Immunoinformatics Methods in Molecular Biology*, vol 409(1): 75-93 2007)

[0711] In some embodiments, the TCR or antigen binding portion thereof may be a recombinantly produced natural protein or mutated form thereof in which one or more property, such as binding characteristic, has been altered. In some embodiments, a TCR may be derived from one of various animal species, such as human, mouse, rat, or other mammal. A TCR may be cell-bound or in soluble form. In some embodiments, for purposes of the provided methods, the TCR is in cell-bound form expressed on the surface of a cell.

[0712] In some embodiments, the TCR is a full-length TCR. In some embodiments, the TCR is an antigen-binding portion. In some embodiments, the TCR is a dimeric TCR (dTCR). In some embodiments, the TCR is a single-chain TCR (sc-TCR). In some embodiments, a dTCR or scTCR have the structures as described in WO 03/020763, WO 04/033685, WO2011/044186.

[0713] In some embodiments, the TCR contains a sequence corresponding to the transmembrane sequence. In some embodiments, the TCR does contain a sequence corresponding to cytoplasmic sequences. In some embodiments, the TCR is capable of forming a TCR complex with CD3. In some embodiments, any of the TCRs, including a dTCR or scTCR, can be linked to signaling domains that yield an active TCR on the surface of a T cell. In some embodiments, the TCR is expressed on the surface of cells.

[0714] In some embodiments a dTCR contains a first polypeptide wherein a sequence corresponding to a TCR α chain variable region sequence is fused to the N terminus of a sequence corresponding to a TCR α chain constant region extracellular sequence, and a second polypeptide wherein a sequence corresponding to a TCR β chain variable region sequence is fused to the N terminus a sequence corresponding to a TCR β chain constant region extracellular sequence, the first and second polypeptides being linked by a disulfide bond. In some embodiments, the bond can correspond to the native inter-chain disulfide bond present in native dimeric $\alpha\beta$ TCRs. In some embodiments, the interchain disulfide bonds are not present in a native TCR. For example, in some embodiments, one or more cysteines can be incorporated into the constant region extracellular sequences of dTCR polypeptide pair. In some cases, both a native and a non-native disulfide bond may be desirable. In some embodiments, the TCR contains a transmembrane sequence to anchor to the membrane.

[0715] In some embodiments, a dTCR contains a TCR α chain containing a variable α domain, a constant α domain and a first dimerization motif attached to the C-terminus of the constant α domain, and a TCR β chain comprising a variable β domain, a constant β domain and a first dimerization motif attached to the C-terminus of the constant β domain, wherein the first and second dimerization motifs easily interact to form a covalent bond between an amino acid in the first dimerization motif and an amino acid in the second dimerization motif linking the TCR α chain and TCR β chain together.

[0716] In some embodiments, the TCR is a scTCR. Typically, a scTCR can be generated using methods known to those of skill in the art, See e.g., Soo Hoo, W. F. et al. *PNAS (USA)* 89, 4759 (1992); Wülfing, C. and Plückthun, A., *J. Mol. Biol.* 242, 655 (1994); Kurucz, I. et al.

PNAS (USA) 90 3830 (1993); International published PCT Nos. WO 96/13593, WO 96/18105, WO99/60120, WO99/18129, WO 03/020763, WO2011/044186; and Schlueter, C. J. et al. *J. Mol. Biol.* 256, 859 (1996). In some embodiments, a scTCR contains an introduced non-native disulfide interchain bond to facilitate the association of the TCR chains (see e.g. International published PCT No. WO 03/020763). In some embodiments, a scTCR is a non-disulfide linked truncated TCR in which heterologous leucine zippers fused to the C-termini thereof facilitate chain association (see e.g. International published PCT No. WO99/60120). In some embodiments, a scTCR contain a TCR α variable domain covalently linked to a TCR β variable domain via a peptide linker (see e.g., International published PCT No. WO99/18129).

[0717] In some embodiments, a scTCR contains a first segment constituted by an amino acid sequence corresponding to a TCR α chain variable region, a second segment constituted by an amino acid sequence corresponding to a TCR β chain variable region sequence fused to the N terminus of an amino acid sequence corresponding to a TCR β chain constant domain extracellular sequence, and a linker sequence linking the C terminus of the first segment to the N terminus of the second segment.

[0718] In some embodiments, a scTCR contains a first segment constituted by an α chain variable region sequence fused to the N terminus of an α chain extracellular constant domain sequence, and a second segment constituted by a β chain variable region sequence fused to the N terminus of a sequence β chain extracellular constant and transmembrane sequence, and, optionally, a linker sequence linking the C terminus of the first segment to the N terminus of the second segment.

[0719] In some embodiments, a scTCR contains a first segment constituted by a TCR β chain variable region sequence fused to the N terminus of a β chain extracellular constant domain sequence, and a second segment constituted by an α chain variable region sequence fused to the N terminus of a sequence α chain extracellular constant and transmembrane sequence, and, optionally, a linker sequence linking the C terminus of the first segment to the N terminus of the second segment.

[0720] In some embodiments, the linker of a scTCRs that links the first and second TCR segments can be any linker capable of forming a single polypeptide strand, while retaining TCR binding specificity. In some embodiments, the linker sequence may, for example, have the formula -P-AA-P- wherein P is proline and AA represents an amino acid sequence wherein the amino acids are glycine and serine. In some embodiments, the first and second segments are paired so that the variable region sequences thereof are orientated for such binding. Hence, in

some cases, the linker has a sufficient length to span the distance between the C terminus of the first segment and the N terminus of the second segment, or vice versa, but is not too long to block or reduces bonding of the scTCR to the target ligand. In some embodiments, the linker can contain from or from about 10 to 45 amino acids, such as 10 to 30 amino acids or 26 to 41 amino acids residues, for example 29, 30, 31 or 32 amino acids. In some embodiments, the linker has the formula -PGGG-(SGGGG)₅-P- wherein P is proline, G is glycine and S is serine (SEQ ID NO: 22). In some embodiments, the linker has the sequence GSADDAKKDAAKKDGKS (SEQ ID NO: 23)

[0721] In some embodiments, the scTCR contains a covalent disulfide bond linking a residue of the immunoglobulin region of the constant domain of the α chain to a residue of the immunoglobulin region of the constant domain of the β chain. In some embodiments, the interchain disulfide bond in a native TCR is not present. For example, in some embodiments, one or more cysteines can be incorporated into the constant region extracellular sequences of the first and second segments of the scTCR polypeptide. In some cases, both a native and a non-native disulfide bond may be desirable.

[0722] In some embodiments of a dTCR or scTCR containing introduced interchain disulfide bonds, the native disulfide bonds are not present. In some embodiments, the one or more of the native cysteines forming a native interchain disulfide bonds are substituted to another residue, such as to a serine or alanine. In some embodiments, an introduced disulfide bond can be formed by mutating non-cysteine residues on the first and second segments to cysteine. Exemplary non-native disulfide bonds of a TCR are described in published International PCT No. WO2006/000830.

[0723] In some embodiments, the TCR or antigen-binding fragment thereof exhibits an affinity with an equilibrium binding constant for a target antigen of between or between about 10⁻⁵ and 10⁻¹² M and all individual values and ranges therein. In some embodiments, the target antigen is an MHC-peptide complex or ligand.

[0724] In some embodiments, nucleic acid or nucleic acids encoding a TCR, such as α and β chains, can be amplified by PCR, cloning or other suitable means and cloned into a suitable expression vector or vectors. The expression vector can be any suitable recombinant expression vector, and can be used to transform or transfect any suitable host. Suitable vectors include those designed for propagation and expansion or for expression or both, such as plasmids and viruses.

[0725] In some embodiments, the vector can a vector of the pUC series (Fermentas Life Sciences), the pBluescript series (Stratagene, LaJolla, Calif.), the pET series (Novagen,

Madison, Wis.), the pGEX series (Pharmacia Biotech, Uppsala, Sweden), or the pEX series (Clontech, Palo Alto, Calif.). In some cases, bacteriophage vectors, such as λ G10, λ GT11, λ ZapII (Stratagene), λ EMBL4, and λ NM1149, also can be used. In some embodiments, plant expression vectors can be used and include pBI01, pBI101.2, pBI101.3, pBI121 and pBIN19 (Clontech). In some embodiments, animal expression vectors include pEUK-C1, pMAM and pMAMneo (Clontech). In some embodiments, a viral vector is used, such as a retroviral vector.

[0726] In some embodiments, the recombinant expression vectors can be prepared using standard recombinant DNA techniques. In some embodiments, vectors can contain regulatory sequences, such as transcription and translation initiation and termination codons, which are specific to the type of host (e.g., bacterium, fungus, plant, or animal) into which the vector is to be introduced, as appropriate and taking into consideration whether the vector is DNA- or RNA-based. In some embodiments, the vector can contain a nonnative promoter operably linked to the nucleotide sequence encoding the TCR or antigen-binding portion (or other MHC-peptide binding molecule). In some embodiments, the promoter can be a non-viral promoter or a viral promoter, such as a cytomegalovirus (CMV) promoter, an SV40 promoter, an RSV promoter, and a promoter found in the long-terminal repeat of the murine stem cell virus. Other promoters known to a skilled artisan also are contemplated.

[0727] In some embodiments, after the T-cell clone is obtained, the TCR alpha and beta chains are isolated and cloned into a gene expression vector. In some embodiments, the TCR alpha and beta genes are linked via a picornavirus 2A ribosomal skip peptide so that both chains are coexpression. In some embodiments, genetic transfer of the TCR is accomplished via retroviral or lentiviral vectors, or via transposons (see, e.g., Baum et al. (2006) *Molecular Therapy: The Journal of the American Society of Gene Therapy*. 13:1050–1063; Frecha et al. (2010) *Molecular Therapy: The Journal of the American Society of Gene Therapy*. 18:1748–1757; and Hackett et al. (2010) *Molecular Therapy: The Journal of the American Society of Gene Therapy*. 18:674–683).

[0728] In some embodiments, to generate a vector encoding a TCR, the α and β chains are PCR amplified from total cDNA isolated from a T cell clone expressing the TCR of interest and cloned into an expression vector. In some embodiments, the α and β chains are cloned into the same vector. In some embodiments, the α and β chains are cloned into different vectors. In some embodiments, the generated α and β chains are incorporated into a retroviral, e.g. lentiviral, vector.

3. Chimeric Auto-Antibody Receptors (CAARs)

[0729] In some embodiments, the recombinant receptor is a chimeric autoantibody receptor (CAAR). In some embodiments, the CAAR is specific for an autoantibody. In some embodiments, a cell expressing the CAAR, such as a T cell engineered to express a CAAR, can be used to specifically bind to and kill autoantibody-expressing cells, but not normal antibody expressing cells. In some embodiments, CAAR-expressing cells can be used to treat an autoimmune disease associated with expression of self-antigens, such as autoimmune diseases. In some embodiments, CAAR-expressing cells can target B cells that ultimately produce the autoantibodies and display the autoantibodies on their cell surfaces, mark these B cells as disease-specific targets for therapeutic intervention. In some embodiments, CAAR-expressing cells can be used to efficiently targeting and killing the pathogenic B cells in autoimmune diseases by targeting the disease-causing B cells using an antigen-specific chimeric autoantibody receptor. In some embodiments, the recombinant receptor is a CAAR, such as any described in U.S. Patent Application Pub. No. US 2017/0051035.

[0730] In some embodiments, the CAAR comprises an autoantibody binding domain, a transmembrane domain, and an intracellular signaling region. In some embodiments, the intracellular signaling region comprises an intracellular signaling domain. In some embodiments, the intracellular signaling domain is or comprises a primary signaling domain, a signaling domain that is capable of inducing a primary activation signal in a T cell, a signaling domain of a T cell receptor (TCR) component, and/or a signaling domain comprising an immunoreceptor tyrosine-based activation motif (ITAM). In some embodiments, the intracellular signaling region comprises a secondary or costimulatory signaling region (secondary intracellular signaling regions).

[0731] In some embodiments, the autoantibody binding domain comprises an autoantigen or a fragment thereof. The choice of autoantigen can depend upon the type of autoantibody being targeted. For example, the autoantigen may be chosen because it recognizes an autoantibody on a target cell, such as a B cell, associated with a particular disease state, e.g. an autoimmune disease, such as an autoantibody-mediated autoimmune disease. In some embodiments, the autoimmune disease includes pemphigus vulgaris (PV). Exemplary autoantigens include desmoglein 1 (Dsg1) and Dsg3.

4. Multi-targeting

[0732] In some embodiments, the cells and methods include multi-targeting strategies, such

as expression of two or more genetically engineered receptors on the cell, each recognizing the same of a different antigen and typically each including a different intracellular signaling component. Such multi-targeting strategies are described, for example, in International Patent Application, Publication No.: WO 2014055668 A1 (describing combinations of activating and costimulatory CARs, e.g., targeting two different antigens present individually on off-target, e.g., normal cells, but present together only on cells of the disease or condition to be treated) and Fedorov et al., *Sci. Transl. Medicine*, 5(215) (2013) (describing cells expressing an activating and an inhibitory CAR, such as those in which the activating CAR binds to one antigen expressed on both normal or non-diseased cells and cells of the disease or condition to be treated, and the inhibitory CAR binds to another antigen expressed only on the normal cells or cells which it is not desired to treat).

[0733] For example, in some embodiments, the cells include a receptor expressing a first genetically engineered antigen receptor (e.g., CAR or TCR) which is capable of inducing an activating or stimulatory signal to the cell, generally upon specific binding to the antigen recognized by the first receptor, e.g., the first antigen. In some embodiments, the cell further includes a second genetically engineered antigen receptor (e.g., CAR or TCR), e.g., a chimeric costimulatory receptor, which is capable of inducing a costimulatory signal to the immune cell, generally upon specific binding to a second antigen recognized by the second receptor. In some embodiments, the first antigen and second antigen are the same. In some embodiments, the first antigen and second antigen are different.

[0734] In some embodiments, the first and/or second genetically engineered antigen receptor (e.g. CAR or TCR) is capable of inducing an activating signal to the cell. In some embodiments, the receptor includes an intracellular signaling component containing ITAM or ITAM-like motifs. In some embodiments, the activation induced by the first receptor involves a signal transduction or change in protein expression in the cell resulting in initiation of an immune response, such as ITAM phosphorylation and/or initiation of ITAM-mediated signal transduction cascade, formation of an immunological synapse and/or clustering of molecules near the bound receptor (e.g. CD4 or CD8, etc.), activation of one or more transcription factors, such as NF- κ B and/or AP-1, and/or induction of gene expression of factors such as cytokines, proliferation, and/or survival.

[0735] In some embodiments, the first and/or second receptor includes intracellular signaling domains or regions of costimulatory receptors such as CD28, CD137 (4-1BB), OX40, and/or ICOS. In some embodiments, the first and second receptor include an intracellular

signaling domain of a costimulatory receptor that are different. In one embodiment, the first receptor contains a CD28 costimulatory signaling region and the second receptor contain a 4-1BB co-stimulatory signaling region or vice versa.

[0736] In some embodiments, the first and/or second receptor includes both an intracellular signaling domain containing ITAM or ITAM-like motifs and an intracellular signaling domain of a costimulatory receptor.

[0737] In some embodiments, the first receptor contains an intracellular signaling domain containing ITAM or ITAM-like motifs and the second receptor contains an intracellular signaling domain of a costimulatory receptor. The costimulatory signal in combination with the activating signal induced in the same cell is one that results in an immune response, such as a robust and sustained immune response, such as increased gene expression, secretion of cytokines and other factors, and T cell mediated effector functions such as cell killing.

[0738] In some embodiments, neither ligation of the first receptor alone nor ligation of the second receptor alone induces a robust immune response. In some aspects, if only one receptor is ligated, the cell becomes tolerized or unresponsive to antigen, or inhibited, and/or is not induced to proliferate or secrete factors or carry out effector functions. In some such embodiments, however, when the plurality of receptors are ligated, such as upon encounter of a cell expressing the first and second antigens, a desired response is achieved, such as full immune activation or stimulation, e.g., as indicated by secretion of one or more cytokine, proliferation, persistence, and/or carrying out an immune effector function such as cytotoxic killing of a target cell.

[0739] In some embodiments, the two receptors induce, respectively, an activating and an inhibitory signal to the cell, such that binding by one of the receptor to its antigen activates the cell or induces a response, but binding by 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 or iCARs. Such a strategy may be used, for example, 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.

[0740] In some embodiments, the multi-targeting strategy is employed in a case where an antigen associated with a particular disease or condition is expressed on a non-diseased cell and/or is expressed on the engineered cell itself, either transiently (e.g., upon stimulation in association with genetic engineering) or permanently. In such cases, by requiring ligation of

two separate and individually specific antigen receptors, specificity, selectivity, and/or efficacy may be improved.

[0741] In some embodiments, the plurality of antigens, e.g., the first and second antigens, are expressed on the cell, tissue, or disease or condition being targeted, such as on the cancer cell. In some aspects, the cell, tissue, disease or condition is multiple myeloma or a multiple myeloma cell. In some embodiments, one or more of the plurality of antigens generally also is expressed on a cell which it is not desired to target with the cell therapy, such as a normal or non-diseased cell or tissue, and/or the engineered cells themselves. In such embodiments, by requiring ligation of multiple receptors to achieve a response of the cell, specificity and/or efficacy is achieved.

B. Vectors and Methods for Genetic Engineering

[0742] Various methods for the introduction of genetically engineered components, e.g., recombinant receptors, e.g., CARs or TCRs, are well known and may be used with the provided methods and compositions. Exemplary methods include those for transfer of nucleic acids encoding the receptors, including via viral, e.g., retroviral or lentiviral, transduction, transposons, and electroporation.

[0743] In some embodiments, gene transfer is accomplished by first stimulating the cell, 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.

[0744] 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. Gene Therapy doi: 10.1038/gt.2014.25 (2014); Carlens et al. *Exp Hematol.*, 28(10): 1137-46 (2000); Alonso-Camino et al. *Mol Ther Nucl Acids*, 2, e93 (2013); Park et al., *Trends Biotechnol.*, November 29(11): 550–557 (2011).

[0745] 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, *BioTechniques*, 7:980-990 (1989); Miller, A. D. *Human Gene Therapy*, 1:5-14 (1990); Scarpa et al. *Virology*, 180:849-852 (1991); Burns et al. *Proc. Natl. Acad. Sci. USA*, 90:8033-8037 (1993); and Boris-Lawrie and Temin, *Cur. Opin. Genet. Develop.*, 3:102-109 (1993).

[0746] Methods of lentiviral transduction are known. Exemplary methods are described in, e.g., Wang et al., *J. Immunother.*, 35(9): 689-701 (2012); Cooper et al. *Blood*. 101:1637-1644 (2003); Verhoeven et al., *Methods Mol Biol.*, 506: 97-114 (2009); and Cavalieri et al., *Blood.*, 102(2): 497-505 (2003).

[0747] In some embodiments, recombinant nucleic acids are transferred into T cells via electroporation (see, e.g., Chicaybam et al, *PLoS ONE* 8(3): e60298 (2013) and Van Tedeloo et al. *Gene Therapy* 7(16): 1431-1437 (2000)). In some embodiments, recombinant nucleic acids are transferred into T cells via transposition (see, e.g., Manuri et al. *Hum Gene Ther* 21(4): 427-437 (2010); Sharma et al. *Molec Ther Nucl Acids* 2, e74 (2013); and Huang et al. *Methods Mol Biol* 506: 115-126 (2009)). 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)).

[0748] 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.

[0749] In some embodiments, the cells, e.g., T cells, may be transfected either during or after expansion e.g. with a T cell receptor (TCR) or 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 CD3/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., *Methods Mol Biol.* 907:645-66 (2012); or Barrett et al., *Chimeric Antigen Receptor Therapy for Cancer Annual Review of Medicine*, Vol. 65: 333-347 (2014).

[0750] 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.

[0751] In some aspects, the cells further are engineered to promote expression of cytokines or other factors. 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.

[0752] In some embodiments, 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 molecule involved in modulating a metabolic pathway 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 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), Thosa 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.

C. Cells and Preparation of Cells for Genetic Engineering

[0753] Among the cells expressing the receptors and administered by the provided methods are engineered cells. The genetic engineering generally involves introduction of a nucleic acid encoding the recombinant or engineered component into a composition containing the cells, such as by retroviral transduction, transfection, or transformation.

[0754] In some embodiments, the nucleic acids are heterologous, *i.e.*, normally not present in a cell or sample obtained from the cell, such as one obtained from another organism or cell, which for example, is not ordinarily found in the cell being engineered and/or an organism from which such cell is derived. In some embodiments, the nucleic acids are not naturally occurring, such as a nucleic acid not found in nature, including one comprising chimeric combinations of nucleic acids encoding various domains from multiple different cell types.

[0755] The cells generally are eukaryotic cells, such as mammalian cells, and typically are human cells. In some embodiments, the cells are derived from the blood, bone marrow, lymph, or lymphoid organs, are cells of the immune system, such as cells of the innate or adaptive immunity, *e.g.*, myeloid or lymphoid cells, including lymphocytes, typically T cells and/or NK cells. Other exemplary cells include stem cells, such as multipotent and pluripotent stem cells, including induced pluripotent stem cells (iPSCs). The cells typically are primary cells, such as those isolated directly from a subject and/or isolated from a subject and frozen. In some embodiments, the cells include one or more subsets of T cells or other cell types, such as whole T cell populations, CD4+ cells, CD8+ cells, and subpopulations thereof, such as those defined by function, activation state, maturity, potential for differentiation, expansion, recirculation, localization, and/or persistence capacities, antigen-specificity, type of antigen receptor, presence in a particular organ or compartment, marker or cytokine secretion profile, and/or degree of differentiation. With reference to the subject to be treated, the cells may be allogeneic and/or autologous. Among the methods include off-the-shelf methods. In some aspects, such as for off-the-shelf technologies, the cells are pluripotent and/or multipotent, such as stem cells, such as induced pluripotent stem cells (iPSCs). In some embodiments, the methods include isolating cells from the subject, preparing, processing, culturing, and/or engineering them, and re-

introducing them into the same subject, before or after cryopreservation.

[0756] Among the sub-types and subpopulations of T cells and/or of CD4+ and/or of CD8+ T cells are naïve T (T_N) cells, effector T cells (T_{EFF}), memory T cells and sub-types thereof, such as stem cell memory T (T_{SCM}), central memory T (T_{CM}), effector memory T (T_{EM}), or terminally differentiated effector memory T cells, tumor-infiltrating lymphocytes (TIL), immature T cells, mature T cells, helper T cells, cytotoxic T cells, mucosa-associated invariant T (MAIT) cells, naturally occurring and adaptive regulatory T (Treg) cells, helper T cells, such as TH1 cells, TH2 cells, TH3 cells, TH17 cells, TH9 cells, TH22 cells, follicular helper T cells, alpha/beta T cells, and delta/gamma T cells.

[0757] In some embodiments, the cells are natural killer (NK) cells. In some embodiments, the cells are monocytes or granulocytes, e.g., myeloid cells, macrophages, neutrophils, dendritic cells, mast cells, eosinophils, and/or basophils.

[0758] 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, the nucleic acids are heterologous, i.e., normally not present in a cell or sample obtained from the cell, such as one obtained from another organism or cell, which for example, is not ordinarily found in the cell being engineered and/or an organism from which such cell is derived. In some embodiments, the nucleic acids are not naturally occurring, such as a nucleic acid not found in nature, including one comprising chimeric combinations of nucleic acids encoding various domains from multiple different cell types.

[0759] 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.

[0760] 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.

[0761] In some aspects, the sample from which the cells are derived or isolated is blood or a blood-derived sample, or is 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.

[0762] In some embodiments, the cells are derived from cell lines, e.g., T cell lines. The cells in some embodiments are obtained from a xenogeneic source, for example, from mouse, rat, non-human primate, and pig.

[0763] In some embodiments, isolation of the cells 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.

[0764] 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.

[0765] 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, Ca⁺⁺/Mg⁺⁺ free PBS. In certain embodiments, components of a blood cell sample are removed and the cells directly resuspended in culture media.

[0766] 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.

[0767] 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.

[0768] 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.

[0769] 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.

[0770] 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.

[0771] 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.

[0772] 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).

[0773] 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.

[0774] 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.

[0775] 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. *Blood*.1:72–82 (2012); Wang et al. *J Immunother.* 35(9):689-701 (2012). In some embodiments, combining TCM-enriched CD8+ T cells and CD4+ T cells further enhances efficacy.

[0776] 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.

[0777] 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 TCM 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 (TCM) 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.

[0778] 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.

[0779] 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-

[0780] 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. 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).

[0781] 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.

[0782] 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.

[0783] 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.

[0784] 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.

[0785] 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.

[0786] 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.

[0787] In some embodiments, the affinity-based selection is via magnetic-activated cell sorting (MACS) (Miltenyi Biotech, 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.

[0788] 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.

[0789] 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.

[0790] 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.

[0791] 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.

[0792] 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. *J Immunother.* 35(9): 651–660 (2012), Terakura et al. *Blood*. 1:72–82 (2012), and Wang et al. *J Immunother.* 35(9):689-701 (2012).

[0793] 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. *Lab Chip* 10, 1567-1573 (2010); and Godin et al. *J Biophoton.* 1(5):355–376 (2008). In both cases, cells can be labeled with multiple markers, allowing for the isolation of well-defined T cell subsets at high purity.

[0794] 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.

[0795] In some embodiments, the preparation methods include steps for freezing, e.g., cryopreserving, the cells, either before or after isolation, incubation, and/or engineering. In some embodiments, the freeze and subsequent thaw step removes granulocytes and, to some extent, monocytes in the cell population. In some embodiments, the cells are suspended in a freezing solution, e.g., following a washing step to remove plasma and platelets. Any of a variety of known freezing solutions and parameters in some aspects may be used. One example involves using PBS containing 20% DMSO and 8% human serum albumin (HSA), or other suitable cell freezing media. This is then diluted 1:1 with media so that the final concentration of DMSO and HSA are 10% and 4%, respectively. The cells are generally then frozen to -80°C . at a rate of 1° per minute and stored in the vapor phase of a liquid nitrogen storage tank.

[0796] In some embodiments, the cells are incubated and/or cultured prior to or in

connection with genetic engineering. The incubation steps can include culture, cultivation, 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 or cultivating cells. 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.

[0797] 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.

[0798] 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.

[0799] 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. *J Immunother.* 35(9): 651–660 (2012), Terakura et al. *Blood*.1:72–82 (2012), and/or Wang et al. *J Immunother.* 35(9):689-701 (2012).

[0800] 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.

[0801] 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, and generally at or about 37 degrees Celsius. Optionally, 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.

[0802] 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.

VII. COMPOSITIONS AND FORMULATIONS

[0803] In some embodiments, the cell therapy is provided as a composition or formulation, such as a pharmaceutical composition or formulation. Such compositions can be used in accord with the provided methods, such as in the prevention or treatment of diseases, conditions, and disorders, or in detection, diagnostic, and prognostic methods.

[0804] The term “pharmaceutical formulation” refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[0805] 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.

[0806] In some embodiments, the T cell therapy, such as engineered T cells (*e.g.* CAR T cells), are formulated with a pharmaceutically acceptable carrier. 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 Sciences 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 dextrins; 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).

[0807] 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 & Wilkins; 21st ed. (May 1, 2005).

[0808] 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 prevented or treated with the cells, including one or more active ingredients where the activities are complementary to the cells and/or 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, vincristine, etc.

[0809] The pharmaceutical composition in some embodiments contain 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.

[0810] The cells 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), it will generally be formulated in a unit dosage injectable form (solution, suspension, emulsion).

[0811] 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.

[0812] 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.

[0813] Sterile injectable solutions can be prepared by incorporating the 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. The compositions can also be lyophilized. The compositions can contain auxiliary substances such as wetting, dispersing, or emulsifying agents (e.g., methylcellulose), pH buffering agents, gelling or viscosity enhancing additives, preservatives, flavoring agents, colors, and the like, depending upon the route of administration and the preparation desired. Standard texts may in some aspects be consulted to prepare suitable preparations.

[0814] 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, sorbic acid, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0815] 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.

[0816] 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.

VIII. KITS AND ARTICLES OF MANUFACTURE

[0817] Also provided are articles of manufacture or kit containing the provided genetically engineered cells, and one or more agents for modulating the expansion, proliferation and/or

activity of the engineered cells, and/or a further therapeutic agent and/or compositions comprising the same, optionally reagents for assessing and/or measuring one or more parameters, e.g., pharmacokinetic parameters and/or patient attributes and/or expression of biomarkers, and optionally instructions for use, for example, instructions for administering and/or assessment, according to the provided methods. The articles of manufacture may include a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, test tubes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. In some embodiments, the container has a sterile access port. Exemplary containers include an intravenous solution bags, vials, including those with stoppers pierceable by a needle for injection.

[0818] The articles of manufacture provided herein contain packaging materials. Packaging materials for use in packaging the provided materials are well known to those of skill in the art. See, for example, U.S. Patent Nos. 5,323,907, 5,052,558 and 5,033,252, each of which is incorporated herein in its entirety. Examples of packaging materials include, but are not limited to, blister packs, bottles, tubes, inhalers, pumps, bags, vials, containers, syringes, disposable laboratory supplies, e.g., pipette tips and/or plastic plates, or bottles. The articles of manufacture or kits can include a device so as to facilitate dispensing of the materials or to facilitate use in a high-throughput or large-scale manner, e.g., to facilitate use in robotic equipment. Typically, the packaging is non-reactive with the compositions contained therein.

[0819] The article of manufacture or kit may further include a package insert indicating that the compositions can be used to treat a particular condition such as a condition described herein (e.g., multiple myeloma). Alternatively, or additionally, the article of manufacture or kit may further include another or the same container comprising a pharmaceutically-acceptable buffer. It may further include other materials such as other buffers, diluents, filters, needles, and/or syringes.

[0820] In some embodiments, the articles of manufacture or kits include one or more containers, typically a plurality of containers, packaging material, and a label or package insert on or associated with the container or containers and/or packaging, generally including instructions for use, e.g., instructions for nucleic acid assembly and/or introduction of the assembled nucleic acid molecules or sets of nucleic acid molecules into of cells, such as transfection or transduction of cells used in the provided methods, such as T cells, T cell lines and/or T cell compositions.

[0821] The container in some embodiments holds a composition which is by itself or

combined with another composition containing one or more agent(s) capable of modulating the expansion, proliferation and/or activity of the engineered cells, such as any described herein. The article of manufacture or kit may include one or more containers with a composition contained therein, wherein the composition includes one or more agent(s) capable of modulating the expansion, proliferation and/or activity of the engineered cells, such as any described herein; wherein the composition optionally includes a further therapeutic agent, and which article or kit further comprises instructions on the label or package insert for treating the subject in an effective amount.

[0822] 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 can be included as a label or package insert accompanying the compositions for administration.

[0823] In some embodiments, the articles of manufacture and/or kits further include one or more reagents for assaying biological samples, e.g., biological samples from subjects who are candidates for administration or who have been administered the therapy, and optionally instructions for use of the reagents or assays, e.g., assessment of one or more parameters, e.g., pharmacokinetic parameters and/or patient attributes and/or expression of biomarkers, and optionally instructions for use, for example, instructions for administering and/or assessment. In some embodiments, the biological sample is or is obtained from a blood, plasma or serum sample.

[0824] In some embodiments, the reagents can be used prior to the administration of the cell therapy or after the administration of cell therapy. For example, in some embodiments, the article of manufacture and/or kits further contain reagents for measuring the level of particular patient attributes and/or inflammatory markers, that are associated with certain pharmacokinetic parameters, response outcome and/or toxicity, and instructions for measuring. In some embodiments, the reagents include components for performing an *in vitro* assay to measure the parameters, such as an immunoassay, an aptamer-based assay, a histological or cytological assay, or an mRNA expression level assay. In some embodiments, the *in vitro* assay is selected from among an enzyme linked immunosorbent assay (ELISA), immunoblotting, immunoprecipitation, radioimmunoassay (RIA), immunostaining, flow cytometry assay, surface plasmon resonance (SPR), chemiluminescence assay, lateral flow immunoassay, inhibition assay and avidity assay. In some aspects, the reagent is a binding reagent that specifically binds the biomarkers. In some cases, the binding reagent is an antibody or antigen-binding fragment

thereof, an aptamer or a nucleic acid probe. In some embodiments, the article of manufacture contains any reagents described herein for assessing the parameters.

[0825] In some embodiments, the articles of manufacture and/or kits comprise one or more reagent capable of detecting one or more parameters, e.g., pharmacokinetic parameters and/or patient attributes and/or expression of biomarkers, for example, instructions for administering and/or assessment, and instructions for using the reagent to assay a biological sample from a subject that is a candidate for treatment, wherein the one or more parameters is selected from among the number or level of CAR+ cells, e.g., CD3+, CD4+ and/or CD8+ CAR+ cells, maximum (peak) plasma concentration (C_{\max}) of CAR+ cells, e.g., CD3+, CD4+ and/or CD8+ CAR+ cells, the peak time (*i.e.* when maximum plasma concentration (C_{\max}) occurs; T_{\max}), the minimum plasma concentration (*i.e.* the minimum plasma concentration between doses of a therapeutic agent, e.g., CAR+ T cells; C_{\min}), the elimination half-life ($T_{1/2}$) and area under the curve (*i.e.* the area under the curve generated by plotting time versus plasma concentration of the therapeutic agent CAR+ T cells; AUC), volumetric measurements of a tumor, e.g., the sum of the products of diameters (SPD), longest tumor diameters (LD), sum of longest tumor diameters (SLD), necrosis, tumor volume, necrosis volume, necrosis-tumor ratio (NTR), peritumoral edema (PTE), and edema-tumor ratio (ETR), erythrocyte sedimentation rate (ESR), albumin, $\beta 2$ microglobulin ($\beta 2$ -M), C-C Motif Chemokine Ligand 13 (CCL13), C-reactive protein (CRP), C-X-C motif chemokine 10 (CXCL10), IL-2, IL-5, IL-6, IL-7, IL-8, IL-10, IL-15, IL-16, interferon gamma ($\text{IFN-}\gamma$), Lymphotoxin-alpha ($\text{LT-}\alpha$), Monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein 1 alpha ($\text{MIP-1}\alpha$), $\text{MIP-1}\beta$, Serum Amyloid A1 (SAA-1), Transforming growth factor beta ($\text{TGF-}\beta$) and tumor necrosis factor alpha ($\text{TNF-}\alpha$). In some embodiments, instructions for assaying presence or absence, level, amount, or concentration of an parameter in the subject compared to a threshold level of the analyte and/or parameters is also included.

IX. DEFINITIONS

[0826] 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.

[0827] 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 immunomodulatory polypeptides, engineered cells, 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.

[0828] 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.

[0829] 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 cancer). This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease. For example, a late stage cancer, such as development of metastasis, may be delayed.

[0830] “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.

[0831] 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 tumor growth reduce the rate of growth of the tumor compared to the rate of growth of the tumor in the absence of the cells.

[0832] 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.

[0833] A “therapeutically effective amount” of an agent, e.g., a pharmaceutical formulation or engineered 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 immunomodulatory polypeptides or engineered cells administered. In some embodiments, the provided methods involve administering the immunomodulatory polypeptides, engineered cells, or compositions at effective amounts, e.g., therapeutically effective amounts.

[0834] 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.

[0835] The term “pharmaceutical formulation” refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[0836] 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.

[0837] As used herein, recitation that nucleotides or amino acid positions “correspond to” nucleotides or amino acid positions in a disclosed sequence, such as set forth in the Sequence listing, refers to nucleotides or amino acid positions identified upon alignment with the disclosed sequence to maximize identity using a standard alignment algorithm, such as the GAP algorithm. By aligning the sequences, one skilled in the art can identify corresponding residues, for example, using conserved and identical amino acid residues as guides. In general, to identify corresponding positions, the sequences of amino acids are aligned so that the highest order match is obtained (see, e.g. : Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I,

Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; Carrillo et al. (1988) SIAM J Applied Math 48: 1073).

[0838] 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.” Among the vectors are viral vectors, such as retroviral, e.g., gammaretroviral and lentiviral vectors.

[0839] The terms “host cell,” “host cell line,” and “host cell culture” are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include “transformants” and “transformed cells,” which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

[0840] 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 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.

[0841] 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 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.

[0842] As used herein, “percent (%) amino acid sequence identity” and “percent identity” when used with respect to an amino acid sequence (reference polypeptide sequence) is defined as the percentage of amino acid residues in a candidate sequence (e.g., the subject antibody or fragment) that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0843] 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.” It is understood that aspects and variations described herein include “consisting” and/or “consisting essentially of” aspects and variations.

[0844] 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.

[0845] 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. For example, description referring to “about X” includes description of “X”.

[0846] 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.

[0847] 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.

[0848] The section heading used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

X. EXEMPLARY EMBODIMENTS

[0849] Among the embodiments provided herein are:

1. A method of treatment, the method comprising:
 - (a) administering, to a subject having a disease or condition, a dose of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR) for treating the disease or condition;
 - (b) after administering the dose of genetically engineered cells, monitoring CAR+ T cells in the blood of the subject to assess if the cells are within a therapeutic range, and
 - (c) if the genetically engineered cells are not within the therapeutic range, administering an agent to the subject capable of modulating, optionally increasing or decreasing, CAR+ T cell expansion or proliferation, in the subject,wherein the therapeutic range is:
 - (i) based upon the range of peak CD3+ CAR+ T cells, or a CD8+CAR+ T cell subset thereof, in the blood among one or more subjects previously treated with the genetically engineered cells that is associated with an estimated probability of response of greater than or greater than about 65% and an estimated probability of a toxicity of less than or about 30%; or

(ii) peak CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 10 cells per microliter and 500 cells per microliter; or

(iii) peak CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 2 cells per microliter and 200 cells per microliter.

2. A method of treatment, the method comprising:

(a) monitoring, in the blood of a subject, the presence of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR) to assess if the cells are within a therapeutic range, wherein the subject has been previously administered a dose of the genetically engineered cells for treating a disease or condition; and

(c) if the genetically engineered cells are not within the therapeutic range, administering an agent to the subject capable of modulating, optionally increasing or decreasing, CAR+ T cell expansion or proliferation, in the subject,

wherein the therapeutic range is:

(i) based upon the range of peak CD3+ CAR+ T cells, or a CD8+CAR+ T cell subset thereof, in the blood among one or more subjects previously treated with the genetically engineered cells that is associated with an estimated probability of response of greater than or greater than about 65% and an estimated probability of a toxicity of less than or about 30%; or

(ii) peak CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 10 cells per microliter and 500 cells per microliter; or

(iii) peak CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 2 cells per microliter and 200 cells per microliter.

3. The method of embodiment 1 or embodiment 2, wherein if the peak number of CAR+ T cells in the blood of the subject is less than the lowest number of peak CAR+ T cells in the therapeutic range, an agent is administered to the subject that is capable of increasing CAR+ T cell expansion or proliferation.

4. The method of embodiment 3, wherein the agent is capable of CAR-specific expansion.

5. The method of embodiment 4, wherein the agent is an anti-idiotypic antibody or antigen-binding fragment thereof specific to the CAR, an immune checkpoint inhibitor, a

modulator of a metabolic pathway, an adenosine receptor antagonist, a kinase inhibitor, an anti-TGF β antibody or an anti-TGF β R antibody or a cytokine.

6. The method of embodiment 1 or embodiment 2, wherein if the peak number of CAR+ T cells in the blood of the subject is greater than the highest number of peak CAR+ T cells in the therapeutic range, an agent is administered to the subject that is capable of decreasing CAR+ T cell expansion or proliferation.

7. The method of embodiment 6, wherein the agent is a steroid.

8. The method of embodiment 7, wherein the steroid is a corticosteroid.

9. The method of embodiment 7 or embodiment 8, wherein the steroid is dexamethasone or methylprednisolone.

10. The method of any of embodiments 7-9, wherein the steroid is administered in an amount that is between or between about 1.0 mg and about 40 mg, between or between about 1.0 mg and about 20 mg, between or between about 2.0 mg and about 20 mg, between or between about 5.0 mg and about 25.0 mg, between or between about 10 mg and about 20 mg dexamethasone or equivalent thereof, each inclusive.

11. The method of any of embodiments 7-10, wherein the steroid is administered in multiple doses over a period of at or more than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more or within a range defined by any of the foregoing.

12. The method of any of embodiments 7-11, wherein the steroid is administered once per day, twice per day, or three times or more per day.

13. The method of any of embodiments 7-12, wherein the steroid is administered in an amount that is between or between about 1.0 mg and about 80 mg, between or between about 1.0 mg and about 60 mg, between or between about 1.0 mg and about 40 mg, between or between about 1.0 mg and about 20 mg, between or between about 1.0 mg and about 10 mg, between or between about 2.0 mg and about 80 mg, between or between about 2.0 mg and about 60 mg, between or between about 2.0 mg and about 40 mg, between or between about 2.0 mg and about 20 mg, between or between about 2.0 mg and about 10 mg, between or between about 5.0 mg and about 80 mg, between or between about 5.0 mg and about 60 mg, between or between about 5.0 mg and about 40 mg, between or between about 5.0 mg and about 20 mg, between or between about 5.0 mg and about 10 mg, between or between about 10 mg and about 80 mg, between or between about 10 mg and about 60 mg, between or between about 10 mg and about 40 mg, between or between about 10 mg and about 20 mg dexamethasone or equivalent

thereof, each inclusive, per day or per 24 hours, or about 10 mg, 20 mg, 40 mg or 80 mg dexamethasone or equivalent thereof, per day or per 24 hours.

14. The method of any of embodiments 1-13, wherein the subject is monitored for CAR+ T cells in the blood at a time that is at least 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days or 21 days after initiation of administration of the genetically engineered cells.

15. The method of any of embodiments 1-14, wherein the subject is monitored for CAR+ T cells in the blood at a time that is between or between about 11 to 22 days, 12 to 18 days or 14 to 16 days, each inclusive, after initiation of administration of the genetically engineered cells.

16. The method of any of embodiment 1-15, wherein the agent is administered at a time that is greater than or greater than about 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days or 21 days after initiation of administration of the genetically engineered cells.

17. The method of any of embodiments 1-16, wherein the agent is administered at a time that is between or between about 11 to 22 days, 12 to 18 days or 14 to 16 days, each inclusive, after initiation of administration of the genetically engineered cells.

18. A method of modulating activity of engineered cells, the method comprising:

(a) selecting a subject in which the level, amount or concentration of a volumetric measure of tumor burden or an inflammatory marker in a sample from the subject is at or above a threshold level, wherein the sample does not comprise genetically engineered T cells expressing a chimeric antigen receptor (CAR) and/or is obtained from the subject prior to receiving administration of genetically engineered T cells expressing a CAR; and

(b) administering to the selected subject an agent that is capable of decreasing expansion or proliferation of genetically engineered T cells expressing a CAR.

19. A method of modulating activity of engineered cells, the method comprising administering to a subject an agent that is capable of decreasing expansion or proliferation of genetically engineered T cells expressing a chimeric antigen receptor (CAR) in a subject, wherein the subject is one in which the level, amount or concentration of a volumetric measure of tumor burden or an inflammatory marker in a sample from the subject is at or above a threshold level.

20. The method of embodiment 18 or embodiment 19, wherein the agent is administered prior to or concurrently with initiation of administration of a dose of genetically engineered cells comprising T cells expressing a chimeric antigen receptor.

21. The method of embodiment 20, wherein the method further comprises administering a dose of the genetically engineered cells.

22. The method of any of embodiments 18-21, wherein the subject has a disease or condition and the genetically engineered cells are for treating the disease or condition.

23. The method of any of embodiments 18-22, wherein, prior to administering the agent, the selected subject is at risk of developing a toxicity following administration of the genetically engineered cells.

24. The method of any of embodiments 17-23, wherein the administration of the agent is sufficient to achieve peak CAR⁺ T cells in a therapeutic range in the subject, or in a majority of selected subjects so treated by the method or in greater than 75% of the selected subjects so treated by the method.

25. The method of embodiment 24, wherein the therapeutic range is:

(i) based upon the range of peak CD3⁺ CAR⁺ T cells, or a CD8⁺CAR⁺ T cell subset thereof, in the blood among one or more subjects previously treated with the genetically engineered cells that is associated with an estimated probability of response of greater than or greater than about 65% and an estimated probability of a toxicity of less than or about 30%; or

(ii) peak CD3⁺CAR⁺ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 10 cells per microliter and 500 cells per microliter; or

(iii) peak CD8⁺CAR⁺ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 2 cells per microliter and 200 cells per microliter.

26. The method of any of embodiments 18-25, wherein a volumetric measure of tumor burden is measured and the volumetric measure is a sum of the products of diameters (SPD), longest tumor diameters (LD), sum of longest tumor diameters (SLD), tumor volume, necrosis volume, necrosis-tumor ratio (NTR), peritumoral edema (PTE), and edema-tumor ratio (ETR).

27. The method of any of embodiments 18-26, wherein the volumetric measure is a sum of the products of diameter (SPD).

28. The method of any of embodiments 18-27, wherein the volumetric measure is measured using computed tomography (CT), positron emission tomography (PET), and/or magnetic resonance imaging (MRI) of the subject.

29. The method of any of embodiments 18-25, wherein an inflammatory marker in a sample from the subject is measured and the inflammatory marker is C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), albumin, ferritin, β 2 microglobulin (β 2-M), lactate dehydrogenase (LDH), a cytokine or a chemokine.

30. The method of any of embodiments 18-25 and 29, wherein the inflammatory marker is LDH.

31. The method of any of embodiments 18-25 and 29, wherein the inflammatory marker is a cytokine or a chemokine that is IL-7, IL15, MIP-1alpha or TNF-alpha.

32. The method of any of embodiments 18-25, 29 and 31, wherein the cytokine or chemokine is associated with macrophage or monocyte activation.

33. The method of any of embodiments 18-25 and 29-32, wherein the sample is or comprises a blood sample, plasma sample, or serum sample.

34. The method of any of embodiments 18-25 and 29-33, wherein the inflammatory marker is assessed using a colorimetric assay or an immunoassay.

35. The method of embodiment 34, wherein the inflammatory marker is assessed using an immunoassay and the immunoassay is selected from enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), radioimmunoassay (RIA), surface plasmon resonance (SPR), Western Blot, Lateral flow assay, immunohistochemistry, protein array or immuno-PCR (iPCR).

36. The method of any of embodiments 18-35, wherein the threshold value is a value that:

i) is within 25%, within 20%, within 15%, within 10%, or within 5% above the average value of the volumetric measure or inflammatory marker and/or is within a standard deviation above the average value of the volumetric measure or the inflammatory marker in a plurality of control subjects;

ii) is above the highest value of the volumetric measure or inflammatory marker, optionally within 50%, within 25%, within 20%, within 15%, within 10%, or within 5% above such highest fold change, measured in at least one subject from among a plurality of control subjects; and/or

iii) is above the highest value of the volumetric measure or inflammatory marker as measured among more than 75%, 80%, 85%, 90%, or 95%, or 98% of subjects from a plurality of control subjects.

37. The method of embodiment 36, wherein the plurality of control subjects are a group of subjects prior to receiving a dose of the genetically engineered cells, wherein:

each of the control subjects of the group exhibited a peak CAR+ T cells in the blood greater than the highest peak CAR+ T cells in the therapeutic range;

each of the control subjects of the group went on to develop at toxicity, optionally a neurotoxicity or cytokine release syndrome (CRS), a grade 2 or grade 3 or higher neurotoxicity or a grade 3 or higher CRS, after receiving a dose of the engineered cells for treating the same disease or condition;

each of the control subjects of the group did not develop a response, optionally a complete response (CR) or partial response (PR), following administration of the dose of genetically engineered cells; and/or

each of the control subjects of the group did not develop a durable response, optionally for at or about or greater than or about 3 months or at or about or greater than or about 6 months, following administration of the dose of genetically engineered cells.

38. The method of any of embodiments 18-37, wherein the volumetric measure is SPD and the threshold value is or is about 30 cm^2 , is or is about 40 cm^2 , is or is about 50 cm^2 , is or is about 60 cm^2 , or is or is about 70 cm^2 .

39. The method of any of embodiments 18-38, wherein the inflammatory marker is LDH and the threshold value is or is about 300 units per liter, is or is about 400 units per liter, is or is about 500 units per liter or is or is about 600 units per liter.

40. The method of any of embodiments 18-39, wherein the agent is a steroid.

41. The method of embodiment 40, wherein the steroid is a corticosteroid.

42. The method of embodiment 40 or embodiment 41, wherein the steroid is dexamethasone or methylprednisolone.

43. The method of any of embodiments 40-42, wherein the steroid is administered in an amount that is between or between about 1.0 mg and about 40 mg, between or between about 1.0 mg and about 20 mg, between or between about 2.0 mg and about 20 mg, between or between about 5.0 mg and about 25.0 mg, between or between about 10 mg and about 20 mg dexamethasone or equivalent thereof, each inclusive.

44. The method of any of embodiments 40-43, wherein the steroid is administered in multiple doses over a period of at or more than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more or within a range defined by any of the foregoing.

45. The method of any of embodiments 40-44, wherein the steroid is administered once per day, twice per day, or three times or more per day.

46. The method of any of embodiments 40-45, wherein the steroid is administered in an amount that is between or between about 1.0 mg and about 80 mg, between or between about 1.0 mg and about 60 mg, between or between about 1.0 mg and about 40 mg, between or between about 1.0 mg and about 20 mg, between or between about 1.0 mg and about 10 mg, between or between about 2.0 mg and about 80 mg, between or between about 2.0 mg and about 60 mg, between or between about 2.0 mg and about 40 mg, between or between about 2.0 mg and about 20 mg, between or between about 2.0 mg and about 10 mg, between or between about 5.0 mg and about 80 mg, between or between about 5.0 mg and about 60 mg, between or between about 5.0 mg and about 40 mg, between or between about 5.0 mg and about 20 mg, between or between about 5.0 mg and about 10 mg, between or between about 10 mg and about 80 mg, between or between about 10 mg and about 60 mg, between or between about 10 mg and about 40 mg, between or between about 10 mg and about 20 mg dexamethasone or equivalent thereof, each inclusive, per day or per 24 hours, or about 10 mg, 20 mg, 40 mg or 80 mg dexamethasone or equivalent thereof, per day or per 24 hours.

47. The method of any of embodiments 18-46, wherein the volumetric measure or inflammatory marker is measured in the subject within 1 day, 2 days, 3 days, 4 days, 6 days, 8 days, 12 days, 16 days, 20 days, 24 days, 28 days or more prior to initiation of administration of the genetically engineered cells.

48. A method of dosing a subject, the method comprising administering, to a subject having a disease or condition, a dose of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR), wherein the dose comprises a number of the genetically engineered cells that is sufficient to achieve peak CAR⁺ cells in the blood within a determined therapeutic range in the subject, or in a majority of subjects so treated by the method or in greater than 75% of the subjects so treated by the method, wherein the therapeutic range is:

(i) based upon the range of peak CD3⁺ CAR⁺ T cells, or a CD8⁺CAR⁺ T cell subset thereof, in the blood among one or more subjects previously treated with the genetically engineered cells that is associated with an estimated probability of response of greater than or greater than about 65% and an estimated probability of a toxicity of less than or about 30%; or

(ii) peak CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 10 cells per microliter and 500 cells per microliter; or

(iii) peak CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 2 cells per microliter and 200 cells per microliter.

49. The method of any of embodiments 1-48, wherein the dose of genetically engineered cells comprises from or from about 1×10^5 to 5×10^8 total CAR-expressing T cells, 1×10^6 to 2.5×10^8 total CAR-expressing T cells, 5×10^6 to 1×10^8 total CAR-expressing T cells, 1×10^7 to 2.5×10^8 total CAR-expressing T cells, 5×10^7 to 1×10^8 total CAR-expressing T cells, each inclusive.

50. The method of any of embodiments 1-49, wherein the dose of genetically engineered cells comprises at least or at least about 1×10^5 CAR-expressing cells, at least or at least about 2.5×10^5 CAR-expressing cells, at least or at least about 5×10^5 CAR-expressing cells, at least or at least about 1×10^6 CAR-expressing cells, at least or at least about 2.5×10^6 CAR-expressing cells, at least or at least about 5×10^6 CAR-expressing cells, at least or at least about 1×10^7 CAR-expressing cells, at least or at least about 2.5×10^7 CAR-expressing cells, at least or at least about 5×10^7 CAR-expressing cells, at least or at least about 1×10^8 CAR-expressing cells, at least or at least about 2.5×10^8 CAR-expressing cells, or at least or at least about 5×10^8 CAR-expressing cells.

51. A method of dosing a subject, the method comprising:

(a) administering, to a subject having a disease or condition, a sub-optimal dose of genetically engineered cells comprising T cells engineered with a chimeric antigen receptor (CAR), wherein the dose comprises a number of the genetically engineered cells that is insufficient to achieve peak CAR+ cells in the blood within a determined therapeutic range in the subject, or in a majority of subjects so treated by the method or in greater than 75% of the subjects so treated by the method; and

(b) subsequent to administering the genetically engineered cells, administering an agent to enhance CAR+ cell expansion or proliferation in the subject to achieve peak CAR+ T cells in the blood within the therapeutic range,

wherein the therapeutic range is:

(i) based upon the range of peak CD3+ CAR+ T cells, or a CD8+CAR+ T cell subset thereof, in the blood among one or more subjects previously treated with the genetically

engineered cells that is associated with an estimated probability of response of greater than or greater than about 65% and an estimated probability of a toxicity of less than or about 30%; or

(ii) peak CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 10 cells per microliter and 500 cells per microliter; or

(iii) peak CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 2 cells per microliter and 200 cells per microliter.

52. The method of embodiment 51, wherein, after administering the dose of genetically engineered cells, the method comprises monitoring the CAR+ T cells in the blood of the subject.

53. The method of embodiment 51 or embodiment 52, wherein, following administration of the agent, the method achieves:

an increased frequency of peak CAR+ cells in the blood within a determined therapeutic range in the subject, compared to a method involving administration of the same dose of genetically engineered cells but without the agent; or

peak CAR+ cells in the blood within a determined therapeutic range in the subject, or in a majority of subjects so treated by the method or in greater than 75% of the subjects so treated by the method.

54. The method of any of embodiments 51-53, wherein the dose of genetically engineered cells is less than or less than about 1×10^7 CAR-expressing cells, less than or less than about 5×10^6 CAR-expressing cells, less than or less than about 2.5×10^6 CAR-expressing cells, less than or less than about 1×10^6 CAR-expressing cells, less than or less than about 5×10^5 CAR-expressing cells, less than or less than about 2.5×10^5 CAR-expressing cells, less than or less than about 1×10^5 CAR-expressing cells.

55. The method of any of embodiments 51-54, wherein the agent is capable of increasing expansion of the CAR+ T cells, optionally CAR-specific expansion.

56. The method of embodiment 55, wherein the agent is an anti-idiotypic antibody or antigen-binding fragment thereof specific to the CAR, an immune checkpoint inhibitor, a modulator of a metabolic pathway, an adenosine receptor antagonist, a kinase inhibitor, an anti-TGF β antibody or an anti-TGF β R antibody or a cytokine.

57. The method of any of embodiments 1-56, wherein, among a plurality of subjects treated, the method achieves an increase in the percentage of subjects achieving a durable

response, optionally a complete response (CR) or objective response (OR) or a partial response (PR), optionally that is durable for at or greater than 3 months or at or greater than 6 months, compared to a method that does not comprise administering the agent.

58. The method of any of embodiments 1-57, wherein the increase is greater than or greater than about 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold or more.

59. The method of any of embodiments 1-58, wherein:

at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40% or at least 50% of subjects treated according to the method achieve a complete response (CR) that is durable for at or greater than 3 months or at or greater than 6 months; and/or

at least 25%, at least 30%, at least 40%, at least 50%, at least 60% or at least 70% of the subjects treated according to the method achieve objective response (OR) that is durable for at or greater than 3 months or at or greater than 6 months.

60. The method of any of embodiments 1-59, wherein:

greater than or greater than about 50%, greater than or greater than about 60%, greater than or greater than about 70%, or greater than or greater than about 80% of the subjects treated according to the method do not exhibit a grade 3 or greater cytokine release syndrome (CRS) and/or do not exhibit a grade 2 or greater or grade 3 or greater neurotoxicity; or

greater than or greater than about 40%, greater than or greater than about 50% or greater than or greater than about 55% of the subjects treated according to the method do not exhibit any neurotoxicity or CRS.

61. The method of any of embodiments 1-60, wherein peak CAR⁺ T cells is determined as the number of CAR⁺ T cells per microliter in the blood of the subject.

62. The method of any of embodiments 1-61, wherein the therapeutic range is the range in which the estimated probability of toxicity is less than 20%, less than 15%, less than 10% or less than 5% and the estimated probability of achieving a response is greater than 65%, 70%, 75%, 80%, 85%, 90%, 95% or more.

63. The method of any of embodiments 1-62, wherein the probability of toxicity is based on a toxicity selected from:

any neurotoxicity or cytokine release syndrome (CRS);

severe toxicity or grade 3 or higher toxicity;

severe CRS or a grade 3 or higher CRS; or

severe neurotoxicity, grade 2 or higher neurotoxicity or grade 3 or higher neurotoxicity.

64. The method of any of embodiments 1-63, wherein the probability of a toxicity is based on the probability of a severe toxicity or a grade 3 or higher toxicity.

65. The method of embodiment 63 or embodiment 64, wherein the severe toxicity is grade 3-5 neurotoxicity.

66. The method of any of embodiments 1-65, wherein the probability of response is based on a response that is a complete response (CR), an objective response (OR) or a partial response (PR), optionally wherein the response is durable, optionally durable for at or at least 3 months or at or at least 6 months.

67. The method of any of embodiments 1-66, wherein the response is a marrow response as determined based on assessment of the presence of a malignant immunoglobulin heavy chain locus (IGH) ad/or an index clone in the bone marrow of the subject.

68. The method of embodiment 67, wherein the malignant IGH and/or index clone is assessed by flow cytometry or IgH sequencing.

69. A method of assessing likelihood of a durable response, the method comprising:

(a) detecting, in a biological sample from a subject, peak levels of one or more inflammatory marker and/or peak levels of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR), wherein the subject has been previously administered a dose of the genetically engineered cells for treating a disease or condition; and

(b) comparing, individually, the peak levels to a threshold value, thereby determining a likelihood that a subject will achieve a durable response to the administration of the genetically engineered cells.

70. The method of embodiment 69, wherein:

the subject is likely to achieve a durable response if the peak levels of the one or more inflammatory marker is below a threshold value and the subject is not likely to achieve a durable response if the peak levels of the one or more inflammatory marker is above a threshold value; or

the subject is likely to achieve a durable response if the peak level of the genetically engineered cells is within a therapeutic range between a lower threshold value and an upper threshold value and the subject is not likely to achieve a durable response if the peak level of the genetically engineered cells is below the lower threshold value or is above the upper threshold value.

71. The method of embodiment 69 or embodiment 70, if the subject is determined not likely to achieve a durable response, further comprising selecting a subject for treatment

with a therapeutic agent or with an alternative therapeutic treatment other than the genetically engineered cells.

72. The method of any of embodiments 69-71, if the subject is determined as not likely to achieve a durable response, further comprising administering a therapeutic agent or an alternative therapeutic treatment other than the genetically engineered cells.

73. A method of treatment, comprising;

(a) selecting a subject having received administration of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR) in which:

peak levels of one or more inflammatory markers in a sample from the subject is above a threshold value; and/or

peak level of T cells comprising a chimeric antigen receptor (CAR) in a sample from the subject is below a lower threshold value or is above an upper threshold value; and

(b) administering to the subject a therapeutic agent or alternative therapeutic treatment other than the genetically engineered cells.

74. The method of any of embodiments 69-72, wherein the response is a complete response (CR), objective response (OR) or partial response (PR).

75. The method of any of embodiments 69-72 and 74, wherein the response is durable for at or greater than 3 months, 4 months, 5 months, or 6 months.

76. The method of any of embodiments 69-75, wherein the peak levels are assessed and/or the sample is obtained from the subject at a time that is at least 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days or 21 days after initiation of administration of the genetically engineered cells.

77. The method of any of embodiments 69-76, wherein the peak levels are assessed and/or the sample is obtained from the subject at a time that is between or between about 11 to 22 days, 12 to 18 days or 14 to 16 days, each inclusive, after initiation of administration of the genetically engineered cells.

78. The method of any of embodiments 69-77, wherein the peak level is a peak level of one or more inflammatory marker and the inflammatory marker is selected from C reactive protein (CRP), IL-2, IL-6, IL-10, IL-15, TNF-alpha, MIP-1alpha, MIP-1beta, MCP-1, CXCL10 or CCL13.

79. The method of any of embodiments 70-78, wherein the peak level of one or more inflammatory marker is assessed and the threshold value is within 25%, within 20%, within 15%, within 10% or within 5% and/or is within a standard deviation of the median or mean of

the peak level of the inflammatory marker as determined among a group of control subjects having received administration of the genetically engineered cells, wherein each of the subjects of the group did not achieve a durable response, optionally a CR and/or PR, optionally at or greater than 3 months or 6 months following administration of the genetically engineered cells.

80. The method of embodiment 79, wherein the control subjects exhibited stable disease (SD) or progressive disease (PD) following administration of the genetically engineered cells, optionally at or greater than 3 months or 6 months following administration of the genetically engineered cells.

81. The method of any of embodiments 69-77, wherein the peak level is a peak level of CAR+ T cells, or a CD8+ T cell subset thereof.

82. The method of any of embodiments 70-77 and 81, the lower threshold value and upper threshold value is the lower and upper end, respectively, of a therapeutic range of peak CD3+ CAR+ T cells, or a CD8+CAR+ T cell subset thereof, in the blood among one or more subjects previously treated with the genetically engineered cells that is associated with an estimated probability of response of greater than or greater than about 65% and an estimated probability of a toxicity of less than or about 30%.

83. The method of any of embodiments 70-77, 81 and 82, wherein the therapeutic range is the range in which the estimated probability of toxicity is less than 20%, less than 15%, less than 10% or less than 5% and the estimated probability of achieving a response is greater than 65%, 70%, 75%, 80%, 85%, 90%, 95% or more.

84. The method of embodiment 82 or embodiment 83, wherein the probability of toxicity is based on a toxicity selected from:

any neurotoxicity or cytokine release syndrome (CRS);

severe toxicity or grade 3 or higher toxicity;

severe CRS or a grade 3 or higher CRS; or

severe neurotoxicity, grade 2 or higher neurotoxicity or grade 3 or higher neurotoxicity.

85. The method of any of embodiments 82-84, wherein the probability of response is based on a response that is a complete response (CR), an objective response (OR) or a partial response (PR), optionally wherein the response is durable, optionally durable for at or at least 3 months or at or at least 6 months.

86. The method of any of embodiments 70-77, and 81-85, wherein peak CAR+ T cells is determined as the number of CAR+ T cells per microliter in the blood of the subject.

87. The method of any of embodiments 70-77 and 81-86, wherein:

the upper threshold value is between or between about 300 cells per microliter and 1000 cells per microliter or 400 cells per microliter and 600 cells per microliter, or is about 300 cells per microliter, 400 cells per microliter, 500 cells per microliter, 600 cells per microliter, 700 cells per microliter, 800 cells per microliter, 900 cells per microliter or 1000 cells per microliter; or

the lower threshold value is less than or less than about 10 cells per microliter, 9 cells per microliter, 8 cells per microliter, 7 cells per microliter, 6 cells per microliter, 5 cells per microliter, 4 cells per microliter, 3 cells per microliter, 2 cells per microliter or 1 cell per microliter.

88. The method of any of embodiments 69-87, wherein the sample is a blood sample or plasma sample.

89. The method of any of embodiments 69-88, wherein the method is carried out ex vivo.

90. The method of any of embodiments 71-89, the peak level of CAR+ T cells is below a lower threshold value and the therapeutic agent is an agent that is capable of decreasing CAR+ T cell expansion or proliferation.

91. The method of embodiment 90, wherein the agent is a steroid.

92. The method of embodiment 91, wherein the steroid is a corticosteroid.

93. The method of embodiment 91 or embodiment 92, wherein the steroid is dexamethasone or methylprednisolone.

94. The method of any of embodiments 91-93, wherein the steroid is administered in an amount that is between or between about 1.0 mg and about 40 mg, between or between about 1.0 mg and about 20 mg, between or between about 2.0 mg and about 20 mg, between or between about 5.0 mg and about 25.0 mg, between or between about 10 mg and about 20 mg dexamethasone or equivalent thereof, each inclusive.

95. The method of any of embodiments 91-94, wherein the steroid is administered in multiple doses over a period of at or more than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more or within a range defined by any of the foregoing.

96. The method of any of embodiments 91-95, wherein the steroid is administered once per day, twice per day, or three times or more per day.

97. The method of any of embodiments 91-96, wherein the steroid is administered in an amount that is between or between about 1.0 mg and about 80 mg, between or between about 1.0 mg and about 60 mg, between or between about 1.0 mg and about 40 mg, between or

between about 1.0 mg and about 20 mg, between or between about 1.0 mg and about 10 mg, between or between about 2.0 mg and about 80 mg, between or between about 2.0 mg and about 60 mg, between or between about 2.0 mg and about 40 mg, between or between about 2.0 mg and about 20 mg, between or between about 2.0 mg and about 10 mg, between or between about 5.0 mg and about 80 mg, between or between about 5.0 mg and about 60 mg, between or between about 5.0 mg and about 40 mg, between or between about 5.0 mg and about 20 mg, between or between about 5.0 mg and about 10 mg, between or between about 10 mg and about 80 mg, between or between about 10 mg and about 60 mg, between or between about 10 mg and about 40 mg, between or between about 10 mg and about 20 mg dexamethasone or equivalent thereof, each inclusive, per day or per 24 hours, or about 10 mg, 20 mg, 40 mg or 80 mg dexamethasone or equivalent thereof, per day or per 24 hours.

98. The method of any of embodiments 71-89, the peak level of CAR+ T cells is above the upper threshold value and the therapeutic agent is an agent that is capable of increasing expansion of the CAR+ T cells, optionally CAR-specific expansion.

99. The method of embodiment 98, wherein the agent is an anti-idiotypic antibody or antigen-binding fragment thereof specific to the CAR, an immune checkpoint inhibitor, a modulator of a metabolic pathway, an adenosine receptor antagonist, a kinase inhibitor, an anti-TGF β antibody or an anti-TGF β R antibody or a cytokine.

100. The method of any of embodiments 1-99, wherein the disease or condition is a cancer.

101. The method of embodiment 100, wherein the cancer is a B cell malignancy.

102. The method of embodiment 101, wherein the cancer is selected from the group consisting of sarcomas, carcinomas, lymphomas, non-Hodgkin lymphomas (NHLs), diffuse large B cell lymphoma (DLBCL), leukemia, CLL, ALL, AML and myeloma.

103. The method of embodiment 102, wherein the cancer is a pancreatic cancer, bladder cancer, colorectal cancer, breast cancer, prostate cancer, renal cancer, hepatocellular cancer, lung cancer, ovarian cancer, cervical cancer, pancreatic cancer, rectal cancer, thyroid cancer, uterine cancer, gastric cancer, esophageal cancer, head and neck cancer, melanoma, neuroendocrine cancers, CNS cancers, brain tumors, bone cancer, or soft tissue sarcoma.

104. The method of any of embodiments 1-103, wherein the subject is a human.

105. The method of any of embodiments 1-104, wherein the CAR specifically binds to an antigen associated with a disease or condition and/or expressed in cells associated with the disease or condition.

106. The method of embodiment 105, wherein the antigen is selected from among $\alpha v\beta 6$ integrin ($\alpha v\beta 6$ integrin), B cell maturation antigen (BCMA), B7-H3, B7-H6, carbonic anhydrase 9 (CA9, also known as CAIX or G250), a cancer-testis antigen, cancer/testis antigen 1B (CTAG, also known as NY-ESO-1 and LAGE-2), carcinoembryonic antigen (CEA), a cyclin, cyclin A2, C-C Motif Chemokine Ligand 1 (CCL-1), CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD138, CD171, epidermal growth factor protein (EGFR), type III epidermal growth factor receptor mutation (EGFR vIII), epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), ephrinB2, ephrine receptor A2 (EPHa2), estrogen receptor, Fc receptor like 5 (FCRL5; also known as Fc receptor homolog 5 or FCRH5), fetal acetylcholine receptor (fetal AchR), a folate binding protein (FBP), folate receptor alpha, ganglioside GD2, O-acetylated GD2 (OGD2), ganglioside GD3, glycoprotein 100 (gp100), G Protein Coupled Receptor 5D (GPRC5D), Her2/neu (receptor tyrosine kinase erb-B2), Her3 (erb-B3), Her4 (erb-B4), erbB dimers, Human high molecular weight-melanoma-associated antigen (HMW-MAA), hepatitis B surface antigen, Human leukocyte antigen A1 (HLA-A1), Human leukocyte antigen A2 (HLA-A2), IL-22 receptor alpha (IL-22Ra), IL-13 receptor alpha 2 (IL-13Ra2), kinase insert domain receptor (kdr), kappa light chain, L1 cell adhesion molecule (L1-CAM), CE7 epitope of L1-CAM, Leucine Rich Repeat Containing 8 Family Member A (LRRC8A), Lewis Y, Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, mesothelin, c-Met, murine cytomegalovirus (CMV), mucin 1 (MUC1), MUC16, natural killer group 2 member D (NKG2D) ligands, melan A (MART-1), neural cell adhesion molecule (NCAM), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME), progesterone receptor, a prostate specific antigen, prostate stem cell antigen (PSCA), prostate specific membrane antigen (PSMA), Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1), survivin, Trophoblast glycoprotein (TPBG also known as 5T4), tumor-associated glycoprotein 72 (TAG72), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor receptor 2 (VEGFR2), Wilms Tumor 1 (WT-1), a pathogen-specific antigen, or an antigen associated with a universal tag, and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV or other pathogens.

107. The method of embodiment 105 or embodiment 106, wherein the antigen is selected from among 5T4, 8H9, $\alpha v\beta 6$ integrin, B7-H6, B cell maturation antigen (BCMA), CA9,

a cancer-testes antigen, carbonic anhydrase 9 (CAIX), CCL-1, CD19, CD20, CD22, CEA, hepatitis B surface antigen, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD138, CD171, carcinoembryonic antigen (CEA), CE7, a cyclin, cyclin A2, c-Met, dual antigen, EGFR, epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), EPHA2, ephrinB2, erb-B2, erb-B3, erb-B4, erbB dimers, EGFR vIII, estrogen receptor, Fetal AchR, folate receptor alpha, folate binding protein (FBP), FCRL5, FCRH5, fetal acetylcholine receptor, G250/CAIX, GD2, GD3, gp100, Her2/neu (receptor tyrosine kinase erbB2), HMW-MAA, IL-22R-alpha, IL-13 receptor alpha 2 (IL-13Ra2), kinase insert domain receptor (kdr), kappa light chain, Lewis Y, L1-cell adhesion molecule (L1-CAM), Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, MART-1, mesothelin, murine CMV, mucin 1 (MUC1), MUC16, NCAM, NKG2D, NKG2D ligands, NY-ESO-1, O-acetylated GD2 (OGD2), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME), PSCA, progesterone receptor, survivin, ROR1, TAG72, VEGF receptors, VEGF-R2, Wilms Tumor 1 (WT-1), a pathogen-specific antigen.

108. The method of any of embodiments 1-107, wherein the chimeric antigen receptor (CAR) comprises an extracellular antigen-recognition domain that specifically binds to the antigen and an intracellular signaling domain comprising an ITAM.

109. The method of embodiment 108, wherein the intracellular signaling domain comprises an intracellular domain of a CD3-zeta (CD3 ζ) chain.

110. The method of embodiment 108 or embodiment 109, wherein the chimeric antigen receptor (CAR) further comprises a costimulatory signaling region.

111. The method of embodiment 110, wherein the costimulatory signaling region comprises a signaling domain of CD28 or 4-1BB.

112. The method of embodiment 110 or embodiment 111, wherein the costimulatory domain is a domain of 4-1BB.

113. The method of any of embodiments 1-112, wherein the cells are T cells.

114. The method of embodiment 113, wherein the T cells are CD4⁺ or CD8⁺.

115. The method of any of embodiments 1-114, wherein the T cells are primary T cells obtained from a subject.

116. The method of any of embodiments 1-115, wherein the cells of the genetically engineered cells are autologous to the subject.

117. The method of any of embodiments 1-115, wherein the cells of the genetically engineered cells are allogeneic to the subject.

118. A kit, comprising a composition comprising genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR) and instructions for administering a dose of the cells to a subject following or based on the results of assessing if peak CAR+ T cells are within a therapeutic range, wherein the therapeutic range is:

(i) based upon the range of peak CD3+ CAR+ T cells, or a CD8+CAR+ T cell subset thereof, in the blood among one or more subjects previously treated with the genetically engineered cells that is associated with an estimated probability of response of greater than or greater than about 65% and an estimated probability of a toxicity of less than or about 30%; or

(ii) peak CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 10 cells per microliter and 500 cells per microliter; or

(iii) peak CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 2 cells per microliter and 200 cells per microliter.

119. The kit of embodiment 118, wherein the instructions specify that if the genetically engineered cells are not within the therapeutic range, administering an agent to the subject capable of modulating, optionally increasing or decreasing, CAR+ T cell expansion or proliferation, in the subject.

120. The kit of embodiment 119, wherein the kit further comprises the agent.

121. A kit, comprising an agent capable of modulating, optionally increasing or decreasing, expansion or proliferation of genetically engineered cells comprising CAR+ T cells in a subject, and instructions for administering the agent to a subject, said subject having been administered the genetically engineered cells, based on results of assessing if peak CAR+ T cells are within a therapeutic range, wherein the therapeutic range is:

(i) based upon the range of peak CD3+ CAR+ T cells, or a CD8+CAR+ T cell subset thereof, in the blood among one or more subjects previously treated with the genetically engineered cells that is associated with an estimated probability of response of greater than or greater than about 65% and an estimated probability of a toxicity of less than or about 30%; or

(ii) peak CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 10 cells per microliter and 500 cells per microliter; or

(iii) peak CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 2 cells per microliter and 200 cells per microliter.

122. The kit of any of embodiments 119-121, wherein the instructions specify that if the peak number of CAR+ T cells in the blood of the subject is less than the lowest number of peak CAR+ T cells in the therapeutic range, an agent is administered to the subject that is capable of increasing CAR+ T cell expansion or proliferation.

123. The kit of embodiment 122, wherein the agent is capable of CAR-specific expansion.

124. The kit of embodiment 122 or embodiment 123, wherein the agent is an anti-idiotypic antibody or antigen-binding fragment thereof specific to the CAR, an immune checkpoint inhibitor, a modulator of a metabolic pathway, an adenosine receptor antagonist, a kinase inhibitor, an anti-TGF β antibody or an anti-TGF β R antibody or a cytokine.

125. The kit of any of embodiments 119-121, wherein if the peak number of CAR+ T cells in the blood of the subject is greater than the highest number of peak CAR+ T cells in the therapeutic range, an agent is administered to the subject that is capable of decreasing CAR+ T cell expansion or proliferation.

126. A kit, comprising an agent capable of decreasing expansion or proliferation of genetically engineered cells comprising CAR+ T cells in a subject, and instructions for assessing a subject the level, amount or concentration of a volumetric measure of tumor burden or an inflammatory marker in a sample from the subject and administering to the subject the agent if the level, amount or concentration is at or above a threshold level, wherein the sample does not comprise genetically engineered T cells expressing a chimeric antigen receptor (CAR) and/or is obtained from the subject prior to receiving administration of genetically engineered T cells expressing a CAR.

127. The kit of embodiment 126, wherein the volumetric measure is a sum of the products of diameters (SPD), longest tumor diameters (LD), sum of longest tumor diameters (SLD), tumor volume, necrosis volume, necrosis-tumor ratio (NTR), peritumoral edema (PTE), and edema-tumor ratio (ETR).

128. The kit of embodiment 126 or embodiment 127, wherein the volumetric measure is a sum of the products of diameter (SPD).

129. The kit of embodiment 126, wherein the inflammatory marker is C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), albumin, ferritin, β 2 microglobulin (β 2-M), lactate dehydrogenase (LDH), a cytokine or a chemokine.

130. The kit of embodiment 129, wherein the inflammatory marker is LDH.

131. The kit of any of embodiments 125-130, wherein the agent is a steroid.

132. The kit of embodiment 131, wherein the steroid is a corticosteroid.

133. The kit of embodiment 131 or embodiment 132, wherein the steroid is dexamethasone or methylprednisolone.

134. The kit of any of embodiments 131-133, wherein the steroid is formulated for administration in an amount that is between or between about 1.0 mg and about 40 mg, between or between about 1.0 mg and about 20 mg, between or between about 2.0 mg and about 20 mg, between or between about 5.0 mg and about 25.0 mg, between or between about 10 mg and about 20 mg dexamethasone or equivalent thereof, each inclusive

135. The kit of any of embodiments 118-134, wherein the CAR specifically binds to an antigen associated with a disease or condition and/or expressed in cells associated with the disease or condition.

136. The kit of any of embodiments 118-135, wherein the genetically engineered cells comprise T cells, optionally CD4+ or CD8+ T cells.

137. An article of manufacture, comprising the kit of any of embodiments 118-136.

138. A method of ameliorating a toxicity, comprising administering, to a subject exhibiting a sign or symptom of a toxicity, a treatment regimen for treating the toxicity, said subject having been administered a dose of genetically engineered cells comprising T cells expressing a recombinant receptor, wherein the treatment regimen is selected from:

(a) if, within 72, 96 or 120 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits a fever and/or one or more first physical signs or symptoms associated with a toxicity, optionally cytokine release syndrome (CRS), and/or one or more physical signs or symptoms associated with grade 1 CRS, administering (i) an agent capable of binding an interleukin-6 receptor (IL-6R), said agent administered no more than once every 24 hours, and (ii) one or more doses of a steroid, said steroid administered about every 12 to 24 hours;

(b) if the subject exhibits one or more physical signs or symptoms associated with grade 2 CRS after receiving a dose of the genetically engineered cells, administering (i) an agent

capable of binding an IL-6R , said agent administered no more than once every 24 hours, and (ii) one or more doses of a steroid, said steroid administered about every 12 to 24 hours;

(c) if the subject exhibits one or more physical signs or symptoms associated with grade 3 CRS after receiving a dose of the genetically engineered cells, administering (i) an agent capable of binding an IL-6R , said agent administered no more than once every 24 hours, and (ii) one or more doses of a steroid, said steroid administered at least twice a day, optionally at least about every 12 hours; or

(d) if the subject exhibits one or more physical signs or symptoms associated with grade 4 CRS after receiving a dose of the genetically engineered cells, administering (i) an agent capable of binding an IL-6R , said agent administered no more than once every 24 hours, and (ii) one or more doses of a steroid, said steroid administered at least twice a day, optionally at least about every 6 hours.

139. The method of embodiment 138, wherein up to two doses of the agent is administered.

140. A method of ameliorating a toxicity, comprising administering, to a subject exhibiting a sign or symptom of a toxicity, a treatment regimen for treating the toxicity, said subject having been administered a dose of genetically engineered cells comprising T cells expressing a recombinant receptor, wherein the treatment regimen is, if, within 72, 96 or 120 hours of administration of the dose of genetically engineered, the subject exhibits a fever and/or one or more first physical signs or symptoms associated with a toxicity, optionally cytokine release syndrome (CRS), and/or one or more physical signs or symptoms associated with grade 1 CRS, administering (i) an agent capable of binding an interleukin-6 receptor (IL-6R) and (ii) one or more doses of a steroid.

141. A method of ameliorating a toxicity, comprising administering, to a subject exhibiting one or more physical signs or symptom of a toxicity, one or more agent capable of reducing and/or ameliorating the one or more physical signs or symptoms associated with the toxicity, said subject having been administered a dose of genetically engineered cells comprising T cells expressing a recombinant receptor, wherein the one or more agent is administered in a treatment regimen comprising:

(a) administering one or more agent if:

(i) at or greater than 72 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits a fever, and exhibits one or more physical signs

or symptoms associated with the toxicity, optionally cytokine release syndrome (CRS), and/or exhibits a rapid progression of the physical signs or symptoms associated with the toxicity; or

(ii) within 48 or 72 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits a fever and/or one or more physical signs or symptoms associated with grade 2 or higher CRS;

(b) administering one or more agent if, within 24, 48 or 72 hours after administration of the one or more agent in (a), the subject does not exhibit an improvement of the fever and/or the one or more physical signs or symptoms associated with the toxicity and/or exhibits a rapid progression of the physical signs or symptoms associated with the toxicity, which one or more agent optionally are different from the one or more agent administered in (a) and/or is administered at the same or higher dose and/or frequency as the one or more agent administered in (a);

(c) administering one or more agent if, within 24, 48 or 72 hours after administration of the one or more agent in (b), the subject does not exhibit an improvement of the fever and/or the one or more physical signs or symptoms associated with the toxicity and/or exhibits a rapid progression of the physical signs or symptoms associated with the toxicity, which one or more agent optionally are different from the one or more agent administered in (a) or (b) and/or is administered at the same or higher dose and/or frequency as the one or more agent administered in (a) or (b); and

(d) administering one or more agent if, after administration of the one or more agent in (c), the subject does not exhibit an improvement of the fever and/or the one or more physical signs or symptoms associated with the toxicity, which one or more agent optionally are different from the one or more agent administered in (a), (b) or (c) and/or is administered at the same or higher dose and/or frequency as the one or more agent administered in (a), (b) or (c).

142. The method of embodiment 141, wherein the one or more agent is selected from an agent capable of binding an interleukin-6 receptor (IL-6R) or one or more steroid, optionally one or more doses of the one or more steroid.

143. A method of ameliorating a toxicity, comprising administering, to a subject exhibiting one or more physical signs or symptom of a toxicity, one or more agent capable of reducing and/or ameliorating the one or more physical signs or symptoms associated with the toxicity, said subject having been administered a dose of genetically engineered cells comprising T cells expressing a recombinant receptor, wherein the one or more agent is administered in a treatment regimen comprising:

(a) administering one or more agent if:

(i) at or greater than 72 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits one or more physical signs or symptoms associated with the toxicity, optionally neurotoxicity (NT); or

(ii) within 48 or 72 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits one or more physical signs or symptoms associated with the toxicity;

(b) administering one or more agent if, within 24, 48 or 72 hours after administration of the one or more agent in (a), the subject does not exhibit an improvement of the one or more physical signs or symptoms associated with the toxicity and/or exhibits a progression of the physical signs or symptoms associated with the toxicity, which one or more agent optionally are different from the one or more agent administered in (a) and/or is administered at the same or higher dose and/or frequency as the one or more agent administered in (a); and

(c) administering one or more agent if, within 24, 48 or 72 hours after administration of the one or more agent in (b), the subject does not exhibit an improvement of the one or more physical signs or symptoms associated with the toxicity and/or exhibits a rapid progression of the physical signs or symptoms associated with the toxicity, which one or more agent optionally are different from the one or more agent administered in (a) or (b) and/or is administered at the same or higher dose and/or frequency as the one or more agent administered in (a) or (b).

144. The method of embodiment 143, wherein the one or more agent is one or more steroid, optionally one or more doses of the one or more steroid.

145. The method of embodiment 140 or 142, wherein the agent capable of binding IL-6R is administered in one or more doses.

146. The method of embodiment 138-145, wherein the dose of the agent capable of binding IL-6R and a dose of steroid is administered simultaneously, or a dose of the steroid is administered within about 1, 2, 3 or 4 hours of the dose of the agent capable of binding IL-6R.

147. The method of any of embodiments 138, 142, 145, and 146, wherein the agent capable of binding IL-6R is administered no more than once every 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or more hours.

148. The method of any of embodiments 139-147, wherein up to two doses of the agent is administered.

149. The method of any of embodiments 138-148, wherein the steroid is administered every 6, 9, 12, 15, 18, 21, 24, 36 or 48 hours, or a range defined by any two of the foregoing values.

150. The method of any of embodiments 138-149, wherein the steroid is or comprises a corticosteroid, which optionally is a glucocorticoid.

151. The method of any of embodiments 138-150, wherein the steroid is selected from among cortisones, dexamethasones, hydrocortisones, methylprednisolones, prednisolones and prednisones.

152. The method of embodiment 151, wherein the steroid is or comprises dexamethasone, prednisone or methylprednisolone.

153. The method of any of embodiments 138-152, wherein the steroid is dexamethasone or methylprednisolone.

154. The method of any of embodiments 138-153, wherein the steroid is for administration at an equivalent dosage amount of from or from about 1.0 mg to at or about 40 mg, from or from about 1.0 mg to at or about 20 mg, from or from about 2.0 mg to at or about 20 mg, from or from about 5.0 mg to at or about 25.0 mg, or from or from about 10 mg to at or about 20 mg dexamethasone or equivalent thereof, each inclusive.

155. The method of any of embodiments 138-154, wherein the steroid is administered at an equivalent dosage amount of between or between about 0.5 mg/kg and about 5 mg/kg, or about 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg or 5 mg/kg methylprednisolone or equivalent thereof, each inclusive.

156. The method of any of embodiments 138-155, wherein multiple doses of the steroid is administered.

157. The method of any of embodiments 138-156, wherein the steroid is administered in multiple doses over a period of at or more than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or within a range defined by any of the foregoing.

158. The method of any of embodiments 138-157, wherein the steroid is administered for 2, 3, 4, 5 or more days.

159. The method of any of embodiments 138-158, wherein the steroid is administered once per day, twice per day, or three times or more per day.

160. The method of any of embodiments 138-159, wherein the steroid is administered at an equivalent dosage amount of between or between about 1.0 mg and about 80 mg, between or between about 1.0 mg and about 60 mg, between or between about 1.0 mg and about 40 mg,

between or between about 1.0 mg and about 20 mg, between or between about 1.0 mg and about 10 mg, between or between about 2.0 mg and about 80 mg, between or between about 2.0 mg and about 60 mg, between or between about 2.0 mg and about 40 mg, between or between about 2.0 mg and about 20 mg, between or between about 2.0 mg and about 10 mg, between or between about 5.0 mg and about 80 mg, between or between about 5.0 mg and about 60 mg, between or between about 5.0 mg and about 40 mg, between or between about 5.0 mg and about 20 mg, between or between about 5.0 mg and about 10 mg, between or between about 10 mg and about 80 mg, between or between about 10 mg and about 60 mg, between or between about 10 mg and about 40 mg, between or between about 10 mg and about 20 mg dexamethasone or equivalent thereof, each inclusive, per day or per 24 hours, or from or from about 10 mg to about 80 mg dexamethasone or equivalent thereof, per day or per 24 hours, or about 10 mg, 20 mg, 40 mg or 80 mg dexamethasone or equivalent thereof, per day or per 24 hours.

161. The method of any of embodiments 156-160 wherein the multiple doses comprise an initial dose of steroids of between about 1 and about 3 mg/kg, such as 2 mg/kg methylprednisolone or equivalent thereof, followed by subsequent doses of between about 1 and about 5 mg/kg, or about 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg or 5 mg/kg methylprednisolone or equivalent thereof, divided between 1, 2, 3, 4 or 5 times over a day or over 24 hours.

162. The method of any of embodiments 138-161, wherein the steroid is formulated for intravenous or oral administration.

163. The method of embodiment 162, wherein the agent capable of binding IL-6R is a recombinant anti-IL-6 receptor antibody or an antigen-binding fragment thereof is or comprises an agent selected from among tocilizumab or sarilumab or an antigen-binding fragment thereof.

164. The method of embodiment 163, wherein the recombinant anti-IL-6R antibody is or comprises tocilizumab or an antigen-binding fragment thereof.

165. The method of embodiment 163 or embodiment 164, wherein the anti-IL-6R antibody is for administration in a dosage amount of from or from about 1 mg/kg to 20 mg/kg, 2 mg/kg to 19 mg/kg, 4 mg/kg to 16 mg/kg, 6 mg/kg to 14 mg/kg or 8 mg/kg to 12 mg/kg, each inclusive, or the anti-IL-6R antibody is administered in a dosage amount of at least or at least about or about 1 mg/kg, 2 mg/kg, 4 mg/kg, 6 mg/kg, 8 mg/kg, 10 mg/kg, 12 mg/kg, 14 mg/kg, 16 mg/kg, 18 mg/kg, 20 mg/kg.

166. The method of any of embodiments 163-165, wherein the anti-IL-6R antibody is formulated for single dosage administration of an amount from or from about 30 mg to about 5000 mg, from about 50 mg to about 1000 mg, from about 50 mg to about 500 mg, from about

50 mg to about 200 mg, from about 50 mg to about 100 mg, from about 100 mg to about 1000 mg, from about 100 mg to about 500 mg, from about 100 mg to about 200 mg, from about 200 mg to about 1000 mg, from about 200 mg to about 500 mg, or from about 500 mg to about 1000 mg.

167. The method of any of embodiments 163-166, wherein the anti-IL-6R antibody is formulated for intravenous administration.

168. The method of any of embodiments 138-167, further comprising, if the subject exhibits one or more first physical signs or symptoms associated with the toxicity, optionally CRS, within 72 hours of administration of the dose of genetically engineered cells, if the physical signs or symptoms associated with the toxicity, optionally CRS, does not improve, if the physical signs or symptoms associated with the toxicity is severe or aggressive and/or if the grade of toxicity, optionally CRS, becomes more severe, administering an additional dose of steroids, optionally at a high dose.

169. The method of embodiment 168, wherein the high dose of steroid is methylprednisolone at about 1 to about 4 mg/kg initial dose followed by about 1 to about 4 mg mg/kg/day divided 2, 3, 4, 5 or 6 times per day, or equivalents thereof.

170. The method of embodiment 169, wherein the high dose of steroid is dexamethasone at dosage amount of at or about 10 mg, 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, 60 mg, 65 mg, 70 mg, 75 mg or 80 mg dexamethasone or equivalent thereof, or a range defined by any of the foregoing, each inclusive.

171. The method of any of embodiments 138-170, further comprising administering to the subject a dose of genetically engineered cells comprising T cells expressing a recombinant receptor for treating a disease or condition prior to administering the treatment regimen

172. The method of any of embodiments 1-171, wherein the recombinant receptor is or comprises a chimeric receptor and/or a recombinant antigen receptor.

173. The method of any of embodiments 1-172, wherein the recombinant receptor is capable of binding to a target antigen that is associated with, specific to, and/or expressed on a cell or tissue of a disease, disorder or condition.

174. The method of embodiment 173, wherein the disease, disorder or condition is an infectious disease or disorder, an autoimmune disease, an inflammatory disease, or a tumor or a cancer.

175. The method of embodiment 173 or embodiment 174, wherein the target antigen is a tumor antigen.

176. The method of any of embodiments 173-175, wherein the target antigen is selected from among $\alpha\text{v}\beta 6$ integrin ($\alpha\text{v}\beta 6$ integrin), B cell maturation antigen (BCMA), B7-H3, B7-H6, carbonic anhydrase 9 (CA9, also known as CAIX or G250), a cancer-testis antigen, cancer/testis antigen 1B (CTAG, also known as NY-ESO-1 and LAGE-2), carcinoembryonic antigen (CEA), a cyclin, cyclin A2, C-C Motif Chemokine Ligand 1 (CCL-1), CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD138, CD171, epidermal growth factor protein (EGFR), type III epidermal growth factor receptor mutation (EGFR vIII), epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), ephrinB2, ephrine receptor A2 (EPHa2), estrogen receptor, Fc receptor like 5 (FCRL5; also known as Fc receptor homolog 5 or FCRH5), fetal acetylcholine receptor (fetal AchR), a folate binding protein (FBP), folate receptor alpha, ganglioside GD2, O-acetylated GD2 (OGD2), ganglioside GD3, glycoprotein 100 (gp100), G Protein Coupled Receptor 5D (GPRC5D), Her2/neu (receptor tyrosine kinase erb-B2), Her3 (erb-B3), Her4 (erb-B4), erbB dimers, Human high molecular weight-melanoma-associated antigen (HMW-MAA), hepatitis B surface antigen, Human leukocyte antigen A1 (HLA-A1), Human leukocyte antigen A2 (HLA-A2), IL-22 receptor alpha (IL-22Ra), IL-13 receptor alpha 2 (IL-13Ra2), kinase insert domain receptor (kdr), kappa light chain, L1 cell adhesion molecule (L1-CAM), CE7 epitope of L1-CAM, Leucine Rich Repeat Containing 8 Family Member A (LRRC8A), Lewis Y, Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, mesothelin, c-Met, murine cytomegalovirus (CMV), mucin 1 (MUC1), MUC16, natural killer group 2 member D (NKG2D) ligands, melan A (MART-1), neural cell adhesion molecule (NCAM), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME), progesterone receptor, a prostate specific antigen, prostate stem cell antigen (PSCA), prostate specific membrane antigen (PSMA), Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1), survivin, Trophoblast glycoprotein (TPBG also known as 5T4), tumor-associated glycoprotein 72 (TAG72), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor receptor 2 (VEGFR2), Wilms Tumor 1 (WT-1), a pathogen-specific antigen, or an antigen associated with a universal tag, and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV or other pathogens.

177. The method of any of embodiments 173-176, wherein the recombinant receptor is or comprises a functional non-TCR antigen receptor or a TCR or antigen-binding fragment thereof.

178. The method of any of embodiments 173-177, wherein the recombinant receptor is a chimeric antigen receptor (CAR).

179. The method of any of embodiments 173-178, wherein the recombinant receptor comprises an extracellular domain comprising an antigen-binding domain.

180. The method of embodiment 179, wherein the antigen-binding domain is or comprises an antibody or an antibody fragment thereof, which optionally is a single chain fragment.

181. The method of embodiment 180, wherein the fragment comprises antibody variable regions joined by a flexible linker.

182. The method of any of embodiments 180 or embodiment 181, wherein the fragment comprises an scFv.

183. The method of any of embodiments 173-182, wherein the recombinant receptor comprises an intracellular signaling region.

184. The method of embodiment 183, wherein the intracellular signaling region comprises an intracellular signaling domain.

185. The method of embodiment 184, wherein the intracellular signaling domain is or comprises a primary signaling domain, a signaling domain that is capable of inducing a primary activation signal in a T cell, a signaling domain of a T cell receptor (TCR) component, and/or a signaling domain comprising an immunoreceptor tyrosine-based activation motif (ITAM).

186. The method of embodiment 185, wherein the intracellular signaling domain is or comprises an intracellular signaling domain of a CD3 chain, optionally a CD3-zeta (CD3 ζ) chain, or a signaling portion thereof.

187. The method of any of embodiments 179-186, wherein the recombinant receptor further comprises a transmembrane domain disposed between the extracellular domain and the intracellular signaling region.

188. The method of any of embodiments 173-187, wherein the intracellular signaling region further comprises a costimulatory signaling domain.

189. The method of embodiment 188, wherein the costimulatory signaling domain comprises an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof.

190. The method of embodiment 188 or embodiment 189, wherein the costimulatory signaling domain comprises an intracellular signaling domain of a CD28, a 4-1BB or an ICOS or a signaling portion thereof.

191. The method of any of embodiments 188-190, wherein the costimulatory signaling domain is between the transmembrane domain and the intracellular signaling domain.

192. The method of any of embodiments 138-191, wherein the cells are T cells.

193. The method of embodiment 192, wherein the T cells are CD4+ or CD8+.

194. The method of any of embodiments 138-193, wherein the T cells are primary T cells obtained from a subject.

195. The method of any of embodiments 138-194, wherein the cells of the genetically engineered cells are autologous to the subject.

196. The method of any of embodiments 138-194, wherein the cells are of the genetically engineered cells allogeneic to the subject.

197. A method of treatment, the method comprising:

(a) administering, to a subject having a disease or condition, a dose of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR) for treating the disease or condition;

(b) after administering the dose of genetically engineered cells, monitoring CAR+ T cells in the blood of the subject to assess if the cells are within a therapeutic range, and

(c) if the genetically engineered cells are not within the therapeutic range, administering to the subject an agent capable of modulating, optionally increasing or decreasing, CAR+ T cell expansion or proliferation, in the subject,

wherein the therapeutic range is:

(i) peak CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 10 cells per microliter and 500 cells per microliter; or

(ii) peak CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 2 cells per microliter and 200 cells per microliter.

198. A method of treatment, the method comprising:

(a) monitoring, in the blood of a subject, the presence of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR) to assess if the cells are within a therapeutic range, wherein the subject has been previously administered a dose of the genetically engineered cells for treating a disease or condition; and

(c) if the genetically engineered cells are not within the therapeutic range, administering to the subject an agent capable of modulating, optionally increasing or decreasing, CAR+ T cell expansion or proliferation, in the subject,

wherein the therapeutic range is:

(i) peak CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 10 cells per microliter and 500 cells per microliter; or

(ii) peak CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 2 cells per microliter and 200 cells per microliter.

199. The method of any of embodiments 197-198, wherein if the peak number of CAR+ T cells in the blood of the subject is less than the lowest number of peak CAR+ T cells in the therapeutic range, an agent that is capable of increasing CAR+ T cell expansion or proliferation is administered to the subject.

200. The method of embodiment 199, wherein the agent is capable of increasing CAR-specific expansion.

201. The method of embodiment 199 or embodiment 200, wherein the agent is an anti-idiotypic antibody or antigen-binding fragment thereof specific to the CAR, an immune checkpoint inhibitor, a modulator of a metabolic pathway, an adenosine receptor antagonist, a kinase inhibitor, an anti-TGF β antibody or an anti-TGF β R antibody or a cytokine.

202. The method of any of embodiments 197-198, wherein if the peak number of CAR+ T cells in the blood of the subject is greater than the highest number of peak CAR+ T cells in the therapeutic range, an agent that is capable of decreasing CAR+ T cell expansion or proliferation is administered to the subject.

203. A method of treatment, the method comprising:

(a) administering, to a subject having a disease or condition, a dose of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR) for treating the disease or condition;

(b) after administering the dose of genetically engineered cells, monitoring CAR+ T cells in the blood of the subject, and

(c) administering to the subject an agent capable of decreasing, CAR+ T cell expansion or proliferation, in the subject if:

(i) the amount of CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, is greater than at or about 500 cells per microliter; or

(ii) the amount of CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, is greater than at or about 200 cells per microliter.

204. A method of treatment, the method comprising:

(a) monitoring, in the blood of a subject, the presence of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR), wherein the subject has been previously administered a dose of the genetically engineered cells for treating a disease or condition; and

(b) administering to the subject an agent capable of decreasing, CAR+ T cell expansion or proliferation, in the subject if: (i) the amount of CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, is greater than at or about 500 cells per microliter; or (ii) the amount of CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, is greater than at or about 200 cells per microliter.

205. The method of any of embodiments 203-204, wherein the agent is one or more steroid.

206. The method of embodiment 205, wherein the steroid is dexamethasone or methylprednisolone.

207. The method of any of embodiments 205-206, wherein the steroid is administered in an amount that is between or between about 1.0 mg and at or about 40 mg, between or between about 1.0 mg and at or about 20 mg, between or between about 2.0 mg and at or about 20 mg, between or between about 5.0 mg and at or about 25.0 mg, between or between about 10 mg and at or about 20 mg dexamethasone or equivalent thereof, each inclusive.

208. The method of any of embodiments 205-207, wherein the steroid is administered in multiple doses over a period of at or more than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more or within a range defined by any of the foregoing.

209. The method of any of embodiments 205-208, wherein the steroid is administered once per day, twice per day, or three times or more per day.

210. The method of any of embodiments 205-209, wherein the steroid is administered in an amount that is between or between about 1.0 mg and at or about 80 mg, between or between about 1.0 mg and at or about 60 mg, between or between about 1.0 mg and at or about 40 mg, between or between about 1.0 mg and at or about 20 mg, between or between about 1.0 mg and at or about 10 mg, between or between about 2.0 mg and at or about 80 mg, between or

between about 2.0 mg and at or about 60 mg, between or between about 2.0 mg and at or about 40 mg, between or between about 2.0 mg and at or about 20 mg, between or between about 2.0 mg and at or about 10 mg, between or between about 5.0 mg and at or about 80 mg, between or between about 5.0 mg and at or about 60 mg, between or between about 5.0 mg and at or about 40 mg, between or between about 5.0 mg and at or about 20 mg, between or between about 5.0 mg and at or about 10 mg, between or between about 10 mg and at or about 80 mg, between or between about 10 mg and at or about 60 mg, between or between about 10 mg and at or about 40 mg, between or between about 10 mg and at or about 20 mg dexamethasone or equivalent thereof, each inclusive, per day or per 24 hours, or about 10 mg, 20 mg, 40 mg or 80 mg dexamethasone or equivalent thereof, per day or per 24 hours.

211. The method of any of embodiments 197-210, wherein the subject is monitored for CAR+ T cells in the blood at a time that is at least 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days or 21 days after initiation of administration of the genetically engineered cells; or at a time that is between or between about 11 to 22 days, 12 to 18 days or 14 to 16 days, each inclusive, after initiation of administration of the genetically engineered cells.

212. The method of any of embodiment 197-211, wherein the agent is administered at a time that is greater than or greater than about 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days or 21 days after initiation of administration of the genetically engineered cells; or at a time that is between or between at or about 11 to at or about 22 days, 12 to 18 days or 14 to 16 days, each inclusive, after initiation of administration of the genetically engineered cells.

213. A method of modulating activity of engineered cells, the method comprising:

(a) selecting a subject in which the level, amount or concentration of a volumetric measure of tumor burden or an inflammatory marker in a sample from the subject is at or above a threshold level, wherein the sample does not comprise genetically engineered T cells expressing a chimeric antigen receptor (CAR) and/or is obtained from the subject prior to receiving administration of genetically engineered T cells expressing a CAR; and

(b) administering to the selected subject an agent that is capable of decreasing expansion or proliferation of genetically engineered T cells expressing a CAR.

214. A method of modulating activity of engineered cells, the method comprising administering to a subject an agent that is capable of decreasing expansion or proliferation of genetically engineered T cells expressing a chimeric antigen receptor (CAR) in a subject,

wherein the subject is one in which the level, amount or concentration of a volumetric measure of tumor burden or an inflammatory marker in a sample from the subject is at or above a threshold level.

215. The method of embodiment 214, wherein the sample does not comprise genetically engineered T cells expressing a CAR and/or is obtained from the subject prior to receiving administration of genetically engineered T cells expressing a CAR.

216. The method of any of embodiments 213-215, wherein the agent is administered prior to or concurrently with initiation of administration of a dose of genetically engineered cells comprising T cells expressing a CAR.

217. The method of embodiment 216, wherein the method further comprises administering a dose of the genetically engineered cells comprising T cells expressing a CAR.

218. The method of any of embodiments 213-217, wherein the subject has a disease or condition and the genetically engineered cells are for treating the disease or condition.

219. The method of any of embodiments 213-218, wherein, prior to administering the agent, the selected subject is at risk of developing a toxicity following administration of the genetically engineered cells.

220. The method of any of embodiments 213-219, wherein the administration of the agent is sufficient to achieve peak CAR+ T cells in a therapeutic range in the subject, or in a majority of selected subjects so treated by the method or in greater than 75% of the selected subjects so treated by the method.

221. The method of embodiment 220, wherein the therapeutic range is:

(i) based upon the range of peak CD3+ CAR+ T cells, or a CD8+CAR+ T cell subset thereof, in the blood among one or more subjects previously treated with the genetically engineered cells that is associated with an estimated probability of response of greater than or greater than about 65% and an estimated probability of a toxicity of less than or about 30%; or

(ii) peak CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 10 cells per microliter and 500 cells per microliter; or

(iii) peak CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 2 cells per microliter and 200 cells per microliter.

222. The method of embodiment 220, wherein the therapeutic range is:

(i) based upon the number or level of CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 10 cells per microliter and 500 cells per microliter; or

(ii) based upon the number or level of CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 2 cells per microliter and 200 cells per microliter.

223. The method of any of embodiments 213-222, wherein a volumetric measure of tumor burden is measured and the volumetric measure is a sum of the products of diameters (SPD), longest tumor diameters (LD), sum of longest tumor diameters (SLD), tumor volume, necrosis volume, necrosis-tumor ratio (NTR), peritumoral edema (PTE), and edema-tumor ratio (ETR).

224. The method of any of embodiments 213-223, wherein the volumetric measure is a sum of the products of diameter (SPD).

225. The method of any of embodiments 213-224, wherein the volumetric measure is measured using computed tomography (CT), positron emission tomography (PET), and/or magnetic resonance imaging (MRI) of the subject.

226. The method of any of embodiments 213-221, wherein an inflammatory marker in a sample from the subject is measured and the inflammatory marker is C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), albumin, ferritin, β 2 microglobulin (β 2-M), lactate dehydrogenase (LDH), a cytokine or a chemokine.

227. The method of any of embodiments 213-221 and 226, wherein the inflammatory marker is LDH.

228. The method of any of embodiments 213-221 and 226, wherein the inflammatory marker is a cytokine or a chemokine that is IL-7, IL15, MIP-1alpha or TNF-alpha.

229. The method of any of embodiments 213-221, 226 and 228, wherein the cytokine or chemokine is associated with macrophage or monocyte activation.

230. The method of any of embodiments 213-221 and 226-229, wherein the sample is or comprises a blood sample, plasma sample, or serum sample.

231. The method of any of embodiments 213-230, wherein the threshold value is a value that:

i) is within 25%, within 20%, within 15%, within 10%, or within 5% above the average value of the volumetric measure or inflammatory marker and/or is within a standard deviation

above the average value of the volumetric measure or the inflammatory marker in a plurality of control subjects;

ii) is above the highest value of the volumetric measure or inflammatory marker, optionally within 50%, within 25%, within 20%, within 15%, within 10%, or within 5% above such highest fold change, measured in at least one subject from among a plurality of control subjects; and/or

iii) is above the highest value of the volumetric measure or inflammatory marker as measured among more than 75%, 80%, 85%, 90%, 95%, or 98% of subjects from a plurality of control subjects.

232. The method of embodiment 231, wherein the plurality of control subjects are a group of subjects prior to receiving a dose of the genetically engineered cells, wherein:

each of the control subjects of the group exhibited a peak CAR+ T cells in the blood greater than the highest peak CAR+ T cells in the therapeutic range;

each of the control subjects of the group went on to develop at toxicity, optionally a neurotoxicity or cytokine release syndrome (CRS), a grade 2 or grade 3 or higher neurotoxicity or a grade 3 or higher CRS, after receiving a dose of the engineered cells for treating the same disease or condition;

each of the control subjects of the group did not develop a response, optionally a complete response (CR) or partial response (PR), following administration of the dose of genetically engineered cells; and/or

each of the control subjects of the group did not develop a durable response, optionally for at or about or greater than at or about 3 months or at or about or greater than at or about 6 months, following administration of the dose of genetically engineered cells.

233. The method of any of embodiments 213-232, wherein the volumetric measure is SPD and the threshold value is or is about 30 cm^2 , is or is about 40 cm^2 , is or is about 50 cm^2 , is or is about 60 cm^2 , or is or is about 70 cm^2 .

234. The method of any of embodiments 213-233, wherein the inflammatory marker is LDH and the threshold value is or is about 300 units per liter, is or is about 400 units per liter, is or is about 500 units per liter or is or is about 600 units per liter.

235. The method of any of embodiments 213-234, wherein the agent is a steroid.

236. The method of embodiment 235, wherein the steroid is dexamethasone or methylprednisolone.

237. The method of any of embodiments 235-236, wherein the steroid is administered in an amount that is between or between about 1.0 mg and about 40 mg, between or between about 1.0 mg and about 20 mg, between or between about 2.0 mg and about 20 mg, between or between about 5.0 mg and about 25.0 mg, between or between about 10 mg and about 20 mg dexamethasone or equivalent thereof, each inclusive.

238. The method of any of embodiments 235-237, wherein the steroid is administered in multiple doses over a period of at or more than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more or within a range defined by any of the foregoing.

239. The method of any of embodiments 235-238, wherein the steroid is administered once per day, twice per day, or three times or more per day.

240. The method of any of embodiments 235-239, wherein the steroid is administered in an amount that is between or between about 1.0 mg and at or about 80 mg, between or between about 1.0 mg and at or about 60 mg, between or between about 1.0 mg and at or about 40 mg, between or between about 1.0 mg and at or about 20 mg, between or between about 1.0 mg and at or about 10 mg, between or between about 2.0 mg and at or about 80 mg, between or between about 2.0 mg and at or about 60 mg, between or between about 2.0 mg and at or about 40 mg, between or between about 2.0 mg and at or about 20 mg, between or between about 2.0 mg and at or about 10 mg, between or between about 5.0 mg and at or about 80 mg, between or between about 5.0 mg and at or about 60 mg, between or between about 5.0 mg and at or about 40 mg, between or between about 5.0 mg and at or about 20 mg, between or between about 5.0 mg and at or about 10 mg, between or between about 10 mg and at or about 80 mg, between or between about 10 mg and at or about 60 mg, between or between about 10 mg and at or about 40 mg, between or between about 10 mg and at or about 20 mg dexamethasone or equivalent thereof, each inclusive, per day or per 24 hours, or from or from about 10 mg to at or about 80 mg dexamethasone or equivalent thereof, per day or per 24 hours, or at or about 10 mg, 20 mg, 40 mg or 80 mg dexamethasone or equivalent thereof, per day or per 24 hours.

241. The method of any of embodiments 213-240, wherein the volumetric measure or inflammatory marker is measured in the subject within 1 day, 2 days, 3 days, 4 days, 6 days, 8 days, 12 days, 16 days, 20 days, 24 days, 28 days or more prior to initiation of administration of the genetically engineered cells.

242. The method of any of embodiments 197-241, wherein the dose of genetically engineered cells comprises at least or at least about 1×10^5 CAR-expressing cells, at least or at least about 2.5×10^5 CAR-expressing cells, at least or at least about 5×10^5 CAR-expressing

cells, at least or at least about 1×10^6 CAR-expressing cells, at least or at least about 2.5×10^6 CAR-expressing cells, at least or at least about 5×10^6 CAR-expressing cells, at least or at least about 1×10^7 CAR-expressing cells, at least or at least about 2.5×10^7 CAR-expressing cells, at least or at least about 5×10^7 CAR-expressing cells, at least or at least about 1×10^8 CAR-expressing cells, at least or at least about 2.5×10^8 CAR-expressing cells, or at least or at least about 5×10^8 CAR-expressing cells or from at or about 1×10^5 to at or about 5×10^8 total CAR-expressing T cells, from at or about 1×10^6 to at or about 2.5×10^8 total CAR-expressing T cells, from at or about 5×10^6 to at or about 1×10^8 total CAR-expressing T cells, from at or about 1×10^7 to at or about 2.5×10^8 total CAR-expressing T cells, from at or about 5×10^7 to at or about 1×10^8 total CAR-expressing T cells, each inclusive.

243. The method of any of embodiments 197-242, wherein, among a plurality of subjects treated, the method achieves an increase in the percentage of subjects achieving a durable response, optionally a complete response (CR) or objective response (OR) or a partial response (PR), optionally that is durable for at or greater than 3 months or at or greater than 6 months, compared to a method that does not comprise administering the agent.

244. The method of embodiment 243, wherein the increase is greater than or greater than about 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold or more.

245. The method of any of embodiments 197-244, wherein:
at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40% or at least 50% of subjects treated according to the method achieve a complete response (CR) that is durable for at or greater than 3 months or at or greater than 6 months; and/or

at least 25%, at least 30%, at least 40%, at least 50%, at least 60% or at least 70% of the subjects treated according to the method achieve objective response (OR) that is durable for at or greater than 3 months or at or greater than 6 months.

246. The method of any of embodiments 197-245, wherein:
greater than or greater than about 50%, greater than or greater than about 60%, greater than or greater than about 70%, or greater than or greater than about 80% of the subjects treated according to the method do not exhibit a grade 3 or greater cytokine release syndrome (CRS) and/or do not exhibit a grade 2 or greater or grade 3 or greater neurotoxicity; or

greater than or greater than about 40%, greater than or greater than about 50% or greater than or greater than about 55% of the subjects treated according to the method do not exhibit any neurotoxicity or CRS.

247. The method of any of embodiments 197-246, wherein the amount or peak CAR+ T cells is determined as the number of CAR+ T cells per microliter in the blood of the subject.

248. The method of any of embodiments 197-247, wherein the therapeutic range is the range in which the estimated probability of a toxicity is less than 20%, less than 15%, less than 10% or less than 5% and the estimated probability of a response is greater than 65%, 70%, 75%, 80%, 85%, 90%, 95% or more.

249. The method of any of embodiments 197-248, wherein the probability of a toxicity is based on a toxicity selected from:

any neurotoxicity or cytokine release syndrome (CRS);

severe toxicity or grade 3 or higher toxicity;

severe CRS or a grade 3 or higher CRS; or

severe neurotoxicity, grade 2 or higher neurotoxicity or grade 3 or higher neurotoxicity.

250. The method of any of embodiments 197-249, wherein the probability of a toxicity is based on the probability of a severe toxicity or a grade 3 or higher toxicity.

251. The method of embodiment 249 or embodiment 250, wherein the severe toxicity is grade 3-5 neurotoxicity.

252. The method of any of embodiments 197-251, wherein the probability of response is based on a response that is a complete response (CR), an objective response (OR) or a partial response (PR), optionally wherein the response is durable, optionally durable for at or at least 3 months or at or at least 6 months.

253. The method of any of embodiments 197-252, wherein the response is a marrow response as determined based on assessment of the presence of a malignant immunoglobulin heavy chain locus (IGH) and/or an index clone in the bone marrow of the subject.

254. The method of embodiment 253, wherein the malignant IGH and/or index clone is assessed by flow cytometry or IgH sequencing.

255. A method of assessing likelihood of a durable response, the method comprising:

(a) detecting, in a biological sample from a subject, peak levels of one or more inflammatory markers and/or peak level of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR), wherein the subject has been previously administered a dose of the genetically engineered cells for treating a disease or condition; and

(b) comparing, individually, the peak levels to a threshold value, thereby determining a likelihood that a subject will achieve a durable response to the administration of the genetically engineered cells.

256. The method of embodiment 255, wherein:

the subject is likely to achieve a durable response if the peak levels of the one or more inflammatory markers is below a threshold value and the subject is not likely to achieve a durable response if the peak levels of the one or more inflammatory markers is above a threshold value; or

the subject is likely to achieve a durable response if the peak level of the genetically engineered cells is within a therapeutic range between a lower threshold value and an upper threshold value and the subject is not likely to achieve a durable response if the peak level of the genetically engineered cells is below the lower threshold value or is above the upper threshold value.

257. The method of embodiment 255 or embodiment 256, if the subject is determined not likely to achieve a durable response, further comprising selecting a subject for treatment with a therapeutic agent or with an alternative therapeutic treatment other than the genetically engineered cells.

258. The method of any of embodiments 255-257, if the subject is determined as not likely to achieve a durable response, further comprising administering a therapeutic agent or an alternative therapeutic treatment other than the genetically engineered cells.

259. A method of treatment, comprising;

(a) selecting a subject having received administration of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR) in which:

peak levels of one or more inflammatory markers in a sample from the subject is above a threshold value; and/or

peak level of T cells comprising a chimeric antigen receptor (CAR) in a sample from the subject is below a lower threshold value or is above an upper threshold value; and

(b) administering to the subject a therapeutic agent or alternative therapeutic treatment other than the genetically engineered cells.

260. The method of any of embodiments 255-258, wherein the response is a complete response (CR), objective response (OR) or partial response (PR).

261. The method of any of embodiments 255-258 and 260, wherein the response is durable for at or greater than 3 months, 4 months, 5 months, or 6 months.

262. The method of any of embodiments 255-261, wherein the peak levels are assessed and/or the sample is obtained from the subject at a time that is at least 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20

days or 21 days after initiation of administration of the genetically engineered cells; or at a time that is between at or about 11 and at or about 22 days, at or about 12 and at or about 18 days or at or about 14 and at or about 16 days, each inclusive, after initiation of administration of the genetically engineered cells.

263. The method of any of embodiments 255-262, wherein the peak level is a peak level of one or more inflammatory marker and the inflammatory marker is selected from C reactive protein (CRP), IL-2, IL-6, IL-10, IL-15, TNF-alpha, MIP-1alpha, MIP-1beta, MCP-1, CXCL10 and CCL13.

264. The method of any of embodiments 256-263, wherein the peak level of one or more inflammatory marker is assessed and the threshold value is within 25%, within 20%, within 15%, within 10% or within 5% and/or is within a standard deviation of the median or mean of the peak level of the inflammatory marker as determined among a group of control subjects having received administration of the genetically engineered cells, wherein each of the subjects of the group did not achieve a durable response, optionally a CR and/or PR, optionally durable for at or greater than 3 months or 6 months following administration of the genetically engineered cells.

265. The method of embodiment 264, wherein the control subjects exhibited stable disease (SD) or progressive disease (PD) following administration of the genetically engineered cells, optionally at or greater than 3 months or 6 months following administration of the genetically engineered cells.

266. The method of any of embodiments 255-262, wherein the peak level of genetically engineered cells is a peak CAR+ T cells, or a CD8+ T cell subset thereof.

267. The method of any of embodiments 256-262 and 266, the lower threshold value and upper threshold value is the lower and upper end, respectively, of a therapeutic range of peak CD3+ CAR+ T cells, or a CD8+CAR+ T cell subset thereof, in the blood among one or more subjects previously treated with the genetically engineered cells that is associated with an estimated probability of response of greater than or greater than about 65% and an estimated probability of a toxicity of less than or less than about 30%.

268. The method of any of embodiments 256-262, 266 and 267, wherein the therapeutic range is the range in which the estimated probability of a toxicity is less than 20%, less than 15%, less than 10% or less than 5% and the estimated probability of a response is greater than 65%, 70%, 75%, 80%, 85%, 90%, 95% or more.

269. The method of embodiment 267 or embodiment 268, wherein the probability of a toxicity is based on a toxicity selected from:

any neurotoxicity or cytokine release syndrome (CRS);

severe toxicity or grade 3 or higher toxicity;

severe CRS or a grade 3 or higher CRS; or

severe neurotoxicity, grade 2 or higher neurotoxicity or grade 3 or higher neurotoxicity.

270. The method of any of embodiments 267-269, wherein the probability of response is based on a response that is a complete response (CR), an objective response (OR) or a partial response (PR), optionally wherein the response is durable, optionally durable for at or at least 3 months or at or at least 6 months.

271. The method of any of embodiments 255-262, and 266-270, wherein peak level of genetically engineered cells is determined as the number of CAR+ T cells per microliter in the blood of the subject.

272. The method of any of embodiments 256-262 and 266-271, wherein:

the upper threshold value is between or between about 300 cells per microliter and at or about 1000 cells per microliter or between or between about 400 cells per microliter and at or about 600 cells per microliter, or is about 300 cells per microliter, 400 cells per microliter, 500 cells per microliter, 600 cells per microliter, 700 cells per microliter, 800 cells per microliter, 900 cells per microliter or 1000 cells per microliter; or

the lower threshold value is less than or less than about 10 cells per microliter, 9 cells per microliter, 8 cells per microliter, 7 cells per microliter, 6 cells per microliter, 5 cells per microliter, 4 cells per microliter, 3 cells per microliter, 2 cells per microliter or 1 cell per microliter.

273. The method of any of embodiments 255-272, wherein the sample is a blood sample or plasma sample.

274. The method of any of embodiments 255-273, wherein the method is carried out *ex vivo*.

275. The method of any of embodiments 257-274, the peak level of genetically engineered cells is above the upper threshold value and the therapeutic agent is an agent that is capable of decreasing CAR+ T cell expansion or proliferation.

276. The method of embodiment 275, wherein the agent is a steroid.

277. The method of embodiment 276, wherein the steroid is dexamethasone or methylprednisolone.

278. The method of any of embodiments 276-277, wherein the steroid is administered in an amount that is between or between about 1.0 mg and at or about 40 mg, between or between about 1.0 mg and at or about 20 mg, between or between about 2.0 mg and at or about 20 mg, between or between about 5.0 mg and at or about 25.0 mg, between or between about 10 mg and at or about 20 mg dexamethasone or equivalent thereof, each inclusive.

279. The method of any of embodiments 276-278, wherein the steroid is administered in multiple doses over a period of at or more than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more or within a range defined by any of the foregoing.

280. The method of any of embodiments 276-279, wherein the steroid is administered once per day, twice per day, or three times or more per day.

281. The method of any of embodiments 276-280, wherein the steroid is administered in an amount that is between or between about 1.0 mg and at or about 80 mg, between or between about 1.0 mg and at or about 60 mg, between or between about 1.0 mg and at or about 40 mg, between or between about 1.0 mg and at or about 20 mg, between or between about 1.0 mg and at or about 10 mg, between or between about 2.0 mg and at or about 80 mg, between or between about 2.0 mg and at or about 60 mg, between or between about 2.0 mg and at or about 40 mg, between or between about 2.0 mg and at or about 20 mg, between or between about 2.0 mg and at or about 10 mg, between or between about 5.0 mg and at or about 80 mg, between or between about 5.0 mg and at or about 60 mg, between or between about 5.0 mg and at or about 40 mg, between or between about 5.0 mg and at or about 20 mg, between or between about 5.0 mg and at or about 10 mg, between or between about 10 mg and at or about 80 mg, between or between about 10 mg and at or about 60 mg, between or between about 10 mg and at or about 40 mg, between or between about 10 mg and at or about 20 mg dexamethasone or equivalent thereof, each inclusive, per day or per 24 hours, or from or from about 10 mg to at or about 80 mg dexamethasone or equivalent thereof, per day or per 24 hours, or at or about 10 mg, 20 mg, 40 mg or 80 mg dexamethasone or equivalent thereof, per day or per 24 hours.

282. The method of any of embodiments 257-274, the peak level of genetically engineered cells is below the lower threshold value and the therapeutic agent is an agent that is capable of increasing expansion or proliferation of the CAR+ T cells, optionally CAR-specific expansion.

283. The method of embodiment 282, wherein the agent is an anti-idiotypic antibody or antigen-binding fragment thereof specific to the CAR, an immune checkpoint inhibitor, a

modulator of a metabolic pathway, an adenosine receptor antagonist, a kinase inhibitor, an anti-TGF β antibody or an anti-TGF β R antibody or a cytokine.

284. The method of any of embodiments 197-283, wherein the disease or condition is a cancer.

285. The method of embodiment 284, wherein the cancer is a B cell malignancy.

286. The method of embodiment 285, wherein the cancer is selected from the group consisting of sarcomas, carcinomas, lymphomas, non-Hodgkin lymphomas (NHLs), diffuse large B cell lymphoma (DLBCL), leukemia, CLL, ALL, AML and myeloma.

287. The method of embodiment 286, wherein the cancer is a pancreatic cancer, bladder cancer, colorectal cancer, breast cancer, prostate cancer, renal cancer, hepatocellular cancer, lung cancer, ovarian cancer, cervical cancer, pancreatic cancer, rectal cancer, thyroid cancer, uterine cancer, gastric cancer, esophageal cancer, head and neck cancer, melanoma, neuroendocrine cancers, CNS cancers, brain tumors, bone cancer, or soft tissue sarcoma.

288. The method of any of embodiments 197-287, wherein the subject is a human.

289. The method of any of embodiments 197-288, wherein the CAR specifically binds to an antigen associated with a disease or condition and/or expressed in cells associated with the disease or condition.

290. The method of embodiment 289, wherein the antigen is selected from among $\alpha\text{v}\beta 6$ integrin ($\alpha\text{v}\beta 6$ integrin), B cell maturation antigen (BCMA), B7-H3, B7-H6, carbonic anhydrase 9 (CA9, also known as CAIX or G250), a cancer-testis antigen, cancer/testis antigen 1B (CTAG, also known as NY-ESO-1 and LAGE-2), carcinoembryonic antigen (CEA), a cyclin, cyclin A2, C-C Motif Chemokine Ligand 1 (CCL-1), CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD138, CD171, epidermal growth factor protein (EGFR), type III epidermal growth factor receptor mutation (EGFR vIII), epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), ephrinB2, ephrine receptor A2 (EPHa2), estrogen receptor, Fc receptor like 5 (FCRL5; also known as Fc receptor homolog 5 or FCRH5), fetal acetylcholine receptor (fetal AchR), a folate binding protein (FBP), folate receptor alpha, ganglioside GD2, O-acetylated GD2 (OGD2), ganglioside GD3, glycoprotein 100 (gp100), G Protein Coupled Receptor 5D (GPRC5D), Her2/neu (receptor tyrosine kinase erb-B2), Her3 (erb-B3), Her4 (erb-B4), erbB dimers, Human high molecular weight-melanoma-associated antigen (HMW-MAA), hepatitis B surface antigen, Human leukocyte antigen A1 (HLA-A1), Human leukocyte antigen A2 (HLA-A2), IL-22 receptor alpha (IL-22Ra), IL-13 receptor alpha 2 (IL-13Ra2), kinase insert domain receptor (kdr), kappa

light chain, L1 cell adhesion molecule (L1-CAM), CE7 epitope of L1-CAM, Leucine Rich Repeat Containing 8 Family Member A (LRRC8A), Lewis Y, Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, mesothelin, c-Met, murine cytomegalovirus (CMV), mucin 1 (MUC1), MUC16, natural killer group 2 member D (NKG2D) ligands, melan A (MART-1), neural cell adhesion molecule (NCAM), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME), progesterone receptor, a prostate specific antigen, prostate stem cell antigen (PSCA), prostate specific membrane antigen (PSMA), Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1), survivin, Trophoblast glycoprotein (TPBG also known as 5T4), tumor-associated glycoprotein 72 (TAG72), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor receptor 2 (VEGFR2), Wilms Tumor 1 (WT-1), a pathogen-specific antigen, or an antigen associated with a universal tag, and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV or other pathogens.

291. The method of any of embodiments 197-290, wherein the chimeric antigen receptor (CAR) comprises an extracellular antigen-recognition domain that specifically binds to the antigen and an intracellular signaling domain comprising an ITAM.

292. The method of embodiment 291, wherein the intracellular signaling domain comprises an intracellular domain of a CD3-zeta (CD3 ζ) chain.

293. The method of embodiment 291 or embodiment 292, wherein the chimeric antigen receptor (CAR) further comprises a costimulatory signaling region.

294. The method of embodiment 293, wherein the costimulatory domain is a signaling domain of 4-1BB.

295. The method of any of embodiments 197-294, wherein the T cells are CD4+ or CD8+.

296. The method of any of embodiments 197-295, wherein the T cells are primary T cells obtained from a subject.

297. The method of any of embodiments 197-296, wherein the cells of the genetically engineered cells are autologous to the subject.

298. The method of any of embodiments 197-296, wherein the cells of the genetically engineered cells are allogeneic to the subject.

XI. EXAMPLES

[0850] The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

Example 1: Probability of Marrow Response Based on Peak CAR T Cell Expansion and Response and Neurotoxicity in High-Risk CLL Patients

[0851] Twenty-four (24) adult human subjects with relapsed or refractory (R/R) CD19+ chronic lymphocytic leukemia (CLL) were administered autologous T cells expressing a chimeric antigen-receptor (CAR) specific for CD19 and evaluated as described below.

[0852] The CAR included an scFv (in a V_L-linker-V_H orientation) specific for CD19, with variable regions derived from FMC63, an IgG hinge region, a transmembrane region, and intracellular signaling domains derived from human 4-1BB and CD3zeta. The construct further encoded a truncated EGFR (EGFRt), which served as a surrogate marker for CAR expression; the EGFRt-coding region was separated from the CAR sequence by a T2A skip sequence. Prior to administration of the cells, patients underwent leukapheresis; CD4+ and CD8+ populations were selected by immunoaffinity-based enrichment methods, transduced with a viral vector with the CAR construct, and expanded in culture over fifteen (15) days.

[0853] Beginning at least forty-eight (48) (and up to ninety-six (96)) hours prior to CAR+ T cell infusion, subjects received a lymphodepleting chemotherapy with either (a) cyclophosphamide (Cy, 60 mg/kg) with or without etoposide (2/13 subjects), or (b) cyclophosphamide (Cy, 60 mg/kg) in combination with fludarabine (flu, 25 mg/m² daily for 3-5 days (cy/flu, 11/13 subjects).

[0854] Cells for administration generally were formulated at a CAR+ CD4+ T cell to CAR+ CD8+ T cell ratio of approximately 1:1. Therapeutic compositions were successfully produced for all subjects. For 1/13 subjects, fewer than the target dose (2×10^6 /kg CAR+) of cells were produced.

[0855] Subjects were infused with a composition having approximately a 1:1 ratio of CD8+ CAR+ T cells to CD4+ CAR-T cells, at one of three different dose levels (2×10^5 (N=4), 2×10^6 (N=8) or 2×10^7 (N=1) CAR+ T cells per kilogram (kg) weight of the subject). Lymphodepleting therapy and T cell infusions were administered out on an outpatient basis.

[0856] The incidence and grade of cytokine release syndrome (CRS) was determined according to Lee et al, Blood. 2014;124(2):188-95. Following treatment, subjects were assessed and monitored for neurotoxicity (neurological complications including symptoms of confusion, aphasia, seizures, convulsions, lethargy, and/or altered mental status), graded based on severity using a Grade 1-5 scale (see, e.g., Guido Cavaletti & Paola Marmiroli *Nature Reviews Neurology* 6, 657-666 (December 2010). Grade 3 (severe symptoms), 4 (life-threatening symptoms) or 5 (death) indicated severe neurotoxicity.

[0857] An estimated probability curve of response and an estimated probability of developing Grade 3-5 neurotoxicity were constructed based on the number of CD4+/EGFRt+ or CD8+/EGFRt+ CAR-T cells in the blood (**FIG. 1**). Generally, as the number of CAR- T cells increased, the probability of response increased then plateaued while the probability of developing Grade 3-5 neurotoxicity increased.

Example 2: Administration of anti-CD19 CAR-Expressing Cells to Subjects

[0858] Twenty eight subjects with relapsed or refractory (R/R) non-Hodgkin lymphoma (NHL) were administered autologous T cells expressing an anti-CD19 chimeric antigen receptor (CAR). Subject demographics and baseline characteristics are set forth in **Table 13**. The CAR contained an anti-CD19 scFv derived from a murine antibody, an immunoglobulin-derived spacer, a transmembrane domain derived from CD28, a costimulatory region derived from 4-1BB, and a CD3-zeta intracellular signaling domain. To generate the autologous CAR-expressing T cells, T cells were isolated by immunoaffinity-based enrichment from leukapheresis samples from individual subjects, activated and transduced with a viral vector encoding an anti-CD19 CAR, followed by expansion (at a target ratio of approximately 1:1 ratio of CD4+ to CD8+ CAR+ T cells).

Table 13. Demographics and Baseline Characteristics	
Characteristic	N=28
Median Age, years (range)	63 (37-79)
≥ 70 years, n (%)	6 (21)
Male/Female, n (%)	19/9 (68/32)
B-NHL Subtype, n (%)	
DLBCL, NOS	15 (54)
Transformed DLBCL	10 (36)
Follicular, Grade 3B	1 (4)
MCL	2 (7)
Disease Status, n (%)	
Refractory [*]	24 (86)
Chemorefractory [†]	23 (82)
Baseline ECOG score, n (%)	
0	14 (50)
1	10 (36)
2	4 (14)

Prior Lines of Therapy	
Median (range)	4 (1-8)
≥ 5, n (%)	7 (25)
Prior Hematopoietic Stem Cell Transplant, n (%)	
Any HSCT	16 (57)
Allogeneic	4 (14)
Autologous	13 (46)

* <CR to last therapy

† SD or PD to last chemo-containing regimen or relapse <12 months after autologous SCT

[0859] Prior to administration of the CAR-expressing T cells, subjects were treated with 30 mg/m² fludarabine daily for 3 days and 300 mg/m² cyclophosphamide daily for 3 days. The cryopreserved cell compositions were thawed prior to intravenous administration. The therapeutic T cell dose was administered as a defined cell composition by administering a formulated CD4+ CAR+ cell population and a formulated CD8+ CAR+ population administered at a target ratio of approximately 1:1. At d=0, subjects were then treated with a single-dose or double-dose schedule of 5 x 10⁷ (DL1) or 1 x 10⁸ (DL2) CAR-expressing T cells by intravenous infusion (each single dose via separate infusions of CD4+ CAR-expressing T cells and CD8+ CAR-expressing T cells, respectively).

[0860] The presence or absence of various treatment-emergent adverse events was assessed in subjects treated with various dose schedules of CAR-T cell therapy (**Table 14** and **Table 15**). As shown in **Table 15**, no severe Cytokine Release Syndrome (sCRS) (Grade 3-5) was observed; Cytokine Release Syndrome (CRS) was observed in 36% (10/28) of the subjects. Grade 3-4 neurotoxicity was observed in 14% (4/28) of the subjects and 18% (5/28) of the subjects exhibited neurotoxicity of any grade. One subject was treated with tocilizumab and four patients received dexamethasone for early onset Grade 2 CRS or neurotoxicity. Six subjects received prophylactic anti-epileptics.

Table 14. Treatment-Emergent Adverse Events				
	DL1-S N=22	DL1-D N=3	DL2-S N=3	Total N=28
Any TEAE	21 (96)	3 (100)	3 (100)	27 (96)
Any Grade 3-5 [*] TEAE	16 (73)	3 (100)	0	19 (68)
Any Related TEAE	14 (64)	2 (67)	1 (33)	17 (61)
Any Related Grade 3-5 [*] TEAE	4 (18)	1 (33)	0	5 (18)

All grade TEAEs reported in ≥15% patients Preferred term, n (%)				
Fatigue	7 (32)	2 (67)	2 (67)	11 (39)
Cytokine release syndrome	8 (36)	2 (67)	0	10 (36)
Decreased appetite	6 (27)	1 (33)	1 (33)	8 (29)
Constipation	5 (23)	1 (33)	1 (33)	7 (25)
Vomiting	5 (23)	1 (33)	1 (33)	7 (25)
Diarrhea	5 (23)	1 (33)	0	6 (21)
Dizziness	6 (27)	0	0	6 (21)
Headache	4 (18)	1 (33)	0	5 (18)
Hypertension	4 (18)	1 (33)	0	5 (18)
Nausea	3 (14)	1 (33)	1 (33)	5 (18)
Peripheral edema	5 (23)	0	0	5 (18)
Lab abnormalities				
Anemia	16 (73)	1 (33)	1 (33)	18 (64)
Neutropenia	22 (100)	3 (100)	2 (67)	27 (96)
Thrombocytopenia	13 (59)	3 (100)	2 (67)	18 (64)

*1 Grade 5 respiratory failure, assessed as possibly related to CAR-T cell therapy, in a patient with MCL who progressed and started on a subsequent therapy

Table 15. Treatment-Emergent Adverse Events of Special Interest				
Preferred Term, n (%)	DL1-S N=22	DL1-D N=3	DL2-S N=3	Total N=28
Cytokine Release Syndrome (CRS), any	8 (36)	2 (67)	0	10 (36)
Grade 3-4	0	0	0	0
Neurotoxicity, any *	4 (18)	1 (33)	0	5 (18)
Grade 3-4	3 (14)	1 (33)	0	4 (14)

* Includes: encephalopathy, confusional state, depressed level of consciousness, lethargy, or seizure

[0861] Subjects among the group were assessed for best overall response, observed over a period of up to a particular time-point in an ongoing study after the last CAR+ T cell infusion of single-dose of DL1. Results of overall responses are shown in **Table 16**. Of the 20 subjects that were treated with the single-dose of DL1 in the Diffuse Large B-Cell Lymphoma (DLBCL) cohort, an overall response rate (ORR) of 80% (16/20) was observed and 60% (12/20) of

subjects showed evidence of complete remission (CR). 20% (4/20) of subjects showed evidence of partial response (PR) and 20% (4/20) of subjects showed evidence of progressive disease (PD). Of the subjects having been chemorefractory (having exhibited stable or progressive disease following last chemo-containing regimen or relapse less than 12 months after autologous SCT) prior to CAR+ T cell administration, the overall response rate was 83% (10 ORR, 7 CR, 3 PR, 2 PD, n=12). Among the subjects having been refractory (having exhibited less than complete remission following last treatment but not deemed chemorefractory), the overall response rate was 77% (13 ORR, 9 CR, 4 PR, 4 PD, n=17).

Table 16. Overall Response			
	DLBCL Cohort, DL1 single-dose schedule		
	All (n=20)	Refractory[*] (n=17)	Chemorefractory[†] (n=12)
ORR, n (%) [95% CI]	16 (80) [56, 94]	13 (77) [50, 93]	10 (83) [52, 98]
CR, n (%) [95% CI]	12 (60) [36, 81]	9 (53) [28, 77]	7 (58) [28, 85]
PR	4 (20)	4 (24)	3 (25)
PD	4 (20)	4 (24)	2 (17)

* <CR to last therapy

[†] SD or PD to last chemo-containing regimen or relapse <12 months after autologous SCT

[0862] Of three DLBCL subjects that at the time of assessment had been treated with two doses of DL1, two (2) exhibited partial response (PR) and one (1) exhibited progressive disease (PD). Among 2 DLBCL subjects that at the time of assessment had been treated with a single-dose of DL2, both subjects were observed to achieve CR. Among a MCL cohort with a total of two subjects treated at the time of assessment with single-dose of DL1, 1 PR and 1 PD were observed. Two subjects with double-hit, three subjects with triple-hit, and four subjects with double-expressor DLBCL were treated and all achieved a response (7 CR, 2 PR).

[0863] The number of CAR⁺ T cells in peripheral blood was determined at certain time points post-treatment by incubating cells with a transgene-specific reagent. The number of CD3⁺/CAR⁺ T cells in peripheral blood measured at certain time points post-infusion is shown for subjects treated with a single dose of DL1 grouped by best overall response in **FIG. 2A**. Higher peak CD3⁺/CAR⁺ T cells were observed in responders (CR/PR) than PD. **FIGS. 2B-2D** shows CD3⁺/CAR⁺ T cells, CD4⁺/CAR⁺ T, and CD8⁺/CAR⁺ T cell levels (cells/μL blood; mean

\pm SEM) in subjects who achieved a response, grouped by continued response (CR/PR) or PD at 3 months.

[0864] The C_{\max} (CAR⁺ cells/ μ L blood) and area under the curve (AUC) for responders (CR/PR) and PD were determined and shown in **Table 17**. The results were consistent with a conclusion that durable responses correlated with higher CD3⁺/CAR⁺ T cell levels in the blood, over time and at peak expansion.

Table 17. C_{\max} and AUC₀₋₂₈ Higher in Patients with CR/PR vs PD						
	CD3		CD4		CD8	
	CR/PR (n=16)	PD (n=4)	CR/PR (n=16)	PD (n=4)	CR/PR (n=16)	PD (n=4)
C_{\max} (CAR⁺ cells/μL blood)						
Mean (SD)	612 (1919)	2 (1)	220 (754)	1 (0.6)	426 (1314)	0.5 (0.5)
Median (Min, Max)	33 (1, 7726)	1 (1, 3)	8 (1, 3040)	1 (0, 2)	4 (0, 5238)	0.3 (0, 1)
Q1, Q3	7, 123	0.7, 2	2, 46	0.6, 2	0.8, 104	0.1, 0.9
AUC₀₋₂₈						
Mean (SD)	5883 (18821)	16 (13)	2369 (8388)	10 (7)	3873 (11963)	6 (6)
Median (Min, Max)	196 (11, 75773)	14 (4, 31)	47 (7, 33740)	9 (3, 17)	23 (1, 47834)	4 (1, 14)
Q1, Q3	52, 781	5, 26	16, 261	4, 16	4, 761	1, 10

AUC₀₋₂₈ = numbers per microliter for the indicated CAR⁺ cell population between days 0 and 28

Example 3: Administration of Anti-CD19 CAR-Expressing Cells to Subjects with Relapsed and Refractory Non-Hodgkin's Lymphoma (NHL)

A. Subjects and Treatment

[0865] Therapeutic CAR⁺ T cell compositions containing autologous T cells expressing a chimeric antigen-receptor (CAR) specific for CD19 were administered to subjects with B cell malignancies. Results are described in this Example for evaluation through a particular time-point in an ongoing study for cohort (full cohort) of fifty-five (55) adult human subjects with relapsed or refractory (R/R) aggressive non-Hodgkin's lymphoma (NHL), including diffuse large B-cell lymphoma (DLBCL), de novo or transformed from indolent lymphoma (NOS), high-grade B-cell lymphoma, with MYC and BCL2 and/or BCL6 rearrangements with DLBCL histology (double/triple hit), DLBCL transformed from chronic lymphocytic leukemia (CLL) or marginal zone lymphomas (MZL), primary mediastinal large b-cell lymphoma (PMBCL), and

follicular lymphoma grade 3b (FL3B) after failure of 2 lines of therapy. Among the subjects treated were those having Eastern Cooperative Oncology Group (ECOG) scores of between 0 and 2 (median follow-up 3.2 months). The 55 subjects did not include subjects with mantle cell lymphoma (MCL). No subjects were excluded based on prior allogeneic stem cell transplantation (SCT) and there was no minimum absolute lymphocyte count (ALC) for apheresis required.

[0866] Outcomes at this time-point for a core subset of the 55 subjects (the subset excluding those subjects with a poor performance status (ECOG 2), DLBCL transformed from marginal zone lymphomas (MZL) and/or chronic lymphocytic leukemia (CLL, Richter's), and subjects with primary mediastinal large b-cell lymphoma (PMBCL), and follicular lymphoma grade 3b (FL3B) (core cohort)). The core cohort includes subjects with DLBCL, NOS and transformed follicular lymphoma (tFL) or high grade B-cell lymphoma (double/triple hit) or high-grade B-cell lymphoma, with MYC and BCL2 and/or BCL6 rearrangements with DLBCL histology (double/triple hit) and with Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1 (core cohort)) were separately assessed.

[0867] The demographics and baseline characteristics of the full and core cohort are set forth in Table 18.

Table 18. Demographics and Baseline Characteristics		
Characteristic	FULL N=55	CORE N=44
Median Age, years (range)	61 (29-82)	61 (29-82)
≥ 65 years, n (%)	22 (40)	17 (39)
Male/Female, n (%)	38/17 (69/31)	28/16 (64/36)
Months from diagnosis, median (range)	17 (3-259)	20 (8-259)
B-NHL Subtype, n (%)		
DLBCL, NOS	40 (73)	35 (80)
Transformed DLBCL	14 (26)	8 (18)
Follicular, Grade 3B	1 (2)	1 (2)
Molecular Subtype, n (%)		
Double/triple hit	15 (27)	12 (27)
Double expressor	6 (11)	4 (9)
Patient Characteristics, n (%)		
Chemorefractory [†]	42 (76)	34 (77)
ECOG 0-1	48 (87)	44 (100)

ECOG 2	7 (13)	0
Prior lines of therapy, median (range)	3 (1-11)	3 (1-8)
< 5 lines of therapy	44 (80)	37 (84)
Any HSCT	27 (49)	22 (50)
Allogeneic	4 (7)	3 (7)
Autologous	24 (44)	20 (45)

* SD or PD to last chemo-containing regimen or relapse <12 months after autologous SCT

[0868] The therapeutic T cell compositions administered had been generated by a process including immunoaffinity-based (e.g., immunomagnetic selection) enrichment of CD4+ and CD8+ cells from leukapheresis samples from the individual subjects to be treated. Isolated CD4+ and CD8+ T cells were separately activated and independently transduced with a viral vector (e.g., lentiviral vector) encoding an anti-CD19 CAR, followed by separate expansion and cryopreservation of the engineered cell populations in a low-volume. The CAR contained an anti-CD19 scFv derived from a murine antibody (variable region derived from FMC63, V_L-linker-V_H orientation), an immunoglobulin-derived spacer, a transmembrane domain derived from CD28, a costimulatory region derived from 4-1BB, and a CD3-zeta intracellular signaling domain. 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.

[0869] The cryopreserved cell compositions were thawed prior to intravenous administration. The therapeutic T cell dose was administered as a defined cell composition by administering a formulated CD4+ CAR+ cell population and a formulated CD8+ CAR+ population administered at a target ratio of approximately 1:1. Subjects were administered a single or double dose of CAR-expressing T cells (each single dose via separate infusions of CD4+ CAR-expressing T cells and CD8+ CAR-expressing T cells, respectively) as follows: a single dose of dose level 1 (DL1) containing 5×10^7 total CAR-expressing T cells (n=30), a double dose of DL1 in which each dose was administered approximately fourteen (14) days apart (n=6 administered on day 1 and day 14, including one subject that inadvertently received two DL2 doses via the two-dose schedule, due to a dosing error), or a single dose of dose level 2 (DL-2) containing 1×10^8 total CAR-expressing T cells (n=18 for subjects assessed in this time point). The target dose level and the numbers of T cell subsets for the administered compositions are set forth in **Table 19**.

Table 19. Target dose levels and number of T cell subsets for cell compositions containing anti-CD19 CAR T cells

Dose level	Helper T cell (T _H) Dose (CD4+CAR+)	Cytotoxic T Cell (T _C) Dose (CD8+CAR+)	Total T Cell Dose (CD3+ CAR+)
1	25 x 10 ⁶	25 x 10 ⁶	50 x 10 ⁶
2	50 x 10 ⁶	50 x 10 ⁶	100 x 10 ⁶

[0870] Beginning at prior to CAR+ T cell infusion, subjects received a lymphodepleting chemotherapy with fludarabine (flu, 30 mg/m²) and cyclophosphamide (Cy, 300mg/m²) for three (3) days. The subjects received CAR-expressing T cells 2-7 days after lymphodepletion.

B. Safety

[0871] The presence or absence of treatment-emergent adverse events (TEAE) of the CAR-T cell therapy was assessed. **FIG. 3** depicts the percentage of subjects who were observed to have experienced laboratory abnormalities and TEAEs, which occurred in $\geq 20\%$ of subjects. In addition to the TEAEs shown in **FIG. 3**, the following event terms were observed at Grade 3-4 in $\geq 5\%$ of patients: white blood cell count decreased (13.6%), encephalopathy (12%), hypertension (7%). Degree of toxicities observed were consistent between dose levels 1 and 2.

[0872] Subjects also were assessed and monitored for neurotoxicity (neurological complications including symptoms of confusion, aphasia, encephalopathy, myoclonus seizures, convulsions, lethargy, and/or altered mental status), graded on a 1-5 scale, according to the National Cancer Institute—Common Toxicity Criteria (CTCAE) scale, version 4.03 (NCI-CTCAE v4.03). Common Toxicity Criteria (CTCAE) scale, version 4.03 (NCI-CTCAE v4.03). See Common Terminology for Adverse Events (CTCAE) Version 4, U.S. Department of Health and Human Services, Published: May 28, 2009 (v4.03: June 14, 2010); and Guido Cavaletti & Paola Marmiroli *Nature Reviews Neurology* 6, 657-666 (December 2010). Cytokine release syndrome (CRS) also was determined and monitored, graded based on severity. See Lee et al, *Blood*. 2014;124(2):188-95. In some cases, adverse events data were reported and collected starting at lymphodepletion to 90 days after CAR+ T cell administration.

[0873] In 84% of the full cohort subjects, severe (grade 3 or higher) cytokine release syndrome (CRS) and severe neurotoxicity were not observed. Additionally, it was observed that 60% of the full cohort subjects did not develop any grade of CRS or neurotoxicity. No differences in incidence of CRS, neurotoxicity (NT), sCRS, or severe neurotoxicity (sNT) were observed between dose levels. **Table 20** summarizes the incidence of cytokine release syndrome (CRS) and neurotoxicity adverse events in patients 28 days after receiving at least one dose of CAR-T cells. As shown in **Table 20**, no sCRS (Grade 3-4) was observed in any subjects

that received a single dose of DL2 or double dose of DL1. Severe neurotoxicity or severe CRS (grade 3-4) was observed in 16% (9/55) of the full cohort of subjects and in 18% (8/44) of the subjects in the core subset. 11% (n=6) of subjects received tocilizumab, 24% (n=13) of subjects received dexamethasone. Among the ECOG2 subjects within the full cohort, observed rates of CRS and neurotoxicity were 71% and 29%, respectively.

Table 20. Assessment of Presence or Absence of CRS and Neurotoxicity Adverse Events					
	FULL				CORE
	All Dose Levels	DL1S	DL2S	DL1D[†]	
Safety, N	55	30	19	6	44
sCRS or sNT, n (%)	9 (16)	6 (20)	2 (11)	1 (17)	8 (18)
CRS or NT, n (%)	22 (40)	12 (40)	7 (37)	3 (50)	15 (34)
CRS					
Grade 1-2, n (%)	18 (33)	10 (33)	5 (26)	3 (50)	12 (27)
Grade 3-4, n (%)	1 (2)	1 (3)	0	0	1 (2)
Neurotoxicity					
Grade 1-2, n (%)	3 (6)	1 (3)	2 (11)	0	2 (5)
Grade 3-4, n (%)	9 (16)	6 (20)	2 (11)	1 (17)	8 (18)

[†] Includes one patient treated at DL2 2-dose schedule due to dosing error

[0874] FIG. 4 shows a Kaplan Meier curve depicting observed time to onset of CRS and/or neurotoxicity. As shown, the observed median times to onset of CRS and to onset of neurotoxicity were 5 and 11 days, respectively, with only 11% of patients experiencing onset of CRS less than 72 hours after initiation of the administration of the cell therapy. The median time to resolution of CRS and neurotoxicity to Grade 1 or better was 5 and 7 days, respectively. The median time to complete resolution of CRS and neurotoxicity was 5 and 11 days, respectively. The results were consistent with a conclusion that there was a low rate of early onset of any CRS or neurotoxicity in the subjects.

C. Response to Treatment

[0875] Subjects were monitored for response, including by assessing tumor burden at 1, 3, 6, 7, 12, 18, and 24 months after administration of the CAR+ T cells. Response rates are listed in Table 21. High durable response rates were observed in the cohort of subjects, which included subjects heavily pretreated or, with poor prognosis and/or with relapsed or refractory disease. For subjects across all doses in the Core (n=44) cohort, the observed overall response rate

(ORR) was 86% and the observed complete response (CR) rate was 59%. At three months for the core cohort, the overall response rate (ORR) was 66%; the three-month CR rate was 50% among the core cohort. In the core cohort, the 3 month ORR was 58% (11/19) at dose level 1 and 78% at dose level 2; the 3 month CR rate was 42% (8/19) for dose level 1 and 56% (5/9) for dose level 2, consistent with a suggested dose response effect on treatment outcome. Additionally, the results were consistent with a relationship between dose and durability of response.

Table 21. Response					
	FULL				CORE
	All Dose Levels	DL1S	DL2S	DL1D^c	All Dose Levels
Best Overall Response, N ^a	54	30	18	6	44
ORR, % (95% CI)	76 (62, 87)	80 (61, 92)	72 (47, 90)	67 (23, 96)	86 (73, 95)
CR, % (95% CI)	52 (38, 66)	53 (34, 72)	50 (26, 74)	50 (12, 88)	59 (43, 74)
≥ 3 mos f/u, n ^b	41	24	11	6	32
3 mo ORR, % (95% CI)	51 (35, 67)	46 (26, 67)	64 (31, 89)	50 (12, 88)	66 (47, 81)
3 mo CR, % (95% CI)	39 (24, 56)	33 (16, 55)	46 (17, 77)	50 (12, 88)	50 (32, 68)

DL1S: DL1 1-dose schedule; DL2S: DL2 1-dose schedule; DL1D: DL1 2-dose schedule;

^a Included patients with event of PD, death, or 28 day restaging scans. Treated patients <28 days prior to data snapshot were not included.

^b The denominator is number of patients who received the CAR T-cell therapy ≥ 3 months : data snapshot date with an efficacy assessment at Month 3 or prior assessment of PD or death.

^c Includes one patient treated at DL2 2-dose schedule due to dosing error

[0876] Overall response rates among various subgroups of subjects in the full and core cohorts are shown in **FIGS. 5A** and **5B**, respectively. In poor-risk DLBCL subgroups, response rates were generally high. An ORR of greater than 50% was observed at 3 months in patients with double/triple hit molecular subtype, that had primary refractory or chemorefractory DLBCL or that never before had achieved a CR. Complete resolution of CNS involvement by lymphoma was observed in 2 patients.

[0877] Among the subjects treated six months or greater prior to the particular time-point of the evaluation, of the ten (10) patients that had been in response at three months, 9 (90%)

remained in response at six months. At the evaluation time-point, 97 % of subjects in the core subset who had responded were alive and in follow-up, median follow-up time 3.2 months.

[0878] Results for the duration of response and overall survival (grouped by best overall response (non-responder, CR/PR, CR and/or PR)) are shown for full and core cohorts of subjects, in **FIGS. 6A** and **6B**, respectively. As shown, prolonged survival was observed in responders, with increased durability of response in subjects with CRs. All patients in response at three months remained alive at the time of evaluation, although 5/6 subjects with poor performance status (ECOG 2) had expired.

C. Assessment of CAR+ T cells in Blood

[0879] Pharmacokinetic analysis was carried out to assess numbers of CAR⁺ T cells in peripheral blood at various time points post-treatment. As shown in **FIG. 7A**, CD4⁺ and CD8⁺ CAR-expressing cells, as measured by the number of cells/ μ L blood (median \pm quartiles) plotted on a log scale, were detected throughout the course of assessment at both administered dose levels.

[0880] An increased median area under the curve (AUC) (CD8⁺ CAR⁺ cell numbers over time in the blood) was observed among subjects administered the higher dose level, as compared to the lower dose level, without an observed increase in toxicity. Higher peak CD8⁺/CAR⁺ T cell exposure was observed in responders (CR/PR) than non-responders (PD) ; persistence of cells over the time of assessment, including out to 3 and 6 months, was observed even in subjects whose disease had progressed (**FIG. 7B**). The results were consistent with a conclusion that treatment resulted in prolonged exposure and persistence of the engineered cells, even in subjects with poor responses. In some embodiments, combination approaches are used, such as administration of an immune checkpoint modulator or other immune modulatory agent, e.g., following relapse or disease progression, at a time at which engineered cells persist in the subject, e.g., as measured by levels of cells in peripheral blood. In some aspects, the cells, having persisted for a prolonged period, re-expand or become activated and/or exhibit anti-tumor function, following administration of the other agent or treatment. Higher median CD4⁺ and CD8⁺ CAR⁺ T cell numbers were generally observed over time in blood of subjects who developed neurotoxicity (**FIG. 7C**).

D. Blood Analytes and Neurotoxicity

[0881] Various pre-treatment blood analytes, including cytokines, were measured in the blood of the subjects prior to administration of the CAR⁺ T cells. Potential correlations to risk of developing neurotoxicity were assessed using statistical analysis. **FIG. 8** shows median

levels of the assessed analytes in units (LDH, U/L; ferritin, ng/mL; CRP, mg/L; cytokines, pg/mL) in subjects that did not develop a neurotoxicity versus subjects that did develop a neurotoxicity following CAR+ T cell therapy. Levels of certain blood analytes, including LDH, Ferritin, CRP, IL-6, IL-8, IL-10, TNF- α , IFN- α 2, MCP-1 and MIP-1 β , were observed to be associated with level of risk of developing neurotoxicity (Wilcoxon p values <0.05, without multiplicity adjustment). In particular, the results were consistent with a conclusion that pre-treatment levels of LDH, which in some embodiments is a surrogate for disease burden, may be useful for potential neurotoxicity risk assessment and/or risk-adapted dosing or adjustment of treatment of certain subjects. In addition, tumor burden measured before administration of the CAR-T cell composition correlated (Spearman p values <0.05) with the risk of developing neurotoxicity. In some aspects, LDH levels may be assessed alone and/or in combination with another pre-treatment parameter, such as another measure or indicator of disease burden, such as a volumetric tumor measurement such as sum of product dimensions (SPD) or other CT-based or MRI-based volumetric measurement of disease burden. In some aspects, one or more parameters indicative of disease burden are assessed, and in some contexts may indicate the presence, absence or degree of risk of developing neurotoxicity following the T cell therapy. In some aspects, the one or more parameters include LDH and/or a volumetric tumor measurement.

[0882] **FIG. 9** shows a graph plotting progression-free time (months) for individual subjects within the full and core cohorts. Each bar represents a single patient. Shading indicates best overall response (in each case, unless otherwise indicated, achieved at 1 month); texture indicates dose (solid=dose level 1 (DL1), single dose; cross-hatched, dose level 2 (DL2), single dose; vertical hatched=dose level 1 (DL1), two-dose). Horizontal arrows indicate an ongoing response. Certain individual subjects were initially assessed (e.g., at 1-month) as exhibiting stable disease (SD) or partial response (PR), and were later observed to have achieved a PR (e.g., conversion of SD to PR) or CR. In such cases, shading of the individual patient bar, as noted, indicates best overall response, and dots (same correspondence of shading to response achieved) along each individual subject bar, indicate when each SD, PR, and/or CR was observed to have occurred in the subject. Complete resolution of CNS involvement by lymphoma was observed in two patients. CAR+ cells in one subject were observed to have expanded following biopsy after relapse.

Example 4: Administration of anti-CD19 CAR-Expressing Cells to Subjects with Mantle Cell Lymphoma (MCL)

[0883] Therapeutic CAR+ T cell compositions containing autologous T cells expressing a chimeric antigen-receptor (CAR) specific for CD19, generated as described in Example 2, were administered to four (4) human subjects with mantle cell lymphoma (MCL) that had failed 1 line of therapy. The cryopreserved cell compositions were thawed prior to intravenous administration. The therapeutic T cell composition was administered as a defined composition cell product with formulated CD4+ and CD8+ populations of CAR+ engineered T cells derived from the same subject administered at a target ratio of approximately 1:1. Subjects were administered a dose of CAR-expressing T cells (as a split dose of the CD4+ and CD8+ CAR-expressing T cells) at a single dose of dose level 1 (DL1) containing 5×10^7 CAR-expressing T cells. Beginning at three (3) days prior to CAR+ T cell infusion, subjects received a lymphodepleting chemotherapy with fludarabine (flu, 30 mg/m²) and cyclophosphamide (Cy, 300mg/m²).

[0884] Subjects were monitored for response and toxicities as described in Example 2. No CRS or neurotoxicity was observed in any of the subjects. Of the 4 subjects that were treated, two (2) subjects achieved PR (not durable) and two (2) patients had progressive disease.

Example 5: Further Assessment of Response, Safety, Pharmacokinetics, Pharmacodynamics and Blood Analytes in Subjects with Relapsed and Refractory Non-Hodgkin's Lymphoma (NHL) After Administration of Anti-CD19 CAR-Expressing Cells

[0885] Response outcomes, safety outcomes, pharmacokinetic and pharmacodynamics parameters, and blood analytes were assessed in patients at a subsequent point in time in the clinical study described in Example 2 above.

A. Subjects and Treatment

[0886] The analysis at this time point presented in this Example is based on assessment of a total of 91 subjects in the full DLBCL cohort (88 (65 from the CORE cohort) assessed for response and 91 (67 from the CORE cohort) assessed for safety) that had been administered the anti-CD19 CAR-expressing cells. The FULL cohort included DLBCL, NOS de novo or transformed from any indolent lymphoma, ECOG 0-2; the CORE cohort for analysis included subjects having DLBCL, NOS (de novo or transformed from follicular lymphoma (tFL)) or high grade B-cell lymphoma and with Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1. Approximately 90% of treated patients in the CORE cohort had at least 1 poor-risk disease feature predictive of short median overall survival (OS) of 3-6 months, such as

double/triple hit expressors, primary refractory disease, refractory to 2 or more lines of therapy, never achieved CR, or never received autologous stem cell transplant (ASCT). In some embodiments a cohort of subjects having Diffuse large B-cell lymphoma (DLBCL), not otherwise specified (NOS; de novo or transformed from follicular lymphoma tFL)) or high-grade B-cell lymphoma, with MYC and BCL2 and/or BCL6 rearrangements with DLBCL histology, and excluding subjects with ECOG score of 2 or subjects who have received prior hematopoietic stem cell transplantation (HSCT), are administered CAR-T compositions as provided herein. In some embodiments, subjects of the CORE cohort are administered anti-CD19 CAR+ T cells at a single dose of DL2 (1×10^8 total CAR-expressing T cells).

[0887] At this time point, a total of 140 subjects had been leukapheresed, of which 10 were awaiting manufactured composition, 2 had withdrawn before manufacturing, and 2 had compositions unavailable. Of another 18 subjects whose products were available, 4 were awaiting treatment, 4 had withdrawn, and 10 had developed progressive disease or had died. A total of 108 subjects had been administered the anti-CD19 CAR-expressing cells, of which 6 were not evaluable and 11 received non-conforming anti-CD19 CAR-expressing cells (compositions not necessarily meeting certain specifications but deemed to be safe for administration). Subjects had received DL1 (n=45), double dose of DL1 (n=6) or DL2 (n=40). Six (6) subjects with mantle cell lymphoma (MCL) had been administered CAR+ cells at DL1 (five treated with conforming product, one treated with non-conforming product), and five (5) had completed 28 days of follow-up. One MCL subject had developed CRS, and none had received tocilizumab or dexamethasone. Product had been available for 98% of apheresed subjects (126/128) in the DLBCL cohort.

[0888] The subjects at this time-point included 5 patients that had been treated in the outpatient setting (including four (4) subjects treated with DL1, one (1) treated with DL2; four (4) of which were included in the CORE cohort). For subjects treated in the outpatient setting, median age was 57 years old (range 26-61), 3 had DLBCL, NOS, 1 had tFL, and 1 had PMBCL. All five (5) subjects had an ECOG scores of 0 or 1. Data on outpatient results included results for three (3) additional subjects that had been treated in the outpatient setting (total of eight (8) subjects) and whose data became available after the time point for the analysis in this Example.

[0889] The demographics and baseline characteristics of the full and core cohort subjects at the timepoint are set forth in **Table 22**.

Table 22. Patient Characteristics: DLBCL Cohort		
Characteristic	FULL	CORE

	(n = 91)	(n = 67)
Median Age, years (range),	61 (20-82)	60 (20-82)
≥ 65 years, n (%)	34 (37)	24 (36)
Male/Female, n (%)	61/30 (67/33)	46/21 (69/31)
B-NHL subtype, n (%)		
DLBCL, NOS de novo	59 (65)	51 (76)
Transformed from FL (tFL)	19 (21)	16 (24)
Transformed from MZL (tMZL) /CLL (tCLL)	6 (7) / 4 (4)	0
Follicular, Grade 3B/PMBCL	1 (1) / 2 (2)	0
Molecular subtype, n (%)		
Double/triple hit [High grade B-cell lymphoma] ^a	18 (20)	16 (24)
Patient characteristics, n (%)		
Chemorefractory ^b	61 (67)	44 (66)
ECOG PS 0-1 / 2 (pre-LD)	81 (89) / 10 (11)	67 (100) / 0
IPI 3-5 / Disease stage 3-4	38 (42) / 70 (77)	24 (36) / 49 (73)
CNS involvement	2 (2)	2 (3)
Prior lines of therapy, median (range)	3 (1-12)	3 (1-8)
Never achieved CR	47 (52)	34 (51)
Any HSCT	39 (43)	28 (42)
Prior Autologous	36 (40)	28 (42)
Prior Allogeneic	5 (5)	0

HSCT, hematopoietic stem cell transplantation; LD, lymphodepletion.

^a At trial initiation, included in DLBCL, NOS histology; based on most recent WHO criteria (Swerdlow et al., (2016) Blood 127(20):2375-2390), are now considered “high-grade B-cell lymphoma, with MYC and BCL2 and/or BCL6 rearrangements with DLBCL histology (double/triple hit).

^b SD or PD to last chemotherapy-containing regimen or relapse <12 months after autologous SCT.

B. Safety and Response Outcomes after Treatment

[0890] As shown in **Table 23**. The objective response rate (ORR) was 74%, including 52% subjects who showed a complete response (CR). The incidence of any grade of cytokine release syndrome (CRS) was 35%, with 1% severe CRS; and the incidence of any grade of neurotoxicity (NT) was 19%, with 1% severe NT.

Table 23. Response and Safety After CAR+ Cell Administration				
	FULL	CORE		
	All Dose Levels	All Dose Levels ^a	DL1S	DL2S
Best Overall Response (BOR), n^b	88	65	34	27
ORR, % (95% CI)	74 (63,83)	80(68, 89)	77 (59,89)	82 (62, 94)
CR, % (95% CI)	52 (41,63)	55(43, 68)	47 (30, 65)	63 (42, 81)
Safety, n^c	91	67	34	29
Any CRS, % (95% CI)	35 (25, 46)	36 (24, 48)	41 (25, 59)	24 (10, 44)
sCRS(grade 3-4), % (95% CI)	1 (0, 6)	1 (0, 8)	38 (0, 15)	0
Any NT, % (95% CI)	19 (11,28)	21 (12, 33)	24 (11, 41)	17 (6, 36)
sNT(grade 3-4), % (95% CI)	12 (6, 21)	15 (7, 26)	21 (9, 38)	7 (1, 23)

^a Four patients treated on DL1D (dose level 1, two-dose schedule) with similar outcomes.

^b Includes patients with event of PD, death, or 28-day restaging scans. One patient did not have restaging scans available.

^c Includes all subjects who have received at least one dose of conforming CAR-expressing cell product 28 days prior to data snapshot date or died.

[0891] As shown in **Table 24**, high rates of response and low severe toxicity was observed in the full DLBCL population.

Table 24. Response After CAR+ Cell Administration					
		By Diagnosis			
	FULL	DLBCL, NOS	tFL	tCLL/MZL	FL3B/PMBCL
BOR, n^a	88	57	19	10	2
ORR, % (95% CI)	74 (63,83)	74 (60, 85)	84 (60, 97)	50 (19, 81)	100 (16, 100)
CR, % (95% CI)	52 (41,63)	51 (37, 64)	63 (38, 84)	30 (7, 65)	100 (16, 100)
Safety, n^b	91	59	19	10	3
Any CRS, % (95% CI)	35 (25, 46)	34 (22, 47)	42 (20, 67)	20 (3, 56)	67 (9, 99)
sCRS (grade 3-4), % (95% CI)	1 (0, 6)	2 (0, 9)	0	0	0
Any NT, % (95% CI)	19 (11,28)	20 (11, 33)	21 (6, 46)	10 (0, 45)	0
sNT (grade 3-4), % (95% CI)	12 (6, 21)	14 (6, 25)	11 (1, 33)	10 (0, 45)	0

^a Includes patients with event of PD, death, or 28-day restaging scans. One patient did not have restaging scans available.

^b Includes all subjects who have received at least one dose of conforming CAR+ expressing cells 28 days prior to data snapshot date or died.

[0892] As shown in **Table 25**, high rate of response and a dose-dependent response was observed in the CORE cohort of subjects.

Table 25. Durable Response After CAR+ Cell Administration			
	Dose Levels^a	DL1S	DL2S
BOR, n^b	65	34	27
ORR (95% CI), %	80(68, 89)	77 (59,89)	82 (62, 94)
CR (95% CI), %	55(43, 68)	47 (30, 65)	63 (42, 81)
≥ 3-mo f/u, n^c	52	29	19
3-mo ORR (95% CI), %	65 (51,78)	59 (39, 77)	74 (49, 91)
3-mo CR (95% CI), %	54(40, 68)	41 (24,61)	68 (43, 87)
≥ 6-mo f/u, n^d	38	20	14
6-mo ORR (95% CI), %	47(31, 64)	40 (19, 64)	50 (23, 77)
6-mo CR (95% CI), %	42 (26, 59)	30 (12, 54)	50 (23, 77)

^a Four patients (CORE) treated on DL1D with similar outcomes.

^b Includes patients with event of PD, death, or 28-day restaging scans. One patient did not have restaging scans available.

^c The denominator is number of patients who received CAR+ cells ≥ 3 months ago, prior to data snapshot date, with an efficacy assessment at month 3 or prior assessment of PD or death.

^d The denominator is number of patients who received CAR+ cells ≥ 6 months ago, prior to data snapshot date, with an efficacy assessment at month 6 or prior assessment of PD or death.

[0893] Three-month objective response rates (ORR) among various subgroups of subjects in the poor-risk DLBCL subgroups, that included all DLBCL patients treated at all dose levels in the core cohort, are shown in **FIG. 24**. The results showed high durable ORR in the poor-risk DLBCL subgroup.

[0894] Results for the duration of response (DOR) and overall survival (grouped by best overall response (non-responder, CR/PR, CR and/or PR)) are shown for the full cohort and the core cohort cohorts of subjects, in **FIGS. 25A-25D**. The results also showed 80% (16/20) of subjects with a CR at 3 months stay in CR at 6 months, and 92% (11/12) of subjects with a response (CR or PR) at 6 months continue to show a response longer term.

[0895] **FIG. 26** depicts the percentage of subjects at this timepoint who were observed to have experienced laboratory abnormalities and treatment-emergent adverse events (TEAEs) (data for 5 patients with MCL treated with conforming product at DL1 with at least 28 days of follow-up are not included). In addition to the TEAEs shown in **FIG. 26**, the following event terms were observed at Grade 3-4 in $\geq 5\%$ of patients: encephalopathy (8%), Pancytopenia (5%) and Febrile neutropenia (7%). Eight patients (9%) had infusional toxicity, defined as AE on day of administration related to CAR+ cell administration, including flushing, headache, fever, pyrexia, chills, rigors, vomiting, rash, hives, pruritis, hypotension, wheezing, bronchospasm, shortness of breath, nausea, vomiting, back pain, cough, and infusion-related reaction. Events included chills (2), pyrexia (5), flushing (1), headache (1), hypotension (1), infusion related reaction (1), rash (1), pruritis (1), and vomiting (1), with 6 grade 1 events, 1 grade 2 (chills), and 1 grade 3 (hypotension) event. TEAE in the core cohort did not differ substantially from those in the full cohort. The most common related TEAEs in the subjects treated in the outpatient setting group were CRS, hypotension, vomiting, anemia, and dyspnea.

[0896] **Table 26** sets forth the TEAEs and neurotoxicity that occurred in 25 percent or more subjects in the FULL or CORE cohort, for subjects who received DL1S and DL2S. No apparent dose-toxicity relationship was observed in the DLBCL population.

Table 26. TEAEs $\geq 25\%$ in FULL cohort, CORE cohort, and CORE cohort by dose level.				
Term, n (%)	FULL (N = 91)	CORE^a (n = 67)	CORE DL1S (n = 34)	CORE DL2S (n = 29)
	85 (93)	63 (94)	33 (97)	26 (90)
Anemia ^b	64 (70)	48 (72)	28 (82)	19 (66)
Thrombocytopenia ^b	48 (53)	41 (61)	20 (59)	19 (66)

Fatigue	34 (37)	25 (37)	11 (32)	12 (41)
CRS	32 (35)	24 (36)	14 (41)	7 (24)
Nausea	25 (27)	19 (28)	12 (35)	5 (17)
Diarrhea	23 (25)	16 (24)	7 (21)	7 (24)

a Includes 4 patients treated at dose level 1, two-dose schedule.

b Laboratory anomalies.

[0897] **FIG. 27** depicts the number and percentage of subjects that were observed to have CRS and/or NT at various time points after administration of CAR+ cells. In this assessment, the median time to onset of first of CRS or NT event was observed to be 5 (range 1-14) or 10 (range 3-23) days, respectively. Within the first 72 hours after CAR+ cell administration, 1 patient had NT (grade 1), and only 14% (13 of 91) had CRS (7 grade 1; 6 grade 2). The median duration (Q1,Q3) of CRS or NT was 5 (4, 8) or 10.5 (7, 19) days, respectively. NT was preceded by CRS in 12 of 17 cases (71%). All evaluable NT events were resolved at the time of analysis except one grade 1 tremor and 2 patients died from progressive disease with ongoing NT (based on safety database of reported events including additional subjects analyzed after the analysis timepoint described in this Example).

[0898] In the full cohort (n=91), selected subjects with onset of CRS or NT were administered anti-cytokine therapy with tocilizumab and/or dexamethasone as follows: Tocilizumab alone, 4% (n = 4); Dexamethasone alone, 9% (n = 8); Tocilizumab and dexamethasone, 8% (n = 7). The median number of dexamethasone doses was 6 (range, 2-99); and the median number of tocilizumab doses was 1 (range, 1-3).

[0899] **Table 27** shows toxicity outcomes in subjects in the CORE cohort that received a single dose at DL1 or DL2. No deaths occurred from CRS or NT. The median time to onset of CRS was 5 days (range, 2 –14) and NT was 11.5 days (range, 5 –23). In the CORE cohort, 13% (n = 9) received tocilizumab, and 18% (n = 12) received dexamethasone to ameliorate toxicity. Eighteen percent of subjects (12 of 67) exhibited neurotoxicity terms consistent with encephalopathy, including encephalopathy (13%), 6% (4 of 67) had aphasia and 3% (2 of 67) had seizures. In **Table 27**, the number of subjects or % of total subjects (parentheses) exhibiting an indicated toxicity outcome is shown at all dose levels or specifically in subjects administered DL1 or DL2. Also shown in brackets is the upper and lower 95% confidence interval.

Table 27. Toxicity in Core Cohort Receiving Different Dose Levels.			
	All Dose Levels^a n=67	DL1S n=34	DL2S n=29
CRS, n (%) [95%CI]			
Any Grade	24 (36) [24, 48]	14 (41) [25, 59]	7 (24) [10, 44]
Grade 1/2	23 (34) [23, 47]	13 (38) [22,56]	7 (24) [10, 44]

Table 27. Toxicity in Core Cohort Receiving Different Dose Levels.

	All Dose Levels ^a n=67	DL1S n=34	DL2S n=29
Grade 3/4 (sCRS)	1 (1) [0, 8]	1 (3) [0, 15]	0
Neurotoxicity^b, n (%) [95% CI]			
Any Grade	14 (21) [12, 33]	8 (24) [11, 41]	5 (17) [6, 36]
Grade 1/2	4 (6) [2, 15]	1 (3) [0, 15]	3 (10) [2, 27]
Grade 3/4 (sNT)	10 (15) [7, 26]	7 (21) [9, 38]	2 (7) [1, 23]
Any, n (%) [95% CI]			
CRS or NT, n (%)	28 (42) [30, 54]	15 (44) [27, 62]	10 (34) [18, 54]
sCRS or sNT, n (%)	10 (15) [7, 26]	7 (21) [9, 38]	2 (7) [1, 23]

^a Four patients treated on DL1D with similar outcomes.

^b Includes confusional state, encephalopathy, aphasia, ataxia, cerebellar syndrome, delirium, depressed level of consciousness, dizziness, flat affect, hand-eye coordination impaired, memory impairment, tremor, agitation, disturbance in attention, dysarthria, mental status changes, muscular weakness, seizure, somnolence, and urinary incontinence.

[0900] Among twelve (12) subjects receiving nonconforming products, 10 at DL1 and 2 at DL2, all had 28-day follow-up. CRS was observed in 33% of the subjects (4/12), and NT was not observed in any of the subjects. Two subjects received tocilizumab and 3 subjects received dexamethasone. The toxicity rates were comparable to those observed in the larger cohort of subjects administered conforming product. In the subjects receiving nonconforming products, pharmacokinetic (PK) expansion was higher in subjects with CRS/NT, subjects with high tumor burden or LDH levels.

C. Assessment of Outpatient Administration

[0901] Data for a total of eight (8) subjects were evaluated at this timepoint that had been treated in the outpatient setting (median age of 58.5 and ECOG of 0 or 1) at multiple clinical sites, including 3 subjects whose data was available subsequent to the time point analyzed for purposes of this Example. The mean length of hospitalization was 15.6 days for subjects treated in the inpatient setting (SD 9.6, n = 86) and 9.3 days for subjects treated in the outpatient setting (SD 11.9, n = 8). A 40% reduction in length of hospitalization was observed in subjects treated in the outpatient setting. The median number of days prior to hospitalization after outpatient CAR+ T cell administration was 5 days (range: 4-22). None required admission to the intensive care unit (ICU) after outpatient administration.

[0902] Among those of the 8 subjects treated in the outpatient setting with more than 28-day post-administration follow-up, 1 remained outpatient throughout the duration of the dose-limiting toxicity period. Seven (7) patients were admitted with fevers (1 on study day 4, the rest on study day \geq 5), 6 patients were admitted with CRS (4 grade 1, 2 grade 2) and 2 patients with grade 1 NT. No patient experienced severe CRS or NT. One (1) patient was treated with

tocilizumab without dexamethasone for CRS (grade 2), and no patients were treated with dexamethasone for CRS or NT. One patient was admitted 3 days after CAR+ T cell administration.

[0903] Among 91 subjects treated in the inpatient and outpatient settings, 11 subjects (12%) required ICU admission for management of toxicity; 8 subjects (9%) required ICU admission for management of CRS or NT; 2 subjects (2%) required ICU admission for management of acute respiratory events (one related to CAR+ T cell administration, one unrelated). Six (6%) subjects were intubated (based on safety database of reported events including additional subjects analyzed after the analysis timepoint described in this Example; n=94); 7 subjects (7%) received vasopressors (based on safety database of reported events, defined as exhibiting hypotension in the first 28 days after CAR+ T cell administration, in the TEAE assessment); and 2 subjects (2%) underwent hemofiltration (based on safety database of reported events). The results showed that very few patients required ICU-level care and associated procedures. The results supported the feasibility of outpatient administration, with safe management of toxicity in the outpatient setting, appropriate education and outpatient monitoring.

[0904] The assessment of outpatient administration supported the feasibility of safe outpatient administration. 30% of the subjects were not re-admitted.

D. Pharmacokinetic Assessment

[0905] Numbers of CAR⁺ T cells in peripheral blood and bone marrow at time points before administration (pre-treatment or pre-lymphodepleting chemotherapy (LDC)) and various time points post-treatment (with day of administration as day 1) in 87 subjects in the DLBCL cohort with evaluable PK, by flow cytometry using an antibody specific for the truncated receptor used as a surrogate marker, and quantitative polymerase chain reaction (qPCR) using primers specific for a woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) present in the vector encoding the chimeric antigen receptor (CAR). The area under the curve plotting numbers per microliter for the indicated CAR+ cell population between days 0 and 28 (AUC₀₋₂₈) and the maximum or peak blood concentration of CAR+ cells (C_{max}; CAR⁺ cells/ μ L blood) were assessed. B-cell aplasia was assessed in peripheral blood by flow cytometry, by staining with CD19. Cytokines were measured using a multiplex cytokine assay. For safety analysis, the data from all subjects receiving different dose levels were pooled. For response analysis, data were stratified by dose level. Statistical analysis was two-sided without multiplicity adjustment.

[0906] **FIG. 10A** shows detected numbers of CAR T cells per microliter of blood at various indicated time-points, as assessed by qPCR or flow cytometry. **FIG 10B** shows CAR+ cells per

microliter of blood versus microliter of bone marrow at day 11 ± 3 . As shown in **FIG. 10A**, levels of CAR-expressing cells in samples from subjects were observed both by flow cytometry-based assays and qPCR-based assays. As shown in **FIG. 10B**, all subjects (n= 86 and 85 for flow cytometry and qPCR, respectively, excluding one patient that did not have flow cytometry results available and 2 patients that did not have qPCR results available) with PK results assessed, showed detectable numbers of the CAR-expressing cells in the blood and bone marrow. Results were consistent with an observation that CAR+ T cells had trafficked similarly to the bone marrow and blood.

[0907] Levels over time of CD4+ and CD8+ CAR-expressing cells (as assessed by AUC_{0-28} and C_{max}) were compared in different patient subgroups receiving dose level 1 (DL1): diffuse large B-cell lymphoma de novo (DLBCL, NOS) or transformed from follicular lymphoma (tFL) (CORE; N=32), DLBCL transformed from marginal zone lymphoma or chronic lymphocytic leukemia (tMZL/tCLL; N=4), or mantle cell lymphoma (MCL; N=5), who had received CAR-expressing T cells at DL1. As shown in **FIGS. 11A** and **11B**, AUC_{0-28} and C_{max} varied among subjects in different disease subgroups, with expansion of CD4+ and CD8+ CAR-expressing cells trending lower in non-CORE subsets. PMBCL (n = 2) and FL3B (n = 1) not shown due to limited patient numbers. Expansion in subjects receiving DL2 was similar to in subjects receiving DL1.

E. Pharmacokinetic Assessment by Dose Level

[0908] AUC_{0-28} and C_{max} for CD3+, CD4+ and CD8+ CAR-expressing cells were also compared for subjects having received dose level 1 (DL1) and those having received dose level 2 (DL2), in the CORE cohort (subjects with DLBCL, NOS or high grade B-cell lymphoma (double/triple hit)). As shown in **FIGS. 12A** and **12B** and in **Table 28**, a higher median AUC_{0-28} was observed for CD3+, CD4+ and CD8+ CAR-expressing cells was observed in subjects that received DL2, compared to subjects who had received DL1. Similarly, a trend of higher expansion in subjects who had received DL2 was observed in the full DLBCL cohort. A higher durability of response (DOR) at 3 months also was observed among subjects who had received DL2 as compared to those having received DL1, without an increase in toxicity. The median time to C_{max} (T_{max}) for CD4+ and CD8+ CAR+ cells was similar between subjects who received DL1 and DL2.

[0909] Increased CAR+ T cell exposure was observed in DL2 versus DL1, corresponding to an increased durability of response without increased toxicity in DL2 subjects.

Table 28. Pharmacokinetics in Subjects Grouped by Dose Levels in Core cohort

	DL1S (n = 32)	DL2S (n = 27)	Total, DL1S and DL2S (n = 59)
CD3⁺			
C_{max}, median (cells/μL)	48.2	96.2	65.8
Q1, Q3	15.6, 151.3	30.2, 219.5	19.0, 204.2
Min, max	0.1, 7726.3	1.1, 1280.9	0.1, 7726.3
T_{max}, median (days)	14.5	15.0	15.0
Q1, Q3	11, 15	11, 15	11, 15
Min, max	9, 24	8, 31	8, 31
AUC₀₋₂₈, median (cells*day/μL)	477.7	823.1	542.4
Q1, Q3	165.9, 999.3	155.8, 3628.3	155.8, 3381.9
Min, max	1.8, 142816.7	16.5, 16087.8	1.8, 142816.7
CD4⁺			
C_{max}, median (cells/μL)	7.0	14.9	7.7
Q1, Q3	2.6, 46.0	2.0, 46.8	2.5, 46.8
Min, max	0.1, 3039.9	0.2, 169.4	0.1, 3039.9
T_{max}, median (days)	14.0	15.0	15.0
Q1, Q3	11, 15	11, 15	11, 15
Min, max	8, 24	8, 31	8, 31
AUC₀₋₂₈, median (cells*day/μL)	71.1	166.1	91.5
Q1, Q3	26.4, 274.7	18.1, 679.0	23.9, 368.8
Min, max	1.2, 68990.3	2.9, 4266.8	1.2, 68990.3
CD8⁺			
C_{max}, median (cells/μL)	26.1	62.8	43.6
Q1, Q3	3.7, 111.2	26.2, 171.7	9.1, 151.6
Min, max	0.0, 5237.6	0.7, 1261.8	0.0, 5237.6
T_{max}, median (days)	15.0	15.0	15.0
Q1, Q3	11, 16	11, 17	11, 16
Min, max	4, 28	8, 31	4, 31
AUC₀₋₂₈, median (cells*day/μL)	347.2	606.6	412.2
Q1, Q3	52.1, 871.4	155.7, 2463.4	72.1, 1852.5
Min, max	0.3, 81865.9	4.7, 15570.0	0.3, 81865.9

F. Persistence

[0910] Persistence of CAR-expressing cells and CD19⁺ B cell aplasia (low numbers or absence of CD19⁺ B cells) was assessed at various time points in evaluable subjects with DLBCL that had been administered CAR⁺ T cells, based on detectable CD3⁺, CD4⁺ or CD8⁺ CAR-expressing cell levels and levels of CD19⁺ B-cells detected in the blood, respectively. The results are set forth in **Table 29**. Among subjects evaluated at progression (time of progression regardless of BOR; n=37), a median of 0.17 CD4⁺ CAR⁺ cells/μL (range, 0-65.5 cells/μL) and a median of 0.15 CD8⁺ CAR⁺ cells/μL (range, 0-131.8 cells/μL) were observed at progression. Among subjects evaluated at relapse (at the time of progression after achieving CR) (n=12), a median of 0.17/μL (range, 0-35.1 cells/μL). CD4⁺ CAR-expressing cells and a median of 0.20

cells/ μL (range, 0-131.8 cells/ μL). CD8⁺ CAR-expressing cells were observed at relapse. Long-term persistence of CAR-expressing cells was observed in 75% of evaluable subjects with DLBCL at 12 months. Long-term persistence of B cell aplasia also was observed in 75% of the subjects at 12 months, and in subjects regardless of relapse status. The results are consistent with a conclusion that the anti-CD19 CAR-expressing cells exhibited long-term persistence in most subjects, and suggest the potential for ongoing, low-level disease control even in relapsed patients.

[0911] Of subjects who relapsed, 91.7% (11/12) had detectable CAR-expressing cells in the blood at the time of relapse. This result is consistent with a conclusion that a combination therapy or other intervention in some embodiments may be used to augment and/or boost CAR-expressing cells such as those that may be exhausted.

Table 29. CAR⁺ Cell Long-Term Persistence and CD19 Aplasia						
	Month 3	Month 6	Month 9	Month 12	At Progression	At Relapse
CAR T persistence in evaluable patients, n	50	30	18	12	37	12
CD3 ⁺ , %	100	80.0	77.8	75.0	91.9	91.7
CD4 ⁺ , %	88.0	63.3	50.0	41.7	83.8	83.3
CD8 ⁺ , %	90.0	70.0	55.6	50.0	83.8	75.0
CD19 ⁺ B-cell aplasia (< 1 cell/ μL), %	96.0	93.3	77.8	75.0	97.3	100

G. Pharmacokinetic Assessment and Toxicity

[0912] AUC₀₋₂₈ and C_{max} of CD4⁺ and CD8⁺ CAR-expressing cells was also compared for subjects in the core cohort with any grade (in this assessment, any of grade 1-4; no grade 5 CRS or NT observed) cytokine release syndrome (CRS) or neurotoxicity (NT) to subjects that were not assessed as exhibiting any grade of CRS or NT. The median CD4⁺ CAR⁺ AUC₀₋₂₈ (Q1, Q3) was 59 (18, 210) for no CRS (grade 0; n=43), and 267 (91, 1510) for any CRS (grades 1-4; n=20) (p = 0.001); the median CD8⁺ CAR⁺ AUC₀₋₂₈ (Q1, Q3) was 310 (36, 900) for no CRS (grade 0; n=43), and 605 (174, 5619) for any CRS (grades 1-4; n=20) (p = 0.021); the median CD4⁺ CAR⁺ AUC₀₋₂₈ (Q1, Q3) was 71 (23, 244) for no NT (grade 0; n=50), and 1269 (184, 3057) for any NT (grades 1-4; n=13) (p = 0.003); the median CD8⁺ CAR⁺ AUC₀₋₂₈ (Q1, Q3) was 304 (43, 799) for no NT (grade 0; n=50), and 2463 (607, 7691) for any NT (grades 1-4; n=13) (p = 0.004). As described above and shown in **FIGS. 13A-13D**, higher CD4⁺ and CD8⁺ CAR-expressing cell levels over time were associated with CRS and NT.

H. Pharmacokinetic Assessment and Response

[0913] The number of peak CD3⁺ CAR⁺ cells/ μ L (CD3+ C_{max}) was assessed over time in subjects who had a best overall response (BOR) of CR, PR or PD or a 3-month (M3) durable response of CR, PR or PD. As shown in **FIGS. 14** and **14B**, a trend towards better BOR was observed in subjects with higher expansion, with variability among subjects.

I. Pharmacokinetic Assessment by Blood Analytes and Patient Parameters

[0914] Pre-CAR⁺ T cell treatment (pre-lymphodepleting chemotherapy) plasma cytokine levels, including interleukin-7 (IL-7), IL-15, macrophage inflammatory protein (MIP-1 α), were assessed in subjects that exhibited a CAR+CD3+ blood C_{max} > 500 CAR+ T cells/ μ L (N=55) as compared to in subjects that exhibited CAR+CD3+ blood C_{max} < 500 CAR+ T cells/ μ L (N=7). As shown in **FIG. 15A**, elevated pre-CAR⁺ T cell treatment cytokine plasma levels were observed to be associated with CAR+CD3+ C_{max} > 500 CAR+ T cells/ μ L (Wilcoxon P values < .05 (without multiplicity of adjustment); except for IL-7 p = 0.07).

[0915] Peak levels of various plasma cytokines (IL-6, IL-10, IL-16, interferon gamma (IFN- γ), tumor necrosis factor alpha (TNF- α), MIP-1 α , MIP-1 β , Monocyte chemoattractant protein-1 (MCP-1), and C-X-C motif chemokine 10 (CXCL10)) were also assessed in subjects that exhibited CAR+ CD3+ blood C_{max} > 500 CAR+ T cells/ μ L (N=68) as compared to subjects that exhibited CAR+ CD3+ blood C_{max} < 500 CAR+ T cells/ μ L; N=9). As shown in **FIG. 15B**, higher peak cytokine levels were observed to be associated with CAR+CD3+ C_{max} > 500 CAR+ T cells/ μ L (Wilcoxon P values < 0.05; without multiplicity of adjustment).

[0916] Relationship between pre-CAR⁺ T cell treatment (pre-lymphodepleting chemotherapy (LDC)) volumetric tumor measurement sum of product dimensions (SPD), as an indicator of tumor burden, and AUC₀₋₂₈ of CD3⁺ CAR⁺ T cells, representing CAR⁺ T exposure over time, was assessed. As shown in **FIG. 16**, a positive correlation was observed between baseline SPD and CD3⁺ AUC₀₋₂₈, with a Spearman correlation of 0.32 and p = 0.019.

J. Pre-treatment patient parameters and response and toxicity outcomes

[0917] Pre-CAR⁺ T cell treatment (pre-LDC) analyte levels, including cytokines and inflammatory markers such as Ferritin, C-reactive protein (CRP), D-dimer (fibrin degradation product), IL-6, IL-10, IL-15, IL-16 TNF- α , MIP-1 α , and MIP-1 β , were compared for subjects with any grade (here, grade 1-4) cytokine release syndrome (CRS) or neurotoxicity (NT) to subjects that did not have any CRS or NT (grade 0). In this cohort, among subjects with CRS grade 1-4, all but one CRS events were determined to be grade 1 or 2. As shown in **FIG. 17A** (CRS) and **FIG. 17B** (NT), higher peak plasma cytokine levels and inflammatory marker levels were observed to be associated with CRS and NT, based on univariate analysis (Wilcoxon P

values < 0.05 for all analytes except ferritin for CRS ($p = 0.14$) and CRP for CRS ($p = 0.09$). For CRS, after adjusting tumor burden in a multivariable analysis, MIP-1 β , IL-10 and TNF had $p < 0.05$; for NT, IL-15, IL-6, MIP-1 α , and TNF had $p < 0.05$.

[0918] Pre-treatment (pre-LDC) patient parameters, such as levels of lactate dehydrogenase (LDH) and a volumetric tumor measurement such as sum of product dimensions (SPD), as an indicator of tumor burden, were compared between subjects that were not observed to have developed CRS or neurotoxicity versus subjects that were observed to have developed CRS or NT. As shown in **FIG. 18**, subjects with CRS or NT exhibited higher levels of pre-treatment patient parameters such as SPD (cm^2) and LDH (U/L) levels; such levels were observed to be correlated with CRS or NT, with univariate statistical analysis. Other patient parameters that were observed to be associated with CRS and NT include shorter time since diagnosis ($p=0.05$ and $p=0.09$, for CRS and NT, respectively). Patient parameters that were observed not to be associated with CRS or NT included age ($p=0.19$ and $p=0.54$, respectively) and prior numbers of therapies ($p=0.67$ and $p=0.59$, respectively), disease stage 0-2 vs 3-4 ($p=0.79$, $p=0.51$), and patient weight ($p=0.35$ and $p=0.44$, respectively).

[0919] **FIG. 19A** shows pre-treatment SPD and LDH levels among individual patients (dots; with shading of individual dots indicating whether the individual patients did or did not exhibit any grade neurotoxicity (left-hand panel) or did or did not exhibit any grade CRS (right-hand panel). In **FIG. 19A**, dotted lines on the y and x axes delineate $\text{SPD} \geq 50 \text{ cm}^2$ and $\text{LDH} \geq 500 \text{ U/L}$, respectively. As shown in **FIG. 19A**, an SPD of approximately 50 cm^2 or higher, and/or an LDH of approximately 500 U/L or higher, were observed to be associated with risk of NT and CRS. Calculated odds ratio estimates for developing CRS or NT in subjects above or below the SPD and LDH levels indicated by dotted lines in **FIG. 19A**, with 95% confidence intervals (CI), are depicted in **FIGS. 19B** and **19C**. An odds ratio over 1 indicated an increased probability or likelihood of developing CRS or NT. As shown, SPD of 50 cm^2 or higher, and LDH of 500 U/L or higher, were observed to be associated with increased risk of developing CRS or NT. SPD of 50 cm^2 or higher and LDH of 500 U/L or higher was observed to be associated with an approximately 8-fold increased risk in developing any grade CRS and NT, and SPD of lower than 50 cm^2 and LDH of lower than 500 U/L showed a reduced risk of any grade CRS and NT. The results were consistent with an association of baseline patient parameters, including high tumor burden and inflammatory biomarkers, with CAR+ T cell expansion and increased rates of CRS and neurotoxicity.

[0920] Various pre-treatment (pre-LDC) patient parameters, including markers associated

with tumor burden (SPD), inflammatory cytokines and other blood analytes, including LDH, ferritin, CRP, D-dimer, SAA-1, IL-6, IL-10, IL-15, IL-16, TNF- α , IFN- γ , MIP-1 α and CXCL10, were compared for subjects with and without a durable response at 3 months, with univariate statistical analysis. As shown in **FIG. 20**, certain markers of tumor burden, markers of inflammation or inflammatory cytokines were observed to be lower in subjects that exhibited a durable response (p value < 0.05 for all parameters except SPD ($p = 0.1274$)). Similar results were observed in subjects receiving DL2, when analyzed alone. An inverse association of baseline patient parameters, including high tumor burden and inflammatory biomarkers, with durable response was observed. In some aspects, such inverse association may be due to higher expansion and exhaustion of CAR+ T cells.

[0921] Relationships between patient factors, clinical correlates and blood analytes to developing of degrees of CRS and NT were assessed using statistical analysis based on univariate nonparametric tests. **Table 30** lists the results of the univariate analysis. In this assessment, age < 40 years and no prior HSCT correlated with incidence of CRS or NT. Subjects with age < 40 years were not observed to have statistically different rates of higher tumor burden than older patients. Subjects with ECOG score of 2 did not have statistically different rates of higher tumor burden compared to subjects with ECOG score 0-1. Those without prior HSCT or double/triple hit or double expressor were not associated with CRS or NT.

Table 30. Univariate Analysis of Key Subgroups						
Variable, n (%)	CRS			NT		
	Any Grade	Grade 1/2	Grade 3/4	Any Grade	Grade 1/2	Grade 3/4
FULL Population (N = 91)						
Age						
< 40 years (n = 8)	5 (63)	4 (50)	1 (13)	3 (38)	0	3 (38)
40-64 years (n = 49)	19 (39)	19 (39)	0	9 (18)	5 (10)	4 (8)
≥ 65 years (n = 34)	8 (24)	8 (24)	0	5 (15)	1 (3)	4 (12)
Pre-LD ECOG PS						
0-1 (n = 81)	28 (35)	27 (33)	1 (1)	15 (19)	4 (5)	11 (14)
2 (n = 10)	4 (40)	4 (40)	0	2 (20)	2 (20)	0
Double/triple hit or double expressor						
Yes (n = 30)	12 (40)	12 (40)	0	6 (20)	3 (10)	3 (10)
No (n = 22)	6 (27)	6 (27)	0	4 (18)	2 (9)	2 (9)
Prior HSCT						
Yes (n = 39)	10 (26)	10 (26)	0	5 (13)	4 (10)	1 (3)
No (n = 52)	22 (42)	21 (40)	1 (2)	12 (23)	2 (4)	10 (19)

K. Peak Blood Analytes, Response and Toxicity

[0922] Peak post-treatment plasma levels of blood analytes, including cytokines and

inflammatory markers such as CRP, Serum Amyloid A1 (SAA-1), IL-2, IL-6, IL-10, IL-15, TNF- α , MIP-1 α , MIP-1 β , MCP-1, CXCL10 and C-C Motif Chemokine Ligand 13 (CCL13) were compared for subjects with grade 1-4 cytokine release syndrome (CRS) or neurotoxicity (NT) to subjects that were not observed to have any CRS or NT. As shown in **FIG. 21A** (CRS; CRS grade 0, n=51; CRS grades 1-4, n=28) and **FIG. 21B** (NT; NT grade 0, n=63; NT grades 1-4, n=16), higher peak plasma cytokine levels and inflammatory marker levels were observed to be associated with CRS and NT (Wilcoxon P values < 0.001 for no CRS vs. any CRS and for no NT vs. any NT, except IL-15 (P= 0.05 and 0.006, respectively)).

[0923] Peak plasma levels of blood analytes, including cytokines and inflammatory markers such as CRP, SAA-1, IL-5, IL-6, IL-7, IL-8, IL-15, Lymphotoxin- α (LT- α), TNF- α , IFN- γ , MIP-1 α , MIP-1 β , MCP-1, CXCL10, and Transforming growth factor beta (TGF- β), were assessed for subjects with a best overall response (BOR) of complete response (CR) or partial response (PR) (N=57) compared to levels in subjects with stable disease (SD) or progressive disease (PD) (N=17); or for subjects with a 3-month SD or PD (SD/PD) (N=31), compared to subjects who exhibited CR/PR at 3-months (N=35). As shown in **FIG. 22A** (best overall response (BOR)) and **FIG. 22B** (month 3 response), lower peak plasma cytokine levels and inflammatory marker levels were observed to be associated with better BOR and response at month 3 (Wilcoxon P values < 0.05 without multiplicity of adjustment).

[0924] In this study, administration of the anti-CD19 CAR+ cell compositions was administered to subjects with relapsed/refractory aggressive non-Hodgkin lymphoma (NHL) that have poor-risk disease features. Responses, including durable responses, were observed, including 81% ORR, 63% CR at DL2, with 80% of patients in CR at 3 months remaining in CR at 6 months at all dose levels, median DOR of subjects treated at all dose levels of 9.2 months, with median duration of CR not having been reached at the time point of analysis in this Example. The results also were consistent with manageable toxicity levels and a favorable safety profile that in some embodiments may be consistent with outpatient administration. Low rates of severe CRS (1%) and severe neurotoxicity (12%) were observed, with few events in first 72 hours. Results were consistent with feasibility of outpatient administration.

[0925] Pharmacokinetic assessments showed that higher expansion of CAR+ T cells was generally associated with increased rates of CRS and NT. Subjects receiving DL2 showed higher CAR T exposure compared to subjects receiving DL1, which generally corresponded to increased durability of response without increased incidence of toxicity. In some aspects, pre-treatment, such as pre-LDC, patient factors, including homeostatic and inflammatory cytokines

and tumor burden, were observed to be associated with and/or drive very high expansion and toxicity. The administered CAR+ T cells were shown to expand in the blood and bone marrow of all patients, with variability among subjects and between disease types. The administered CAR+ T cells also exhibited long-term persistence, with 75% (9/12) of evaluable patients having detectable CAR T cells at 12 months. CAR T cells and B cell aplasia were observed to be still present at time of relapse (11/12 and 12/12 patients, respectively), supporting that tumors may evade CAR T cell action and that combination strategies may be effective to prevent relapse or augment, boost or enhance exhausted CAR T cells. In general, a trend of higher response was observed with higher expansion, with variability among subjects, supporting that other patient factors and/or disease characteristics, e.g., tumor burden, may contribute to determining response.

Example 6: Probability of Response, Durable Response and Toxicity Based on Peak CAR T Cell Numbers

[0926] Probabilities of response, durable response and toxicity were calculated based on the peak number of CAR+ expressing cells, in evaluable subjects in the core DLBCL population, after administration of anti-CD19 CAR-expressing cells, from the clinical study described in Examples 2-5 above. Subjects included those analyzed in the time point in Example 5.

[0927] An estimated probability curve of response (overall response rate, ORR; including subjects with complete response (CR) and partial response (PR)), 3-month response (M3 response; including CR and PR at month 3 after administration), any NT, any CRS, Grade 3-4 NT, Grade 3-5 NT or Grade 2-5 CRS, based on the maximum blood concentration of CD3+, CD4+ or CD8+ CAR-expressing cells (C_{\max} ; cells/ μ L blood). For the probability curves, linear logistic regression model fit was used, except CR/PR at month 3 for CD3+ and CR/PR at month 3 for CD8+, where quadratic model fit was used.

[0928] As shown in **FIG. 23A** (CD3+), **FIG. 23B** (CD8+) and **FIG. 23C** (CD4+), higher CD3+, CD8+ and CD4+ expansion was observed to correlate with increased rates of CRS, NT and response (ORR). Higher CD3+ and CD8+ expansion, was observed to result in a reduced probability of durable response (CR/PR at 3 months) at high C_{\max} .

[0929] The results are consistent with a conclusion that certain pre-treatment patient characteristics, including high tumor burden and high levels of inflammatory biomarkers, were associated with increased CRS and neurotoxicity, and increased CAR T cell expansion. Lower durable response was associated with very high levels of CAR+ cell numbers or expansion,

consistent with an observation that certain high degrees of CAR expansion may lead to exhaustion of highly expanding CAR+ cells.

[0930] In some embodiments provided herein, a therapeutic range or window of CAR+ T cell exposure or peak CAR+ T cell levels, is targeted and/or achieved by the methods or compositions or dosages administered, that does or is designed to minimize risk of toxicity and/or maximize or optimize likelihood of response and/or durability of response. In some embodiments, subjects with expansion or exposure below a certain level may be administered one or more additional interventions, such as to boost CAR-T function; in some embodiments, subjects exhibiting high levels of exposure or expansion (such as those associated with risk of toxicity and/or decreased likelihood of durability of response) may be administered one or more interventions, such as early or prophylactic measures, such as those for the purpose of reducing or limiting CAR+ T cell expansion and/or reduce toxicity or improve durability of response, such as based on one or more of the observed parameters.

[0931] In this study at this time-point, increased CAR+ T cell exposure and higher median expansion was observed in DL2 versus DL1, corresponding to an increased durability of response (DOR) without increased toxicity in DL2 subjects. The results are consistent with the conclusion that increased CAR+ cell expansion over a range correlated with durable responses, but that a very high degree of expansion can be associated with a higher risk of toxicity and/or lower durability of response. Certain patient-specific factors such as baseline patient factors, such as homeostatic and inflammatory cytokine levels and parameters indicative of tumor burden, can in some embodiments be associated with higher degrees of expansion and with increased risk of toxicity.

Example 7: Management of Symptoms of Toxicity

[0932] In the clinical studies described above, **FIG. 27** depicts the number and percentage of subjects that were observed to have cytokine release syndrome (CRS) and/or neurotoxicity (NT) at various time points after administration of CAR+ cells. In the assessment performed at the time point described in the clinical study described in Examples 2-6 above, in the full cohort (n=91), the median time to onset of first CRS or NT event was observed to be 5 (range 1-14) or 10 (range 3-23) days, respectively. Within the first 72 hours after CAR+ cell administration, 1 patient had NT (grade 1), and only 14% (13 of 91) had CRS (7 grade 1; 6 grade 2). The median duration (Q1,Q3) of CRS or NT was 5 (4, 8) or 10.5 (7, 19) days, respectively. NT was preceded by CRS in 12 of 17 cases (71%). All evaluable NT events were resolved at the time of

analysis except one grade 1 tremor and 2 patients died from progressive disease with ongoing NT (based on safety database of reported events including additional subjects analyzed after the analysis timepoint described in Examples 2-6 above). In the full cohort (n=91), certain subjects with onset of CRS or NT were administered anti-cytokine therapy with tocilizumab and/or dexamethasone as follows: Tocilizumab alone, 4% (n = 4); Dexamethasone alone, 9% (n = 8); Tocilizumab and dexamethasone, 8% (n = 7). The median number of dexamethasone doses was 6 (range, 2-99); and the median number of tocilizumab doses was 1 (range, 1-3).

[0933] In the core cohort (n=67), the median time to onset of CRS was 5 days (range, 2 –14) and NT was 11.5 days (range, 5 – 23). 13% (n = 9) received tocilizumab, and 18% (n = 12) received dexamethasone to ameliorate toxicity. Eighteen percent of subjects (12 of 67) exhibited neurotoxicity terms consistent with encephalopathy, including encephalopathy (13%), 6% (4 of 67) exhibited had aphasia and 3% (2 of 67) had seizures.

[0934] In some cases, subjects who show signs or symptoms of CRS or NT were treated according to the toxicity management algorithm described in **Table 31** below.

Table 31. Toxicity Management Algorithms		
	Management	
CRS^a	Tocilizumab	Dexamethasone
Any Grade		
Admit to hospital for observation, infectious work-up; antibiotics per institutional guidelines; symptomatic support; recommend seizure prophylaxis (e.g., Levetiracetam)		
Grade 1	None	None
≥ 96 hours after CAR+ T cell administration		
Grade 1	8 mg/kg every 24 hours x 1-2 doses	10 mg every 12-24 hours ≥ 3 days
≤ 96 hours after CAR+ T cell administration		
Grade 2	8 mg/kg every 24 hours x 1-2 doses	10 mg every 12-24 hours ≥ 3 days
Grade 3	8 mg/kg every 24 hours x 1-2 doses	10-20 mg every 12 hours ≥ 3 days
Grade 4	8 mg/kg every 24 hours x 1-2 doses	20 mg every 6 hours ≥ 3 days
	Management	
Neurotoxicity^b		
Grade 1		
Mild Symptoms	Admit to hospital for observation: <ul style="list-style-type: none"> • Rule out other causes of neurologic symptoms • Start anti-seizure medicines (e.g., levetiracetam) for seizure prophylaxis 	
Grade 2		
Moderate symptoms and/or limiting age-appropriate instrumental ADL	<ul style="list-style-type: none"> • Consider dexamethasone 10 mg IV every 12-24 hours • Continue dexamethasone use until the event is ≤ Grade 1, then taper over 3 days, if necessary 	
Grade 3		

Severe or Medically significant and/or limiting self care ADL	<ul style="list-style-type: none"> • Administer dexamethasone 10 mg IV every 12 hours • Continue dexamethasone use until the event is \leq Grade 1, then taper over at least 3 days
Grade 4	
Life-threatening symptoms	<ul style="list-style-type: none"> • Administer dexamethasone 10-20 mg IV every 6-12 hours • Continue dexamethasone use until the event is \leq Grade 1, then taper over at least 3 days
Any grade CRS or NT: In cases with very early onset (< 72 hours) and/or rapid progression, aggressive intervention with high dose steroids (20 mg every 6-12 hours or methylprednisolone) is recommended	

^a Grading per Lee et al, Blood. 2014;124(2):188-95.

^b CTCAE v4.03.

Example 8: Further Assessment of Response and Safety Outcomes in Subjects with Relapsed and Refractory Non-Hodgkin's Lymphoma (NHL) After Administration of Anti-CD19 CAR-Expressing Cells

[0935] Response and safety outcomes were assessed in patients at a subsequent point in time in the clinical study described in Examples 2-6 above.

A. Subjects and Treatment

[0936] The analysis at this time point presented in this example is based on assessment of a total of 102 subjects in the FULL cohort (73 in the CORE cohort) that had been administered the anti-CD19 CAR-expressing cells. The FULL cohort included subjects who had DLBCL (DLBCL, NOS; de novo or transformed from follicular lymphoma); high grade B-cell lymphoma (double/triple hit); DLBCL transformed from CLL or MZL; PMBCL; and FL3B, ECOG 0-2, after 2 lines of therapy; the CORE cohort for analysis included subjects having DLBCL, NOS (de novo or transformed from follicular lymphoma (tFL)) or high grade B-cell lymphoma (double/triple hit) and with Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1. Approximately 90% of treated patients in the FULL and the CORE cohort had at least one poor-risk disease feature predictive of short median overall survival (OS) of 3-6 months (see Crump et al., Blood (2017) 130:1800-1808 and Van de Neste et al., Bone Marrow Transplant. (2016) 51(1):51-7), such as double/triple hit expressors, primary refractory disease, refractory to 2 or more lines of therapy, never achieved CR, never received autologous stem cell transplant (ASCT) or an ECOG PS of 2.

[0937] At this time point, a total of 134 subjects had been leukapheresed, of which 2 had compositions unavailable. Product was available for 99% of apheresed subjects (132/134) in the DLBCL cohort. Of another 18 subjects whose products were available, 5 had withdrawn, and

13 had developed progressive disease or had died. A total of 114 subjects had been administered the anti-CD19 CAR-expressing cells, of which 12 received non-conforming anti-CD19 CAR-expressing cells (compositions not necessarily meeting certain specifications but deemed to be safe for administration). Subjects had received DL1 (n=45), double dose of DL1 (n=6) or DL2 (n=51). Seven (7) subjects with mantle cell lymphoma (MCL) had been administered CAR⁺ cells at DL1. At this time point, eight (8) subjects were treated in an outpatient setting.

[0938] The demographics and baseline characteristics of the FULL and CORE cohort subjects at the timepoint are set forth in **Table 32**.

Table 32. Patient Characteristics: DLBCL Cohort		
Characteristic	FULL (n = 102)	CORE (n = 73)
Median age (range), years	61 (20-82)	60 (20-82)
≥0 (20-82) (range)	37 (36)	24 (33)
B-NHL Subtype, n (%)		
DLBCL, NOS de novo	63 (62)	53 (73)
Transformed from FL (tFL)	23 (23)	20 (27)
Transformed from MZL (tMZL) /CLL (tCLL)	6 (6)/6 (6)	0
Follicular, grade 3B/PMBCL	1 (1)/3 (3)	0
Molecular Subtype, n (%)		
Double/triple hit ^a	19 (19)	16 (22)
Patient Characteristics, n (%)		
ECOG PS 0-1	93 (91)	73 (100)
IPI 3-5	43 (42)	26 (36)
CNS involvement	2 (2)	1 (1)
Chemorefractory ^b	71 (70)	49 (67)
Prior lines of therapy, median (range)	3 (1-8)	3 (2-8)
Never achieved CR	49 (48)	36 (49)
Any HSCT	41 (40)	28 (38)
Prior autologous	38 (37)	28 (38)
Prior allogeneic	5 (5)	0

HSCT, hematopoietic stem cell transplant. IPI, International Prognostic Index; SD, stable disease; WHO, World Health Organization.

^a At trial initiation, included in DLBCL, NOS histology; based on most recent WHO criteria (Swerdlow et al., (2016) Blood 127(20):2375-2390), are now considered high-grade B-cell lymphoma, with *myc* and *bcl2* and/or *bcl6* rearrangements with DLBCL histology (double/triple hit).

^b SD or PD to last chemotherapy-containing regimen or relapse < 12 months after autologous SCT.

B. Safety and Response Outcomes after Treatment

[0939] **Table 33** shows the safety outcome of the FULL and CORE cohort. As shown, no

deaths from CRS or NT were observed. In the FULL cohort, the median time to onset of CRS was 5 days (range, 2–12 days) and NT was 10 days (range, 3–23 days). In the FULL cohort, 17% (n = 17) received tocilizumab and 21% (n = 21) received corticosteroids as a toxicity intervention. In the CORE cohort, no increase in CRS or NT was observed at DL2, compared to DL1. In the full cohort, 19 (19%) subjects had Grade 1 CRS, 18 (18%) subjects had Grade 2 CRS, 0 (0%) subjects had Grade 3 CRS, and 1 (1%) subject had Grade 4 CRS.

Table 33. Safety Outcomes After CAR⁺ Cell Administration

	FULL	CORE		
	All Dose Levels n = 102	All Dose Levels ^a n = 73	DL1S n = 33	DL2S n = 37
CRS, n (%)				
Any grade	38 (37)	27 (37)	14 (42)	11 (30)
Grade 1/2	37 (36)	26 (36)	13 (39)	11 (30)
Grade 3/4 (sCRS)	1 (1)	1 (1)	1 (3)	0
Neurotoxicity, n (%)				
Any grade	23 (23)	18 (25)	8 (24)	9 (24)
Grade 1/2	10 (10)	7 (10)	1 (3)	6 (16)
Grade 3/4 (sNT)	13 (13)	11 (15)	7 (21)	3 (8)
Any, n (%)				
CRS or NT	44 (43)	32 (44)	15 (45)	15 (41)
sCRS or sNT	13 (13)	11 (15)	7 (21)	3 (8)

^a Three patients treated on DL1D (dose level 1, two-dose schedule) with similar outcomes.

[0940] FIG. 28 depicts the percentage of subjects in the FULL cohort at this timepoint (n = 102) who were observed to have experienced laboratory abnormalities and treatment-emergent adverse events (TEAEs) (data for 6 subjects with MCL treated with conforming product at DL1 with at least 28 days of follow-up are not included; showing TEAEs and laboratory abnormalities occurring in 20% or more of the subjects).

[0941] As shown in Table 34, high rates of response was observed in subjects with relapsed or refractory (R/R) DLBCL. The results are consistent with a dose response effect on treatment outcome in the CORE cohort. Subjects with a tumor burden above a threshold (as indicated by the volumetric tumor measurement of sum of product dimensions (SPD) of more than 50 cm²) was similarly distributed between subjects receiving DL1 and DL2 (approximately 1/3 of the subjects in each group).

Table 34. Response After CAR⁺ Cell Administration

	FULL	CORE		
	All Dose Levels	All Dose Levels ^a (n=73)	DL1S (n=33)	DL2S (n=37)

	(n=102)			
ORR (95% CI), %	75 (65-83)	80 (68-88)	79 (61-91)	78 (62-90)
CR (95% CI), %	55 (45-65)	59 (47-70)	55 (36-72)	62 (45-78)
3-mo ORR (95% CI), %	51 (41-61)	59 (47-70)	52 (34-69)	65 (48-80)
3-mo CR (95% CI), %	38 (29-48)	45 (34-57)	36 (20-55)	51 (34-68)
6-mo ORR (95% CI), %	40 (31-50)	47 (35-59)	42 (26-61)	49 (32-66)
6-mo CR (95% CI), %	34 (25-44)	41 (30-53)	33 (18-52)	46 (30-63)

^a Three patients treated on DL1D (dose level 1, two-dose schedule) with similar outcomes.

[0942] Six-month objective response rates (ORR) among various subgroups of subjects in the poor-risk DLBCL subgroups, that included all DLBCL patients treated at all dose levels in the CORE cohort, are shown in **FIG. 29**. The results showed high durable ORR in the poor-risk DLBCL subgroup for anti-CD19 CAR+ T cell administration.

[0943] Results for the duration of response (DOR, with median follow-up of 8 months) and overall survival (grouped by best overall response (non-responder, CR/PR, CR and/or PR), with median follow-up of 12 months) are shown for the full cohort and the core cohort cohorts of subjects, in **FIGS. 30A-30D**. The results showed that in the CORE cohort, 88% of subjects with CR at 3 months continued to show CR at 6 months, and 93% of subjects who exhibited CR at 6 months continued to show a response longer term.

[0944] The results were consistent with an observation that administration of anti-CD19 CAR+ cell compositions that contains a precise and consistent dose of CD4⁺ and CD8⁺ CAR+ T cells results in durable response in subjects with R/R aggressive NHL with poor prognosis and/or heavy pretreatment. The results showed a favorable durable response rate in the CORE cohort, with 49% ORR and 46% CR rate at 6 months, and 93% of the subjects (at all dose levels) in CR at 6 months remained in response at this time point. The results also were consistent with manageable toxicity and a favorable safety profile, including low rates of severe CRS (1%) and severe neurotoxicity (13%), which, in some aspects, supports outpatient administration.

Example 9: Early Intervention with High-dose Steroids for Management of Neurotoxicity

[0945] A total of 38 subjects were administered a T cell composition containing autologous T cells expressing an anti-CD19 chimeric antigen receptor (CAR) for treatment of adult ALL. At the start of treatment, of the 38 subjects, thirty-two (32) had exhibited morphologic evidence of disease in bone marrow (at least 5% blasts) and six (6) had exhibited molecularly detectable

disease by PCR. The therapeutic T cell compositions administered had been generated by a process including immunoaffinity-based selection of T cells (including CD4+ and CD8+ cells) from leukapheresis samples from the individual subjects, followed by activation and transduction with a viral vector encoding the anti-CD19 CAR, expansion and cryopreservation. The CAR contained an anti-CD19 scFv derived from a murine antibody, a region of CD28 including an extracellular region, a transmembrane domain and a costimulatory region, and a CD3-zeta intracellular signaling domain. The cryopreserved cell compositions were thawed at bedside prior to intravenous administration.

[0946] Cells were administered at a first target dose of approximately 1.0×10^6 CD3+CAR+ cells/kg (subject body weight), followed approximately 14-28 days later by a second dose of approximately 3.0×10^6 CD3+CAR+ cells/kg (subject body weight).

[0947] Before administration of autologous CAR-expressing cells, subjects had been administered a preconditioning lymphodepleting chemotherapy containing either a single dose of cyclophosphamide (about $1\text{-}3 \text{ g/m}^2$) only or cyclophosphamide ($30\text{-}60 \text{ mg/kg}$) and fludarabine ($25 \text{ mg/m}^2\text{-}30 \text{ mg/m}^2$, administered daily over three days).

[0948] Subjects were monitored for response and other outcomes, including by examination of bone marrow, peripheral blood and cerebrospinal fluid (CSF). Subjects also were assessed and monitored for neurotoxicity (neurological complications including symptoms of confusion, aphasia, encephalopathy, myoclonus seizures, convulsions, lethargy, and/or altered mental status), graded on a 1-5 scale, according to the National Cancer Institute—Common Toxicity Criteria (CTCAE) scale, version 4.03 (NCI-CTCAE v4.03). Common Toxicity Criteria (CTCAE) scale, version 4.03 (NCI-CTCAE v4.03). See Common Terminology for Adverse Events (CTCAE) Version 4, U.S. Department of Health and Human Services, Published: May 28, 2009 (v4.03: June 14, 2010); and Guido Cavaletti & Paola Marmioli *Nature Reviews Neurology* 6, 657-666 (December 2010). Cytokine release syndrome (CRS) also was determined and monitored, graded based on severity.

[0949] Samples derived from blood from subjects obtained prior to each dose (Day 1) and at various time points following administration of the first dose (on Days 2, 4, 7, and 14 after first dose) and the second dose (on Days 21 and 28 and Months 2, 3, 6 and 12 after second dose) were analyzed by flow cytometry, for pharmacokinetic parameters, including the presence and number of CD3+ CAR-expressing cells in the blood, maximum (peak) plasma concentrations (C_{\max}) and the time point at which C_{\max} is achieved (T_{\max}), of CD3+CAR+ cells.

[0950] A total of 34 subjects had detectable CD3+ CAR+ T cells in the blood following

administration of the cells, and 29 of the 34 subjects exhibited maximal cell expansion (C_{\max}) of CD3+ CAR+ T cells after the first administration. Four of the five subjects who had maximal expansion after the second administration exhibited neurotoxicity of lower than Grade 2.

[0951] One of the subject who had maximal expansion after the second dose exhibited severe neurotoxicity (prolonged Grade 3 neurotoxicity, as determined by duration of symptoms observed for 10 days or longer) after the second dose. Before administration of the CAR+ T cells, this subject exhibited morphological disease, and had received 2 prior lines of therapy. The subject received high-dose fludarabine/cyclophosphamide lymphodepleting chemotherapy and a first dose of 1.1×10^6 CD3+ cells/kg. The subject did not respond to the first dose of CAR+ T cell administration, as shown by tumor burden (30% blasts) at Day 14 after administration of the first dose, at which point the subject did not exhibit symptoms of neurotoxicity or severe cytokine release syndrome (CRS). The subject then received a fludarabine/high dose cyclophosphamide lymphodepleting therapy and a second dose of 3.3×10^6 cells/kg.

[0952] On Day 7 after administration of the second dose, the subject exhibited a rapid increase in CAR+ T cell levels (see **FIG. 31**), to levels similar to those observed in subjects with prolonged Grade 3 and Grade 5 neurotoxicity following administration of a first dose of the CAR+ T cells. The subject exhibited grade 2 cytokine release syndrome (CRS), and exhibited a fever of 39°C, 2 days after administration of the second dose. Other toxicities observed included Grade 3 confusion (duration: 11 days), Grade 3 expressive aphasia (duration: 6 days) and Grade 3 encephalopathy (duration: 4 days). cm_{\max} of CD3+CAR+ for this subject was observed to be 75.3 cells/ μ L, and the T_{\max} was at Day 29 after administration of the second dose.

[0953] As an early intervention to manage symptoms of the cytokine release syndrome (CRS) and neurotoxicity (NT), on various days after administration of the second dose, dexamethasone was administered at a dose ranging from 10 mg to 40 mg daily, as shown in **FIG. 31**. Administration of a high dose (up to 40 mg daily) dexamethasone and maintaining administration of dexamethasone until Day 14 was associated with a subsequent attenuation of expansion of CAR+ T cells (see **FIG. 31**). Dexamethasone was tapered down and discontinued after Day 14, and an increase in CAR+ T cell expansion was observed after the tapering of dexamethasone. The subject's severe neurotoxicity resolved completely and did not develop cerebral edema.

[0954] The results are consistent with an observation that early intervention with high-dose dexamethasone may reduce excessive expansion of administered CAR+ T cells and may prevent

certain toxicities, such as fatal cerebral edema.

Example 10: Management of Symptoms of Toxicities, including Cytokine Release

Syndrome and Neurotoxicity

[0955] In the clinical studies described above in Example 8, management of cytokine release syndrome (CRS) in some cases were handled generally as described in **Table 35** below.

Table 35. Toxicity Management Algorithms		
	Management	
CRS^a	Tocilizumab	Dexamethasone
Grade 1 Onset < 72 hours after CAR+ T cell administration	8 mg/kg every 24 hours	Optionally 10 mg every 24 hours
Grade 2 Onset < 72 hours after CAR+ T cell administration	8 mg/kg every 12-24 hours	10 mg every 12-24 hours
Grade 2 Onset ≥ 72 hours after CAR+ T cell administration	8 mg/kg every 12-24 hours	Optionally 10 mg every 24 hours
Grade 3	8 mg/kg every 12 hours	10 mg every 12 hours
Grade 4	8 mg/kg every 6 hours	10 mg every 6 hours

^a Grading per Lee et al, Blood. 2014;124(2):188-95.

[0956] In some cases, management of CRS and neurotoxicity (NT) were handled based on an exemplary toxicity management algorithm generally as described in **Table 36** (CRS) and **Table 37** (NT) below.

Table 36. Exemplary Guidelines for Administering Agents for Modulating Cell Therapy for Cytokine Release Syndrome (CRS)	
After CAR+ T cell administration	Monitoring: <ul style="list-style-type: none"> • Monitor for CRS symptoms (fever, hemodynamic instability, hypoxia) with neurologic evaluations • Follow serum CRP, ferritin, and coagulation parameters • Consider hospitalization for close monitoring
If onset of fever ≥ 38 °C/ 100.4 °F ≥ 72 hours post CAR+ T cell administration:	Monitoring: <ul style="list-style-type: none"> • Check absolute neutrophil count (ANC), evaluate fever, rule out infection (surveillance cultures) • Admit for/continue close monitoring of cardiac and organ function, including routine neurologic exams • Follow serum C-reactive protein (CRP), ferritin and coagulation parameters (international normalized ratio (INR), partial thromboplastin time (PTT), fibrinogen) • Symptomatic support (e.g. antipyretics, analgesics), antibiotics as per institutional guidelines (febrile neutropenia)
If rapid onset of CRS signs or symptoms (defined as fever ≥ 38.5 °C/ 101.3°F seen < 72 hours post-CAR+ T cell administration or any signs or symptoms defining	First line treatment: <p><u>Grade 1:</u></p> <ul style="list-style-type: none"> • If slow onset (≥ 72h), treat symptomatically • If rapid onset(< 72h), consider tocilizumab 8 mg/kg IV ± dexamethasone 10 mg q24h <p><u>Grade 2:</u></p>

CRS Grade ≥ 2 ; or If clinical progression of CRS or rapid deterioration when monitoring after onset of fever, initiate 1st line treatment	<ul style="list-style-type: none"> • If slow onset (≥ 72h), give tocilizumab 8 mg/kg IV \pm dexamethasone 10 mg IV q12-24h if needed • If rapid onset (< 72h), give tocilizumab 8mg/kg IV and dexamethasone 10 mg IV q12-24h <p><u>Grade 3:</u></p> <ul style="list-style-type: none"> • Give tocilizumab 8 mg/kg IV and dexamethasone 10 mg IV q12h <p><u>Grade 4:</u></p> <ul style="list-style-type: none"> • Give tocilizumab 8 mg/kg IV and dexamethasone 20 mg IV q6
If no improvement with 1st line treatment within 24 hours or rapid progression of CRS, initiate 2nd line treatment	<p>Second line Treatment:</p> <ul style="list-style-type: none"> • Give 2nd dose of tocilizumab 8 mg/kg IV and dexamethasone 20 mg IV q6-12h • Consider other causes for clinical deterioration (e.g. sepsis, adrenal insufficiency)
If no improvement with 2nd line treatment within 24 hours or rapid progression of CRS, initiate 3rd line treatment	<p>Third line treatment:</p> <ul style="list-style-type: none"> • Methylprednisolone 2 mg/kg followed by 2 mg/kg divided 4 times per day (taper within 7 days) • Consider other anti-IL-6 agents
If ongoing CRS despite prior therapies initiate 4th line treatment	<p>Fourth line treatment:</p> <ul style="list-style-type: none"> • Consider anti-T cell therapies such as cyclophosphamide (1.5 mg/m²) or others
<p>Other considerations:</p> <ul style="list-style-type: none"> • Once dexamethasone is initiated, give for a minimum of 3 doses or until resolution of CRS and any associated neurological symptoms • Grade 1: consider seizure prophylaxis (e.g. levetiracetam) • Grade 2: frequent inpatient monitoring until fever and symptom resolution, include neurologic evaluations and symptomatic support (supplemental oxygen, IV fluids with aggressive electrolyte replacement, antipyretics, low-dose vasopressor support); initiate seizure prophylaxis (e.g. levetiracetam) and consider electroencephalogram (EEG) monitoring if concurrent neurotoxicity (NT); also see Table 37 below for NT management algorithms • Grade ≥ 3: ICU-level monitoring and symptomatic, hemodynamic, and respiratory support, include neurologic exams; initiate seizure prophylaxis (e.g. levetiracetam) and consider EEG monitoring if concurrent NT; also see Table 37 below for NT management algorithms 	

Table 37. Exemplary Guidelines for Administering Agents for Modulating Cell Therapy for Neurotoxicity (NT)

After CAR+ T cell administration	<p>Monitoring:</p> <ul style="list-style-type: none"> • Monitor for NT symptoms (aphasia, confusion, altered mental status) • Consider seizure prophylaxis (e.g., levetiracetam) for subjects at high risk of NT
Early onset NT (event onset < 72 hours):	<p>First line treatment:</p> <ul style="list-style-type: none"> • Start seizure prophylaxis • <u>Grade 1</u>: consider dexamethasone 10 mg q8-12h • <u>Grade 2</u>: dexamethasone 10 mg q8-12h • <u>Grade 3</u>: dexamethasone 20 mg q6-8h • <u>Grade 4</u>: dexamethasone 20 mg q6h

Late Onset NT (event onset \geq 72 hours)	First line treatment: <ul style="list-style-type: none"> • Start seizure prophylaxis • <u>Grade 1</u>: Observe • <u>Grade 2</u>: Consider dexamethasone 10 mg q12-24h • <u>Grade 3</u>: Give dexamethasone 10-20 mg q8-12h; use lower doses/longer interval for aphasia or confusion and higher doses/longer interval for events leading to depressed level of consciousness. High-dose corticosteroids are not recommended for isolated Grade 3 headaches • <u>Grade 4</u>: Give dexamethasone 10-20 mg q6-8h; use higher dose/shorter interval for events requiring respiratory support or seizures
If no improvement with 1st line treatment within 24 hours or worsening of NT, initiate 2nd line treatment	Second line treatment: <ul style="list-style-type: none"> • Increase dose and/or frequency of dexamethasone • Consider methylprednisolone (2 mg/kg loading dose followed by 2 mg/ kg divided 4 times per day (taper within 7 days)) if life-threatening complications arise (require respiratory support or if seizures)
If no improvement with 2nd line treatment within 24 hours or symptoms progress rapidly initiate 3rd line treatment	Third line treatment: <ul style="list-style-type: none"> • Further increase dose and/or frequency of dexamethasone • Give methylprednisolone if at maximum doses of dexamethasone
If cerebral edema occurs	Cerebral edema: <ul style="list-style-type: none"> • Give high-dose methylprednisolone (1-2 g, repeat q24 if needed). Taper as clinically indicated. Consider hyperventilation and hyperosmolar therapy
Other considerations: <ul style="list-style-type: none"> • Hospitalize for monitoring if subject is an outpatient upon start of event; initiate neurologic consultation • If concurrent with CRS, treat CRS per CRS management algorithms (e.g., as indicated in Table 36 above) in addition to NT recommendations; use the most aggressive interventions recommended between the two algorithms • Consider other causes of neurologic symptoms (e.g., infection, metabolic syndrome, disease progression, medications) • Steroids could be continued for a minimum of 48 hours; consider longer course with potential taper for a total of 5 to 7 days for higher grade or persistent/recurrent symptoms • Imaging (magnetic resonance imaging (MRI) or computed tomography (CT) scan), electroencephalogram (EEG) and lumbar puncture (LP) should be done and imaging repeated if no clinical improvement; continuous monitoring by EEG should be considered • For subjects who have seizures or seizure-like activity, antiepileptic drugs are recommended; antiepileptic drug combinations may be required for multiple or refractory seizure activity • intensive care unit (ICU) monitoring may be required; mechanical ventilation for airway protection may be indicated 	

[0957] 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	ESKYGPPCPPCPGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHY TQKSLSLSLGK	Hinge-CH3 spacer Homo sapiens
4	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSQEDPE VQFNWYVDGVEVHNAKTKPREEQFNSTYRVSVLTVQLHQLDNLNGKEYCKVSNK GLPSSIEKTIISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHN HYTQKSLSLSLGK	Hinge-CH2-CH3 spacer Homo sapiens
5	RWPESPKAQASSVPTAQQAEGSLAKATTAPATTRNTGRGGEEKKKEKEKEEQE ERETKTPECPSTQPLGVYLLTPAVQDLWLRDKATFTCFVVGSDLKDAHLTWEV AGKVPTGGVEEGLLERHSNGSQSHSRLTLPRSLWNAGTSVTCTLNHPSLPPQR LMALREPAAQAPVKLSLNLLASSDPPEAASWLLCEVSGFSPPNILLMWLEDQRE VNTSGFAPARPPPQPGSTTFWAWSVLRVPAPPSPQPATYTCVVSHEDSRTLLNA SRSLEVSIVTDH	IgD-hinge-Fc Homo sapiens
6	LEGGGEGRGSLLTCGDVEENPGPR	T2A artificial
7	MLLLVTSLLLCELPHPAFLLIPRKVCNGIGIGIEFKDSLSINATNIKHFKNCTSI SGDLHILPVAFRGDSFTHTPPLDPQELDILKTVEKITGFLLIQAWPENRTDLHA FENLEIIRGRTKQHGGFSLAVVSLNITSLGLRSLKEISDGDVVISGNKNLCYAN TINWKKLFGTSGQTKIISNRGENSCKATGQVCHALCSPEGCWGPEPRDCVSCR NVSRGRECVDKCNLLEGEPRFVENSECIQCHPECLPQAMNITCTGRGPDNCIQ CAHYIDGPHCVKTCPAGVMGENNTLVWKYADAGHVCHLCHPNCTYGCTGPGLEG CPTNGPKIPSIATGMVGALLLLLVVALGIGLFM	tEGFR artificial
8	FWVLVVVGVLACYSLLVTVAFIIFWV	CD28 (amino acids 153-179 of Accession No. P10747) Homo sapiens
9	IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFLFPGPSKPFWVLVVVGVLACYS LLVTVAFIIFWV	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	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL	4-1BB (amino acids 214-255 of Q07011.1) Homo sapiens
13	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPRKPNP QEGLYNELQDKMAEAYSEIGMKGERRRGKGDGLYQGLSTATKDTYDALHMQA LPPR	CD3 zeta Homo sapiens

14	RVKFSRSAEPPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP QEGLYNELQKDKMAEAYSEIGMKGERRRGKGDGLYQGLSTATKDTYDALHMQA LPPR	CD3 zeta Homo sapiens
15	RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP QEGLYNELQKDKMAEAYSEIGMKGERRRGKGDGLYQGLSTATKDTYDALHMQA LPPR	CD3 zeta Homo sapiens
16	RKVCNGIGIGIEFKDLSINATNIKHFKNCTISISGDLHILPVAFRGDSFTHTPPL DPQELDILKTVEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGGQFSLAVV SLNITSLGLRSLKEISDGDVVISGNKNLCYANTINWKKLFGTSGQKTKIISNRG ENSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLEGEPREF VENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVMGEN NTLVWKYADAGHVCHLCHPNCTYGCTGPGLEGCPNTPGKIPSIATGMVGALLLL LVVALGIGLFM	tEGFR artificial
17	EGRGSLTTCGDVEENPGP	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	GSADDAKKDAAKKGKS	Linker
24	GSTSGSGKPGSGEGSTKG	Linker
25	gacatccagatgacccagaccacctccagcctgagcgccagcctgggcgacccgg gtgaccatcagctgccgggcccagccaggacatcagcaagtacctgaactggtat cagcagaagccccgacggcaccgtcaagctgctgatctaccacaccagccggctg cacagcggcgctgccagccgggttagcggcagcggctccggcaccgactacagc ctgaccatctccaacctggaacaggaagatatcgccacctacttttgccagcag ggcaacacactgacctacacctttggcgggcggaacaaagctggaaatcacccggc agcacctccggcagcggcaagcctggcagcggcgagggcagcaccaagggcgag gtgaagctgcaggaaagcggccctggcctggtggccccccagccagagcctgagc gtgacctgcaccgtgagcggcggtgagcctgccgactacggcggtgagctggatc cggcagccccccaggaaggcgctggaatggctggcggtgatctggggcagcgag accacctactacaacagcgccctgaagagcggcgtgaccatcatcaaggacaac agcaagagccaggtgttctgaagatgaacagcctgcagaccgacgacaccgcc atctactactgcgccaagcactactactacggcggcagctacgccatggactac tggggccagggcaccagcgtgaccgtgagcagc	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	ELKTPLGDTHTCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTP PPCPRCP	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	EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGS ETTYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMD YWGQGTSTVTVSS	VH
42	DIQMTQTSSLSASLGDRVTISCRASQDISKYLWYQQKPDGTVKLLIYHTSRL HSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGKLEIT	VL
43	DIQMTQTSSLSASLGDRVTISCRASQDISKYLWYQQKPDGTVKLLIYHTSRL HSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGKLEITG STSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWI RQPPRKGLEWLGVIWGSSETTYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTA IYYCAKHYYYGGSYAMDYWGQGTSTVTVSS	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	EVKLQQSGAELVRPGSSVKISCKASGYAFSSYWMNWVKQRPQGQLEWIGQIYPG DGDITNYNGKFKGQATLTADKSSSTAYMQLSGLTSEDSAVYFCARKTISSVVDY FDYWGQGTITVTVSS	VH
51	DIELTQSPKFMSTSVGDRVSVTCKASQNVGTNVAWYQQKPGQSPKPLIYSATYR NSGVPDRFTGSGSGTDFTLTITNVQSKDLADYFCQQYNRYPYTSGGGTKLEIKR	VL
52	GGGSGGGSGGGGS	Linker
53	EVKLQQSGAELVRPGSSVKISCKASGYAFSSYWMNWVKQRPQGQLEWIGQIYPG DGDITNYNGKFKGQATLTADKSSSTAYMQLSGLTSEDSAVYFCARKTISSVVDY FDYWGQGTITVTVSSGGGSGGGSGGGSGGGSDIELTQSPKFMSTSVGDRVSVTCKA SQNVGTNVAWYQQKPGQSPKPLIYSATYRNSGVPDRFTGSGSGTDFTLTITNVQ SKDLADYFCQQYNRYPYTSGGGTKLEIKR	scFv
54	HYYYGGSYAMDY	CDR H3
55	HTSRLHS	CDR L2
56	QQGNTLPYT	CDR L3
57	ACACGGCCTCGTGTATTACTGT	IGH primer
58	ACCTGAGGAGACGGTGACC	IGH Primer

CLAIMS

WHAT IS CLAIMED:

1. A method of ameliorating a toxicity, comprising administering, to a subject exhibiting one or more physical signs or symptom of a toxicity, one or more agent capable of reducing and/or ameliorating the one or more physical signs or symptoms associated with the toxicity, said subject having been administered a dose of genetically engineered cells comprising T cells expressing a recombinant receptor, wherein the one or more agent is administered in a treatment regimen comprising:

(a) administering one or more agent if:

(i) at or greater than 72 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits a fever, and exhibits one or more physical signs or symptoms associated with the toxicity, optionally cytokine release syndrome (CRS), and/or exhibits a rapid progression of the physical signs or symptoms associated with the toxicity; or

(ii) within 48 or 72 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits a fever and/or one or more physical signs or symptoms associated with grade 2 or higher CRS;

(b) administering one or more agent if, within 24, 48 or 72 hours after administration of the one or more agent in (a), the subject does not exhibit an improvement of the fever and/or the one or more physical signs or symptoms associated with the toxicity and/or exhibits a rapid progression of the physical signs or symptoms associated with the toxicity, which one or more agent optionally are different from the one or more agent administered in (a) and/or is administered at the same or higher dose and/or frequency as the one or more agent administered in (a);

(c) administering one or more agent if, within 24, 48 or 72 hours after administration of the one or more agent in (b), the subject does not exhibit an improvement of the fever and/or the one or more physical signs or symptoms associated with the toxicity and/or exhibits a rapid progression of the physical signs or symptoms associated with the toxicity, which one or more agent optionally are different from the one or more agent administered in (a) or (b) and/or is administered at the same or higher dose and/or frequency as the one or more agent administered in (a) or (b); and

(d) administering one or more agent if, after administration of the one or more agent in (c), the subject does not exhibit an improvement of the fever and/or the one or more physical

signs or symptoms associated with the toxicity, which one or more agent optionally are different from the one or more agent administered in (a), (b) or (c) and/or is administered at the same or higher dose and/or frequency as the one or more agent administered in (a), (b) or (c).

2. The method of claim 1, wherein the one or more agent is selected from an agent capable of binding an interleukin-6 receptor (IL-6R) or one or more steroid, optionally one or more doses of the one or more steroid.

3. The method of claim 1 or claim 2, wherein, in (a), the treatment regimen comprises:

(1) if, within 48 or 72 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits a fever and/or one or more first physical signs or symptoms associated with grade 1 CRS, administering (i) an agent capable of binding an interleukin-6 receptor (IL-6R) , said agent administered no more than once every 24 hours, and optionally (ii) one or more doses of a steroid, said steroid administered no more than once every 24 hours;

(2) if within 48 or 72 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits one or more physical signs or symptoms associated with grade 2 CRS, administering (i) an agent capable of binding an IL-6R , said agent administered about every 12 to 24 hours, and (ii) one or more doses of a steroid, said steroid administered about every 12 to 24 hours; and if at or greater than 72 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits one or more physical signs or symptoms associated with grade 2 CRS; administering (i) an agent capable of binding an IL-6R , said agent administered about every 12 to 24 hours, and optionally (ii) one or more doses of a steroid, said steroid administered about every 12 to 24 hours;

(3) if the subject exhibits one or more physical signs or symptoms associated with grade 3 CRS after receiving a dose of the genetically engineered cells, administering (i) an agent capable of binding an IL-6R , said agent administered at least twice a day, optionally at least about every 12 hours, and (ii) one or more doses of a steroid, said steroid administered at least twice a day, optionally at least about every 12 hours; and

(4) if the subject exhibits one or more physical signs or symptoms associated with grade 4 CRS after receiving a dose of the genetically engineered cells, administering (i) an agent capable of binding an IL-6R , said agent administered at least twice a day, optionally at least

about every 6 hours, and (ii) one or more doses of a steroid, said steroid administered at least twice a day, optionally at least about every 6 hours.

4. The method of any of claims 1-3, wherein, in (b), the treatment regimen comprises administering an additional dose of the agent capable of binding an IL-6R and one or more additional doses of the steroid, said steroid administered at least twice a day, optionally at least about every 6 hours.

5. The method of any of claims 1-4, wherein, in (c), the treatment regimen comprises administering an additional steroid that is different from the one or more agent administered in (a) or (b) and/or administering an agent capable of binding an IL-6R or an IL-6, that is different from the one or more agent administered (a) or (b).

6. The method of any of claims 1-5, wherein, in (d), the treatment regimen further comprises administering an anti-T cell therapy, optionally cyclophosphamide.

7. The method of any of claims 1-6, wherein the agent capable of binding IL-6R is administered in one or more doses.

8. A method of ameliorating a toxicity, comprising administering, to a subject exhibiting one or more physical signs or symptom of a toxicity, one or more agent capable of reducing and/or ameliorating the one or more physical signs or symptoms associated with the toxicity, said subject having been administered a dose of genetically engineered cells comprising T cells expressing a recombinant receptor, wherein the one or more agent is administered in a treatment regimen comprising:

(a) administering one or more agent if:

(i) at or greater than 72 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits one or more physical signs or symptoms associated with the toxicity, optionally neurotoxicity (NT); or

(ii) within 48 or 72 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits one or more physical signs or symptoms associated with the toxicity;

(b) administering one or more agent if, within 24, 48 or 72 hours after administration of the one or more agent in (a), the subject does not exhibit an improvement of the one or more physical signs or symptoms associated with the toxicity and/or exhibits a progression of the physical signs or symptoms associated with the toxicity, which one or more agent optionally are different from the one or more agent administered in (a) and/or is administered at the same or higher dose and/or frequency as the one or more agent administered in (a); and

(c) administering one or more agent if, within 24, 48 or 72 hours after administration of the one or more agent in (b), the subject does not exhibit an improvement of the one or more physical signs or symptoms associated with the toxicity and/or exhibits a rapid progression of the physical signs or symptoms associated with the toxicity, which one or more agent optionally are different from the one or more agent administered in (a) or (b) and/or is administered at the same or higher dose and/or frequency as the one or more agent administered in (a) or (b).

9. The method of claim 8, wherein the one or more agent is one or more steroid, optionally one or more doses of the one or more steroid.

10. The method of claim 8 or claim 9, wherein, in (a)(i), the treatment regimen comprises:

(1) if the subject exhibits one or more physical signs or symptoms associated with grade 2 NT after receiving a dose of the genetically engineered cells, optionally administering one or more doses of a steroid, said steroid administered about every 12 to 24 hours;

(2) if the subject exhibits one or more physical signs or symptoms associated with grade 3 NT after receiving a dose of the genetically engineered cells, administering one or more doses of a steroid, said steroid administered about every 8 to 12 hours, wherein a lower dose and/or frequency of the steroid is administered if the subject exhibits aphasia or confusion, and a higher dose and/or frequency of the steroid is administered if the subject exhibits events leading to depressed level of consciousness; and

(3) if the subject exhibits one or more physical signs or symptoms associated with grade 4 NT after receiving a dose of the genetically engineered cells, administering one or more doses of a steroid, said steroid administered about every 6 to 8 hours, wherein a higher dose and/or frequency of the steroid is administered if the subject exhibits events requiring respiratory support or seizures.

11. The method of any of claims 8-10, wherein, in (a)(ii), the treatment regimen comprises:

(1) if the subject exhibits one or more physical signs or symptoms associated with grade 1 NT after receiving a dose of the genetically engineered cells, optionally administering one or more doses of a steroid, said steroid administered about every 8 to 12 hours;

(2) if the subject exhibits one or more physical signs or symptoms associated with grade 2 NT after receiving a dose of the genetically engineered cells, administering one or more doses of a steroid, said steroid administered about every 8 to 12 hours;

(3) if the subject exhibits one or more physical signs or symptoms associated with grade 3 NT after receiving a dose of the genetically engineered cells, administering one or more doses of a steroid, said steroid administered about every 6 to 8 hours; and

(4) if the subject exhibits one or more physical signs or symptoms associated with grade 4 NT after receiving a dose of the genetically engineered cells, administering one or more doses of a steroid, said steroid administered about at least twice a day, optionally at least about every 6 hours.

12. The method of any of claims 8-11, wherein, in (b), the treatment regimen comprises administering a higher dose and/or frequency of the steroid compared to the doses of the steroid administered in (a)(i) or (a)(ii), and optionally administering an additional steroid that is different from the one or more agent administered in (a)(i) or (a)(ii) if the subject exhibits events requiring respiratory support or seizures.

13. The method of any of claims 8-12, wherein, in (c), the treatment regimen comprises administering a higher dose and/or frequency of the steroid compared to the doses of the steroid administered in (a) or (b), and optionally administering an additional steroid that is different from the one or more agent administered in (a) and (b) at a higher dose and/or frequency of the additional steroid administered in (b).

14. The method of any of claims 8-13, wherein if the subject exhibits a cerebral edema, administering one or more doses of an additional steroid that is different from the one or more agent administered in (a) and (b), optionally every 24 hours.

15. A method of ameliorating a toxicity, comprising administering, to a subject exhibiting one or more physical signs or symptom of a toxicity, one or more agent capable of reducing and/or ameliorating the one or more physical signs or symptoms associated with the toxicity, said subject having been administered a dose of genetically engineered cells comprising T cells expressing a recombinant receptor, wherein the one or more agent is administered in a treatment regimen comprising:

(a) if, within 48 or 72 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits a fever and/or one or more first physical signs or symptoms associated with grade 1 CRS, administering (i) an agent capable of binding an interleukin-6 receptor (IL-6R) , said agent administered no more than once every 24 hours, and optionally (ii) one or more doses of a steroid, said steroid administered no more than once every 24 hours;

(b) if within 48 or 72 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits one or more physical signs or symptoms associated with grade 2 CRS, administering (i) an agent capable of binding an IL-6R , said agent administered about every 12 to 24 hours, and (ii) one or more doses of a steroid, said steroid administered about every 12 to 24 hours; and if at or greater than 72 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits one or more physical signs or symptoms associated with grade 2 CRS; administering (i) an agent capable of binding an IL-6R , said agent administered about every 12 to 24 hours, and optionally (ii) one or more doses of a steroid, said steroid administered no more than once every 24 hours;

(c) if the subject exhibits one or more physical signs or symptoms associated with grade 3 CRS after receiving a dose of the genetically engineered cells, administering (i) an agent capable of binding an IL-6R , said agent administered at least twice a day, optionally at least about every 12 hours, and (ii) one or more doses of a steroid, said steroid administered at least twice a day, optionally at least about every 12 hours; and

(d) if the subject exhibits one or more physical signs or symptoms associated with grade 4 CRS after receiving a dose of the genetically engineered cells, administering (i) an agent capable of binding an IL-6R , said agent administered at least twice a day, optionally at least about every 6 hours, and (ii) one or more doses of a steroid, said steroid administered at least twice a day, optionally at least about every 6 hours.

16. A method of ameliorating a toxicity, comprising administering, to a subject exhibiting a sign or symptom of a toxicity, a treatment regimen for treating the toxicity, said

subject having been administered a dose of genetically engineered cells comprising T cells expressing a recombinant receptor, wherein the treatment regimen comprises:

(a) if, within 72, 96 or 120 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits a fever and/or one or more first physical signs or symptoms associated with a toxicity, optionally cytokine release syndrome (CRS), and/or one or more physical signs or symptoms associated with grade 1 CRS, administering (i) an agent capable of binding an interleukin-6 receptor (IL-6R) , said agent administered no more than once every 24 hours, and (ii) one or more doses of a steroid, said steroid administered about every 12 to 24 hours;

(b) if the subject exhibits one or more physical signs or symptoms associated with grade 2 CRS after receiving a dose of the genetically engineered cells, administering (i) an agent capable of binding an IL-6R , said agent administered no more than once every 24 hours, and (ii) one or more doses of a steroid, said steroid administered about every 12 to 24 hours;

(c) if the subject exhibits one or more physical signs or symptoms associated with grade 3 CRS after receiving a dose of the genetically engineered cells, administering (i) an agent capable of binding an IL-6R , said agent administered no more than once every 24 hours, and (ii) one or more doses of a steroid, said steroid administered at least twice a day, optionally at least about every 12 hours; and

(d) if the subject exhibits one or more physical signs or symptoms associated with grade 4 CRS after receiving a dose of the genetically engineered cells, administering (i) an agent capable of binding an IL-6R , said agent administered no more than once every 24 hours, and (ii) one or more doses of a steroid, said steroid administered at least twice a day, optionally at least about every 6 hours.

17. The method of claim 15 or claim 16, wherein up to two doses of the agent is administered.

18. A method of ameliorating a toxicity, comprising administering, to a subject exhibiting a sign or symptom of a toxicity, a treatment regimen for treating the toxicity, said subject having been administered a dose of genetically engineered cells comprising T cells expressing a recombinant receptor, wherein the treatment regimen is, if, within 72, 96 or 120 hours of administration of the dose of genetically engineered, the subject exhibits a fever and/or one or more first physical signs or symptoms associated with a toxicity, optionally cytokine

release syndrome (CRS), and/or one or more physical signs or symptoms associated with grade 1 CRS, administering (i) an agent capable of binding an interleukin-6 receptor (IL-6R) and (ii) one or more doses of a steroid.

19. A method of ameliorating a toxicity, comprising administering, to a subject exhibiting a sign or symptom of a toxicity, a treatment regimen for treating the toxicity, said subject having been administered a dose of genetically engineered cells comprising T cells expressing a recombinant receptor, wherein the treatment regimen comprises:

(a) if the subject exhibits one or more physical signs or symptoms associated with grade 2 neurotoxicity (NT) after receiving a dose of the genetically engineered cells, administering one or more doses of a steroid, said steroid administered about every 12 to 24 hours until the subject exhibits physical signs or symptoms associated with grade 1 NT or the subject does not exhibit any physical signs or symptoms associated with neurotoxicity, optionally further administering one or more reduced doses of the steroid;

(b) if the subject exhibits one or more physical signs or symptoms associated with grade 3 NT after receiving a dose of the genetically engineered cells, administering one or more doses of a steroid, said steroid administered at least twice a day, optionally at least about every 12 hours until the subject exhibits physical signs or symptoms associated with grade 1 NT or the subject does not exhibit any physical signs or symptoms associated with neurotoxicity, and further administering one or more reduced doses of the steroid; and

(c) if the subject exhibits one or more physical signs or symptoms associated with grade 4 NT after receiving a dose of the genetically engineered cells, administering one or more doses of a steroid, said steroid administered at least twice a day, optionally at least about every 6 hours until the subject exhibits physical signs or symptoms associated with grade 1 NT or the subject does not exhibit any physical signs or symptoms associated with neurotoxicity, and further administering one or more reduced doses of the steroid.

20. The method of any of claims 16-19, wherein if, within 72 hours after receiving administration of the dose of genetically engineered cells, the subject exhibits one or more first physical signs or symptoms associated with the toxicity and/or if the one or more physical signs or symptoms associated with the toxicity and/or exhibits a rapid progression of the physical signs or symptoms associated with the toxicity, administering one or more doses of a steroid, said steroid administered at least twice a day, optionally at least about every 6 hours.

21. The method of claim 2-8, 16-18 and 20, wherein the dose of the agent capable of binding IL-6R and a dose of steroid or the additional steroid is administered simultaneously, or a dose of the steroid or the additional steroid is administered within about 1, 2, 3 or 4 hours of the dose of the agent capable of binding IL-6R.

22. The method of any of claims 2-8, 16-18, 20 and 21, wherein the agent capable of binding IL-6R is administered no more than once every 4, 5, 6, 7, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or more hours.

23. The method of any of claims 1-22, wherein up to two doses of the one or more agent is administered.

24. The method of any of claims 2-8 and 10-23, wherein the steroid or the additional steroid is administered at or about every 3, 6, 9, 12, 15, 18, 21, 24, 36 or 48 hours, or a range defined by any two of the foregoing values.

25. The method of any of claims 2-8 and 10-24, wherein the steroid or the additional steroid is or comprises a corticosteroid, which optionally is a glucocorticoid.

26. The method of any of claims 2-8 and 10-25, wherein the steroid or the additional steroid is selected from among cortisones, dexamethasones, hydrocortisones, methylprednisolones, prednisolones and prednisones.

27. The method of claim 26, wherein the steroid or the additional steroid is or comprises dexamethasone, prednisone or methylprednisolone.

28. The method of any of claims 2-8 and 10-27, wherein the steroid or the additional steroid is dexamethasone or methylprednisolone.

29. The method of any of claims 2-8 and 10-28, wherein the steroid or the additional steroid is for administration at an equivalent dosage amount of from at or about 1.0 mg to at or about 40 mg, from at or about 1.0 mg to at or about 20 mg, from at or about 2.0 mg to at or

about 20 mg, from at or about 5.0 mg to at or about 25.0 mg, or from at or about 10 mg to at or about 20 mg dexamethasone or equivalent thereof, each inclusive.

30. The method of any of claims 2-8 and 10-28, wherein the steroid or the additional steroid is administered at an equivalent dosage amount of between or between about 0.5 mg/kg and at or about 5 mg/kg, or at or about 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg or 5 mg/kg methylprednisolone or equivalent thereof, each inclusive.

31. The method of any of claims 2-8 and 10-30, wherein multiple doses of the steroid or the additional steroid is administered, optionally wherein at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or more doses of the steroid or the additional steroid is administered.

32. The method of any of claims 2-8 and 10-31, wherein the steroid or the additional steroid is administered in multiple doses over a period of at or more than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or within a range defined by any of the foregoing.

33. The method of any of claims 2-8 and 10-32, wherein the steroid or the additional steroid is administered for 2, 3, 4, 5 or more days.

34. The method of any of claims 2-8 and 10-33, wherein the steroid or the additional steroid is administered once per day, twice per day, or three times or more per day.

35. The method of any of claims 2-8 and 10-34, wherein the steroid or the additional steroid is administered at an equivalent dosage amount of between or between about 1.0 mg and at or about 80 mg, between or between about 1.0 mg and at or about 60 mg, between or between about 1.0 mg and at or about 40 mg, between or between about 1.0 mg and at or about 20 mg, between or between about 1.0 mg and at or about 10 mg, between or between about 2.0 mg and at or about 80 mg, between or between about 2.0 mg and at or about 60 mg, between or between about 2.0 mg and at or about 40 mg, between or between about 2.0 mg and at or about 20 mg, between or between about 2.0 mg and at or about 10 mg, between or between about 5.0 mg and at or about 80 mg, between or between about 5.0 mg and at or about 60 mg, between or between about 5.0 mg and at or about 40 mg, between or between about 5.0 mg and at or about 20 mg, between or between about 5.0 mg and at or about 10 mg, between or between about 10 mg and

at or about 80 mg, between or between about 10 mg and at or about 60 mg, between or between about 10 mg and at or about 40 mg, between or between about 10 mg and at or about 20 mg dexamethasone or equivalent thereof, each inclusive, per day or per 24 hours, or from or from about 10 mg to at or about 80 mg dexamethasone or equivalent thereof, per day or per 24 hours, or at or about 10 mg, 20 mg, 40 mg or 80 mg dexamethasone or equivalent thereof, per day or per 24 hours.

36. The method of any of claims 31-35, wherein the multiple doses comprise an initial dose of the steroid or the additional steroid of between at or about 1 and at or about 3 mg/kg, such as at or about 2 mg/kg methylprednisolone or equivalent thereof, followed by subsequent doses of between at or about 1 and at or about 5 mg/kg, or at or about 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg or 5 mg/kg methylprednisolone or equivalent thereof, divided between 1, 2, 3, 4 or 5 times over a day or over 24 hours.

37. The method of any of claims 2-8 and 10-36, wherein the steroid or the additional steroid is formulated for intravenous or oral administration.

38. The method of claim 2-8, 16-18 and 20-47, wherein the agent capable of binding IL-6R is a recombinant anti-IL-6 receptor antibody or an antigen-binding fragment thereof is or comprises an agent selected from among tocilizumab or sarilumab or an antigen-binding fragment thereof.

39. The method of claim 38, wherein the recombinant anti-IL-6R antibody is or comprises tocilizumab or an antigen-binding fragment thereof.

40. The method of claim 38 or claim 39, wherein the anti-IL-6R antibody is for administration in a dosage amount of from or from about 1 mg/kg to at or about 20 mg/kg, from at or about 2 mg/kg to at or about 19 mg/kg, from at or about 4 mg/kg to at or about 16 mg/kg, from at or about 6 mg/kg to at or about 14 mg/kg or 8 mg/kg to at or about 12 mg/kg, each inclusive, or the anti-IL-6R antibody is administered in a dosage amount of at least or at least about or about 1 mg/kg, 2 mg/kg, 4 mg/kg, 6 mg/kg, 8 mg/kg, 10 mg/kg, 12 mg/kg, 14 mg/kg, 16 mg/kg, 18 mg/kg, 20 mg/kg.

41. The method of any of claims 38-40, wherein the anti-IL-6R antibody is formulated for single dosage administration of an amount from at or about 30 mg to at or about 5000 mg, from at or about 50 mg to at or about 1000 mg, from at or about 50 mg to at or about 500 mg, from at or about 50 mg to at or about 200 mg, from at or about 50 mg to at or about 100 mg, from at or about 100 mg to at or about 1000 mg, from at or about 100 mg to at or about 500 mg, from at or about 100 mg to at or about 200 mg, from at or about 200 mg to at or about 1000 mg, from at or about 200 mg to at or about 500 mg, or from at or about 500 mg to at or about 1000 mg.

42. The method of any of claims 38-41, wherein the anti-IL-6R antibody is formulated for intravenous administration.

43. The method of any of claims 1-42, further comprising, if the subject exhibits one or more first physical signs or symptoms associated with the toxicity, optionally CRS, within 72 hours of administration of the dose of genetically engineered cells, if the physical signs or symptoms associated with the toxicity, optionally CRS, does not improve, if the physical signs or symptoms associated with the toxicity is severe or aggressive and/or if the grade of toxicity, optionally CRS, becomes more severe, administering an additional dose of steroids, optionally at a high dose and/or a dose of an additional steroid, optionally at a high dose.

44. The method of claim 43, wherein the high dose of the steroid or the additional steroid is methylprednisolone at or about 1 to at or about 4 mg/kg initial dose followed by at or about 1 to at or about 4 mg mg/kg/day divided 2, 3, 4, 5 or 6 times per day, or equivalents thereof.

45. The method of claim 43, wherein the high dose of the steroid or the additional steroid is dexamethasone at dosage amount of at or about 10 mg, 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, 60 mg, 65 mg, 70 mg, 75 mg or 80 mg dexamethasone or equivalent thereof, or a range defined by any of the foregoing, each inclusive.

46. The method of any of claims 1-45, wherein, prior to administering the treatment regimen, the method further comprises administering to the subject a dose of genetically

engineered cells comprising T cells expressing a recombinant receptor for treating a disease or condition.

47. The method of any of claims 1-46, wherein the recombinant receptor is or comprises a chimeric receptor and/or a recombinant antigen receptor.

48. The method of any of claims 1-47, wherein the recombinant receptor is capable of binding to a target antigen that is associated with, specific to, and/or expressed on a cell or tissue of a disease, disorder or condition.

49. The method of claim 48, wherein the disease, disorder or condition is an infectious disease or disorder, an autoimmune disease, an inflammatory disease, or a tumor or a cancer.

50. The method of claim 48 or claim 49, wherein the target antigen is a tumor antigen.

51. The method of any of claims 48-50, wherein the target antigen is selected from among $\alpha v\beta 6$ integrin ($\alpha v\beta 6$ integrin), B cell maturation antigen (BCMA), B7-H3, B7-H6, carbonic anhydrase 9 (CA9, also known as CAIX or G250), a cancer-testis antigen, cancer/testis antigen 1B (CTAG, also known as NY-ESO-1 and LAGE-2), carcinoembryonic antigen (CEA), a cyclin, cyclin A2, C-C Motif Chemokine Ligand 1 (CCL-1), CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD138, CD171, epidermal growth factor protein (EGFR), type III epidermal growth factor receptor mutation (EGFR vIII), epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), ephrinB2, ephrine receptor A2 (EPHa2), estrogen receptor, Fc receptor like 5 (FCRL5; also known as Fc receptor homolog 5 or FCRH5), fetal acetylcholine receptor (fetal AchR), a folate binding protein (FBP), folate receptor alpha, ganglioside GD2, O-acetylated GD2 (OGD2), ganglioside GD3, glycoprotein 100 (gp100), G Protein Coupled Receptor 5D (GPCR5D), Her2/neu (receptor tyrosine kinase erb-B2), Her3 (erb-B3), Her4 (erb-B4), erbB dimers, Human high molecular weight-melanoma-associated antigen (HMW-MAA), hepatitis B surface antigen, Human leukocyte antigen A1 (HLA-A1), Human leukocyte antigen A2 (HLA-A2), IL-22 receptor alpha (IL-22Ra), IL-13 receptor alpha 2 (IL-13Ra2), kinase insert domain receptor (kdr), kappa

light chain, L1 cell adhesion molecule (L1-CAM), CE7 epitope of L1-CAM, Leucine Rich Repeat Containing 8 Family Member A (LRRC8A), Lewis Y, Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, mesothelin, c-Met, murine cytomegalovirus (CMV), mucin 1 (MUC1), MUC16, natural killer group 2 member D (NKG2D) ligands, melan A (MART-1), neural cell adhesion molecule (NCAM), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME), progesterone receptor, a prostate specific antigen, prostate stem cell antigen (PSCA), prostate specific membrane antigen (PSMA), Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1), survivin, Trophoblast glycoprotein (TPBG also known as 5T4), tumor-associated glycoprotein 72 (TAG72), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor receptor 2 (VEGFR2), Wilms Tumor 1 (WT-1), a pathogen-specific antigen, or an antigen associated with a universal tag, and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV or other pathogens.

52. The method of any of claims 48-51, wherein the recombinant receptor is or comprises a functional non-TCR antigen receptor or a TCR or antigen-binding fragment thereof.

53. The method of any of claims 48-52, wherein the recombinant receptor is a chimeric antigen receptor (CAR).

54. The method of any of claims 48-53, wherein the recombinant receptor comprises an extracellular domain comprising an antigen-binding domain.

55. The method of claim 54, wherein the antigen-binding domain is or comprises an antibody or an antibody fragment thereof, which optionally is a single chain fragment.

56. The method of claim 55, wherein the fragment comprises antibody variable regions joined by a flexible linker.

57. The method of any of claims 55 or 56, wherein the fragment comprises an scFv.

58. The method of any of claims 48-57, wherein the recombinant receptor comprises an intracellular signaling region.

59. The method of claim 58, wherein the intracellular signaling region comprises an intracellular signaling domain.

60. The method of claim 59, wherein the intracellular signaling domain is or comprises a primary signaling domain, a signaling domain that is capable of inducing a primary activation signal in a T cell, a signaling domain of a T cell receptor (TCR) component, and/or a signaling domain comprising an immunoreceptor tyrosine-based activation motif (ITAM).

61. The method of claim 60, wherein the intracellular signaling domain is or comprises an intracellular signaling domain of a CD3 chain, optionally a CD3-zeta (CD3 ζ) chain, or a signaling portion thereof.

62. The method of any of claims 58-61, wherein the recombinant receptor further comprises a transmembrane domain disposed between the extracellular domain and the intracellular signaling region.

63. The method of any of claims 46-62, wherein the intracellular signaling region further comprises a costimulatory signaling domain.

64. The method of claim 63, wherein the costimulatory signaling domain comprises an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof.

65. The method of claim 63 or claim 64, wherein the costimulatory signaling domain comprises an intracellular signaling domain of a CD28, a 4-1BB or an ICOS or a signaling portion thereof.

66. The method of any of claims 63-65, wherein the costimulatory signaling domain is between the transmembrane domain and the intracellular signaling domain.

67. The method of any of claims 1-66, wherein the cells are T cells.

68. The method of claim 67, wherein the T cells are CD4+ or CD8+.

69. The method of any of claims 1-68, wherein the T cells are primary T cells obtained from a subject.

70. The method of any of claims 1-69, wherein the cells of the genetically engineered cells are autologous to the subject.

71. The method of any of claims 1-69, wherein the cells are of the genetically engineered cells allogeneic to the subject.

72. A method of treatment, the method comprising:

(a) administering, to a subject having a disease or condition, a dose of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR) for treating the disease or condition;

(b) after administering the dose of genetically engineered cells, monitoring CAR+ T cells in the blood of the subject to assess if the cells are within a therapeutic range, and

(c) if the genetically engineered cells are not within the therapeutic range, administering to the subject an agent capable of modulating, optionally increasing or decreasing, CAR+ T cell expansion or proliferation, in the subject,

wherein the therapeutic range is:

(i) peak CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 10 cells per microliter and 500 cells per microliter; or

(ii) peak CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 2 cells per microliter and 200 cells per microliter.

73. A method of treatment, the method comprising:

(a) monitoring, in the blood of a subject, the presence of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR) to assess if the cells are within a therapeutic range, wherein the subject has been previously administered a dose of the genetically engineered cells for treating a disease or condition; and

(c) if the genetically engineered cells are not within the therapeutic range, administering to the subject an agent capable of modulating, optionally increasing or decreasing, CAR⁺ T cell expansion or proliferation, in the subject,

wherein the therapeutic range is:

(i) peak CD3⁺CAR⁺ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 10 cells per microliter and 500 cells per microliter; or

(ii) peak CD8⁺CAR⁺ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 2 cells per microliter and 200 cells per microliter.

74. The method of any of claims 72-73, wherein if the peak number of CAR⁺ T cells in the blood of the subject is less than the lowest number of peak CAR⁺ T cells in the therapeutic range, an agent that is capable of increasing CAR⁺ T cell expansion or proliferation is administered to the subject.

75. The method of claim 74, wherein the agent is capable of increasing CAR-specific expansion.

76. The method of claim 74 or claim 75, wherein the agent is an anti-idiotypic antibody or antigen-binding fragment thereof specific to the CAR, an immune checkpoint inhibitor, a modulator of a metabolic pathway, an adenosine receptor antagonist, a kinase inhibitor, an anti-TGF β antibody or an anti-TGF β R antibody or a cytokine.

77. The method of any of claims 72-73, wherein if the peak number of CAR⁺ T cells in the blood of the subject is greater than the highest number of peak CAR⁺ T cells in the therapeutic range, an agent that is capable of decreasing CAR⁺ T cell expansion or proliferation is administered to the subject.

78. A method of treatment, the method comprising:

(a) administering, to a subject having a disease or condition, a dose of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR) for treating the disease or condition;

(b) after administering the dose of genetically engineered cells, monitoring CAR+ T cells in the blood of the subject, and

(c) administering to the subject an agent capable of decreasing, CAR+ T cell expansion or proliferation, in the subject if:

(i) the amount of CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, is greater than at or about 500 cells per microliter; or

(ii) the amount of CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, is greater than at or about 200 cells per microliter.

79. A method of treatment, the method comprising:

(a) monitoring, in the blood of a subject, the presence of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR), wherein the subject has been previously administered a dose of the genetically engineered cells for treating a disease or condition; and

(b) administering to the subject an agent capable of decreasing, CAR+ T cell expansion or proliferation, in the subject if: (i) the amount of CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, is greater than at or about 500 cells per microliter; or (ii) the amount of CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, is greater than at or about 200 cells per microliter.

80. The method of any of claims 78-79, wherein the agent is one or more steroid.

81. The method of claim 80, wherein the steroid is dexamethasone or methylprednisolone.

82. The method of any of claims 80-81, wherein the steroid is administered in an amount that is between or between about 1.0 mg and at or about 40 mg, between or between about 1.0 mg and at or about 20 mg, between or between about 2.0 mg and at or about 20 mg, between or between about 5.0 mg and at or about 25.0 mg, between or between about 10 mg and at or about 20 mg dexamethasone or equivalent thereof, each inclusive.

83. The method of any of claims 80-82, wherein the steroid is administered in multiple doses over a period of at or more than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more or within a range defined by any of the foregoing.

84. The method of any of claims 80-83, wherein the steroid is administered once per day, twice per day, or three times or more per day.

85. The method of any of claims 80-84, wherein the steroid is administered in an amount that is between or between about 1.0 mg and at or about 80 mg, between or between about 1.0 mg and at or about 60 mg, between or between about 1.0 mg and at or about 40 mg, between or between about 1.0 mg and at or about 20 mg, between or between about 1.0 mg and at or about 10 mg, between or between about 2.0 mg and at or about 80 mg, between or between about 2.0 mg and at or about 60 mg, between or between about 2.0 mg and at or about 40 mg, between or between about 2.0 mg and at or about 20 mg, between or between about 2.0 mg and at or about 10 mg, between or between about 5.0 mg and at or about 80 mg, between or between about 5.0 mg and at or about 60 mg, between or between about 5.0 mg and at or about 40 mg, between or between about 5.0 mg and at or about 20 mg, between or between about 5.0 mg and at or about 10 mg, between or between about 10 mg and at or about 80 mg, between or between about 10 mg and at or about 60 mg, between or between about 10 mg and at or about 40 mg, between or between about 10 mg and at or about 20 mg dexamethasone or equivalent thereof, each inclusive, per day or per 24 hours, or about 10 mg, 20 mg, 40 mg or 80 mg dexamethasone or equivalent thereof, per day or per 24 hours.

86. The method of any of claims 72-85, wherein the subject is monitored for CAR+ T cells in the blood at a time that is at least 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days or 21 days after initiation of administration of the genetically engineered cells; or at a time that is between or between about 11 to 22 days, 12 to 18 days or 14 to 16 days, each inclusive, after initiation of administration of the genetically engineered cells.

87. The method of any of claim 72-86, wherein the agent is administered at a time that is greater than or greater than about 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days or 21 days after initiation of

administration of the genetically engineered cells; or at a time that is between or between at or about 11 to at or about 22 days, 12 to 18 days or 14 to 16 days, each inclusive, after initiation of administration of the genetically engineered cells.

88. A method of modulating activity of engineered cells, the method comprising:

(a) selecting a subject in which the level, amount or concentration of a volumetric measure of tumor burden or an inflammatory marker in a sample from the subject is at or above a threshold level, wherein the sample does not comprise genetically engineered T cells expressing a chimeric antigen receptor (CAR) and/or is obtained from the subject prior to receiving administration of genetically engineered T cells expressing a CAR; and

(b) administering to the selected subject an agent that is capable of decreasing expansion or proliferation of genetically engineered T cells expressing a CAR.

89. A method of modulating activity of engineered cells, the method comprising administering to a subject an agent that is capable of decreasing expansion or proliferation of genetically engineered T cells expressing a chimeric antigen receptor (CAR) in a subject, wherein the subject is one in which the level, amount or concentration of a volumetric measure of tumor burden or an inflammatory marker in a sample from the subject is at or above a threshold level.

90. The method of claim 89, wherein the sample does not comprise genetically engineered T cells expressing a CAR and/or is obtained from the subject prior to receiving administration of genetically engineered T cells expressing a CAR.

91. The method of any of claims 88-90, wherein the agent is administered prior to or concurrently with initiation of administration of a dose of genetically engineered cells comprising T cells expressing a CAR.

92. The method of claim 91, wherein the method further comprises administering a dose of the genetically engineered cells comprising T cells expressing a CAR.

93. The method of any of claims 88-92, wherein the subject has a disease or condition and the genetically engineered cells are for treating the disease or condition.

94. The method of any of claims 88-93, wherein, prior to administering the agent, the selected subject is at risk of developing a toxicity following administration of the genetically engineered cells.

95. The method of any of claims 88-94, wherein the administration of the agent is sufficient to achieve peak CAR+ T cells in a therapeutic range in the subject, or in a majority of selected subjects so treated by the method or in greater than 75% of the selected subjects so treated by the method.

96. The method of claim 95, wherein the therapeutic range is:

(i) based upon the range of peak CD3+ CAR+ T cells, or a CD8+CAR+ T cell subset thereof, in the blood among one or more subjects previously treated with the genetically engineered cells that is associated with an estimated probability of response of greater than or greater than about 65% and an estimated probability of a toxicity of less than or about 30%; or

(ii) peak CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 10 cells per microliter and 500 cells per microliter; or

(iii) peak CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 2 cells per microliter and 200 cells per microliter.

97. The method of claim 95, wherein the therapeutic range is:

(i) based upon the number or level of CD3+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 10 cells per microliter and 500 cells per microliter; or

(ii) based upon the number or level of CD8+CAR+ T cells in the blood, following administration of the genetically engineered cells, that is between or between about 2 cells per microliter and 200 cells per microliter.

98. The method of any of claims 88-97, wherein a volumetric measure of tumor burden is measured and the volumetric measure is a sum of the products of diameters (SPD),

longest tumor diameters (LD), sum of longest tumor diameters (SLD), tumor volume, necrosis volume, necrosis-tumor ratio (NTR), peritumoral edema (PTE), and edema-tumor ratio (ETR).

99. The method of any of claims 88-98, wherein the volumetric measure is a sum of the products of diameter (SPD).

100. The method of any of claims 88-99, wherein the volumetric measure is measured using computed tomography (CT), positron emission tomography (PET), and/or magnetic resonance imaging (MRI) of the subject.

101. The method of any of claims 88-96, wherein an inflammatory marker in a sample from the subject is measured and the inflammatory marker is C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), albumin, ferritin, $\beta 2$ microglobulin ($\beta 2$ -M), lactate dehydrogenase (LDH), a cytokine or a chemokine.

102. The method of any of claims 88-96 and 101, wherein the inflammatory marker is LDH.

103. The method of any of claims 88-96 and 101, wherein the inflammatory marker is a cytokine or a chemokine that is IL-7, IL15, MIP-1 α or TNF- α .

104. The method of any of claims 88-96, 101 and 103, wherein the cytokine or chemokine is associated with macrophage or monocyte activation.

105. The method of any of claims 88-96 and 101-104, wherein the sample is or comprises a blood sample, plasma sample, or serum sample.

106. The method of any of claims 88-105, wherein the threshold value is a value that:
i) is within 25%, within 20%, within 15%, within 10%, or within 5% above the average value of the volumetric measure or inflammatory marker and/or is within a standard deviation above the average value of the volumetric measure or the inflammatory marker in a plurality of control subjects;

ii) is above the highest value of the volumetric measure or inflammatory marker, optionally within 50%, within 25%, within 20%, within 15%, within 10%, or within 5% above such highest fold change, measured in at least one subject from among a plurality of control subjects; and/or

iii) is above the highest value of the volumetric measure or inflammatory marker as measured among more than 75%, 80%, 85%, 90%, 95%, or 98% of subjects from a plurality of control subjects.

107. The method of claim 106, wherein the plurality of control subjects are a group of subjects prior to receiving a dose of the genetically engineered cells, wherein:

each of the control subjects of the group exhibited a peak CAR⁺ T cells in the blood greater than the highest peak CAR⁺ T cells in the therapeutic range;

each of the control subjects of the group went on to develop at toxicity, optionally a neurotoxicity or cytokine release syndrome (CRS), a grade 2 or grade 3 or higher neurotoxicity or a grade 3 or higher CRS, after receiving a dose of the engineered cells for treating the same disease or condition;

each of the control subjects of the group did not develop a response, optionally a complete response (CR) or partial response (PR), following administration of the dose of genetically engineered cells; and/or

each of the control subjects of the group did not develop a durable response, optionally for at or about or greater than at or about 3 months or at or about or greater than at or about 6 months, following administration of the dose of genetically engineered cells.

108. The method of any of claims 88-107, wherein the volumetric measure is SPD and the threshold value is or is about 30 cm², is or is about 40 cm², is or is about 50 cm², is or is about 60 cm², or is or is about 70 cm².

109. The method of any of claims 88-108, wherein the inflammatory marker is LDH and the threshold value is or is about 300 units per liter, is or is about 400 units per liter, is or is about 500 units per liter or is or is about 600 units per liter.

110. The method of any of claims 88-109, wherein the agent is a steroid.

111. The method of claim 110, wherein the steroid is dexamethasone or methylprednisolone.

112. The method of any of claims 110-111, wherein the steroid is administered in an amount that is between or between about 1.0 mg and about 40 mg, between or between about 1.0 mg and about 20 mg, between or between about 2.0 mg and about 20 mg, between or between about 5.0 mg and about 25.0 mg, between or between about 10 mg and about 20 mg dexamethasone or equivalent thereof, each inclusive.

113. The method of any of claims 110-112, wherein the steroid is administered in multiple doses over a period of at or more than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more or within a range defined by any of the foregoing.

114. The method of any of claims 110-113, wherein the steroid is administered once per day, twice per day, or three times or more per day.

115. The method of any of claims 110-114, wherein the steroid is administered in an amount that is between or between about 1.0 mg and at or about 80 mg, between or between about 1.0 mg and at or about 60 mg, between or between about 1.0 mg and at or about 40 mg, between or between about 1.0 mg and at or about 20 mg, between or between about 1.0 mg and at or about 10 mg, between or between about 2.0 mg and at or about 80 mg, between or between about 2.0 mg and at or about 60 mg, between or between about 2.0 mg and at or about 40 mg, between or between about 2.0 mg and at or about 20 mg, between or between about 2.0 mg and at or about 10 mg, between or between about 5.0 mg and at or about 80 mg, between or between about 5.0 mg and at or about 60 mg, between or between about 5.0 mg and at or about 40 mg, between or between about 5.0 mg and at or about 20 mg, between or between about 5.0 mg and at or about 10 mg, between or between about 10 mg and at or about 80 mg, between or between about 10 mg and at or about 60 mg, between or between about 10 mg and at or about 40 mg, between or between about 10 mg and at or about 20 mg dexamethasone or equivalent thereof, each inclusive, per day or per 24 hours, or from or from about 10 mg to at or about 80 mg dexamethasone or equivalent thereof, per day or per 24 hours, or at or about 10 mg, 20 mg, 40 mg or 80 mg dexamethasone or equivalent thereof, per day or per 24 hours.

116. The method of any of claims 88-115, wherein the volumetric measure or inflammatory marker is measured in the subject within 1 day, 2 days, 3 days, 4 days, 6 days, 8 days, 12 days, 16 days, 20 days, 24 days, 28 days or more prior to initiation of administration of the genetically engineered cells.

117. The method of any of claims 72-116, wherein the dose of genetically engineered cells comprises at least or at least about 1×10^5 CAR-expressing cells, at least or at least about 2.5×10^5 CAR-expressing cells, at least or at least about 5×10^5 CAR-expressing cells, at least or at least about 1×10^6 CAR-expressing cells, at least or at least about 2.5×10^6 CAR-expressing cells, at least or at least about 5×10^6 CAR-expressing cells, at least or at least about 1×10^7 CAR-expressing cells, at least or at least about 2.5×10^7 CAR-expressing cells, at least or at least about 5×10^7 CAR-expressing cells, at least or at least about 1×10^8 CAR-expressing cells, at least or at least about 2.5×10^8 CAR-expressing cells, or at least or at least about 5×10^8 CAR-expressing cells or from at or about 1×10^5 to at or about 5×10^8 total CAR-expressing T cells, from at or about 1×10^6 to at or about 2.5×10^8 total CAR-expressing T cells, from at or about 5×10^6 to at or about 1×10^8 total CAR-expressing T cells, from at or about 1×10^7 to at or about 2.5×10^8 total CAR-expressing T cells, from at or about 5×10^7 to at or about 1×10^8 total CAR-expressing T cells, each inclusive.

118. The method of any of claims 72-117, wherein, among a plurality of subjects treated, the method achieves an increase in the percentage of subjects achieving a durable response, optionally a complete response (CR) or objective response (OR) or a partial response (PR), optionally that is durable for at or greater than 3 months or at or greater than 6 months, compared to a method that does not comprise administering the agent.

119. The method of claim 118, wherein the increase is greater than or greater than about 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold or more.

120. The method of any of claims 72-119, wherein:
at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40% or at least 50% of subjects treated according to the method achieve a complete response (CR) that is durable for at or greater than 3 months or at or greater than 6 months; and/or

at least 25%, at least 30%, at least 40%, at least 50%, at least 60% or at least 70% of the subjects treated according to the method achieve objective response (OR) that is durable for at or greater than 3 months or at or greater than 6 months.

121. The method of any of claims 72-120, wherein:

greater than or greater than about 50%, greater than or greater than about 60%, greater than or greater than about 70%, or greater than or greater than about 80% of the subjects treated according to the method do not exhibit a grade 3 or greater cytokine release syndrome (CRS) and/or do not exhibit a grade 2 or greater or grade 3 or greater neurotoxicity; or

greater than or greater than about 40%, greater than or greater than about 50% or greater than or greater than about 55% of the subjects treated according to the method do not exhibit any neurotoxicity or CRS.

122. The method of any of claims 72-121, wherein the amount or peak CAR⁺ T cells is determined as the number of CAR⁺ T cells per microliter in the blood of the subject.

123. The method of any of claims 72-122, wherein the therapeutic range is the range in which the estimated probability of a toxicity is less than 20%, less than 15%, less than 10% or less than 5% and the estimated probability of a response is greater than 65%, 70%, 75%, 80%, 85%, 90%, 95% or more.

124. The method of any of claims 72-123, wherein the probability of a toxicity is based on a toxicity selected from:

any neurotoxicity or cytokine release syndrome (CRS);
severe toxicity or grade 3 or higher toxicity;
severe CRS or a grade 3 or higher CRS; or
severe neurotoxicity, grade 2 or higher neurotoxicity or grade 3 or higher neurotoxicity.

125. The method of any of claims 72-124, wherein the probability of a toxicity is based on the probability of a severe toxicity or a grade 3 or higher toxicity.

126. The method of claim 124 or claim 125, wherein the severe toxicity is grade 3-5 neurotoxicity.

127. The method of any of claims 72-126, wherein the probability of response is based on a response that is a complete response (CR), an objective response (OR) or a partial response (PR), optionally wherein the response is durable, optionally durable for at or at least 3 months or at or at least 6 months.

128. The method of any of claims 72-127, wherein the response is a marrow response as determined based on assessment of the presence of a malignant immunoglobulin heavy chain locus (IGH) and/or an index clone in the bone marrow of the subject.

129. The method of claim 128, wherein the malignant IGH and/or index clone is assessed by flow cytometry or IgH sequencing.

130. A method of assessing likelihood of a durable response, the method comprising:
(a) detecting, in a biological sample from a subject, peak levels of one or more inflammatory markers and/or peak level of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR), wherein the subject has been previously administered a dose of the genetically engineered cells for treating a disease or condition; and
(b) comparing, individually, the peak levels to a threshold value, thereby determining a likelihood that a subject will achieve a durable response to the administration of the genetically engineered cells.

131. The method of claim 130, wherein:
the subject is likely to achieve a durable response if the peak levels of the one or more inflammatory markers is below a threshold value and the subject is not likely to achieve a durable response if the peak levels of the one or more inflammatory markers is above a threshold value; or

the subject is likely to achieve a durable response if the peak level of the genetically engineered cells is within a therapeutic range between a lower threshold value and an upper threshold value and the subject is not likely to achieve a durable response if the peak level of the genetically engineered cells is below the lower threshold value or is above the upper threshold value.

132. The method of claim 130 or claim 131, if the subject is determined not likely to achieve a durable response, further comprising selecting a subject for treatment with a therapeutic agent or with an alternative therapeutic treatment other than the genetically engineered cells.

133. The method of any of claims 130-132, if the subject is determined as not likely to achieve a durable response, further comprising administering a therapeutic agent or an alternative therapeutic treatment other than the genetically engineered cells.

134. A method of treatment, comprising;
(a) selecting a subject having received administration of genetically engineered cells comprising T cells expressing a chimeric antigen receptor (CAR) in which:
peak levels of one or more inflammatory markers in a sample from the subject is above a threshold value; and/or
peak level of T cells comprising a chimeric antigen receptor (CAR) in a sample from the subject is below a lower threshold value or is above an upper threshold value; and
(b) administering to the subject a therapeutic agent or alternative therapeutic treatment other than the genetically engineered cells.

135. The method of any of claims 130-133, wherein the response is a complete response (CR), objective response (OR) or partial response (PR).

136. The method of any of claims 130-133 and 135, wherein the response is durable for at or greater than 3 months, 4 months, 5 months, or 6 months.

137. The method of any of claims 130-136, wherein the peak levels are assessed and/or the sample is obtained from the subject at a time that is at least 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days or 21 days after initiation of administration of the genetically engineered cells; or at a time that is between at or about 11 and at or about 22 days, at or about 12 and at or about 18 days or at or about 14 and at or about 16 days, each inclusive, after initiation of administration of the genetically engineered cells.

138. The method of any of claims 130-137, wherein the peak level is a peak level of one or more inflammatory marker and the inflammatory marker is selected from C reactive protein (CRP), IL-2, IL-6, IL-10, IL-15, TNF-alpha, MIP-1alpha, MIP-1beta, MCP-1, CXCL10 and CCL13.

139. The method of any of claims 131-138, wherein the peak level of one or more inflammatory marker is assessed and the threshold value is within 25%, within 20%, within 15%, within 10% or within 5% and/or is within a standard deviation of the median or mean of the peak level of the inflammatory marker as determined among a group of control subjects having received administration of the genetically engineered cells, wherein each of the subjects of the group did not achieve a durable response, optionally a CR and/or PR, optionally durable for at or greater than 3 months or 6 months following administration of the genetically engineered cells.

140. The method of claim 139, wherein the control subjects exhibited stable disease (SD) or progressive disease (PD) following administration of the genetically engineered cells, optionally at or greater than 3 months or 6 months following administration of the genetically engineered cells.

141. The method of any of claims 130-137, wherein the peak level of genetically engineered cells is a peak CAR+ T cells, or a CD8+ T cell subset thereof.

142. The method of any of claims 131-137 and 141, the lower threshold value and upper threshold value is the lower and upper end, respectively, of a therapeutic range of peak CD3+ CAR+ T cells, or a CD8+CAR+ T cell subset thereof, in the blood among one or more subjects previously treated with the genetically engineered cells that is associated with an estimated probability of response of greater than or greater than about 65% and an estimated probability of a toxicity of less than or less than about 30%.

143. The method of any of claims 131-137, 141 and 142, wherein the therapeutic range is the range in which the estimated probability of a toxicity is less than 20%, less than 15%, less than 10% or less than 5% and the estimated probability of a response is greater than 65%, 70%, 75%, 80%, 85%, 90%, 95% or more.

144. The method of claim 142 or claim 143, wherein the probability of a toxicity is based on a toxicity selected from:

any neurotoxicity or cytokine release syndrome (CRS);
severe toxicity or grade 3 or higher toxicity;
severe CRS or a grade 3 or higher CRS; or
severe neurotoxicity, grade 2 or higher neurotoxicity or grade 3 or higher neurotoxicity.

145. The method of any of claims 142-144, wherein the probability of response is based on a response that is a complete response (CR), an objective response (OR) or a partial response (PR), optionally wherein the response is durable, optionally durable for at or at least 3 months or at or at least 6 months.

146. The method of any of claims 130-137 and 141-145, wherein peak level of genetically engineered cells is determined as the number of CAR+ T cells per microliter in the blood of the subject.

147. The method of any of claims 131-137 and 141-146, wherein:
the upper threshold value is between or between about 300 cells per microliter and at or about 1000 cells per microliter or between or between about 400 cells per microliter and at or about 600 cells per microliter, or is about 300 cells per microliter, 400 cells per microliter, 500 cells per microliter, 600 cells per microliter, 700 cells per microliter, 800 cells per microliter, 900 cells per microliter or 1000 cells per microliter; or

the lower threshold value is less than or less than about 10 cells per microliter, 9 cells per microliter, 8 cells per microliter, 7 cells per microliter, 6 cells per microliter, 5 cells per microliter, 4 cells per microliter, 3 cells per microliter, 2 cells per microliter or 1 cell per microliter.

148. The method of any of claims 130-147, wherein the sample is a blood sample or plasma sample.

149. The method of any of claims 130-148, wherein the method is carried out ex vivo.

150. The method of any of claims 132-149, the peak level of genetically engineered cells is above the upper threshold value and the therapeutic agent is an agent that is capable of decreasing CAR⁺ T cell expansion or proliferation.

151. The method of claim 150, wherein the agent is a steroid.

152. The method of claim 151, wherein the steroid is dexamethasone or methylprednisolone.

153. The method of any of claims 151-152, wherein the steroid is administered in an amount that is between or between about 1.0 mg and at or about 40 mg, between or between about 1.0 mg and at or about 20 mg, between or between about 2.0 mg and at or about 20 mg, between or between about 5.0 mg and at or about 25.0 mg, between or between about 10 mg and at or about 20 mg dexamethasone or equivalent thereof, each inclusive.

154. The method of any of claims 151-153, wherein the steroid is administered in multiple doses over a period of at or more than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more or within a range defined by any of the foregoing.

155. The method of any of claims 151-154, wherein the steroid is administered once per day, twice per day, or three times or more per day.

156. The method of any of claims 151-155, wherein the steroid is administered in an amount that is between or between about 1.0 mg and at or about 80 mg, between or between about 1.0 mg and at or about 60 mg, between or between about 1.0 mg and at or about 40 mg, between or between about 1.0 mg and at or about 20 mg, between or between about 1.0 mg and at or about 10 mg, between or between about 2.0 mg and at or about 80 mg, between or between about 2.0 mg and at or about 60 mg, between or between about 2.0 mg and at or about 40 mg, between or between about 2.0 mg and at or about 20 mg, between or between about 2.0 mg and at or about 10 mg, between or between about 5.0 mg and at or about 80 mg, between or between about 5.0 mg and at or about 60 mg, between or between about 5.0 mg and at or about 40 mg, between or between about 5.0 mg and at or about 20 mg, between or between about 5.0 mg and at or about 10 mg, between or between about 10 mg and at or about 80 mg, between or between

about 10 mg and at or about 60 mg, between or between about 10 mg and at or about 40 mg, between or between about 10 mg and at or about 20 mg dexamethasone or equivalent thereof, each inclusive, per day or per 24 hours, or from or from about 10 mg to at or about 80 mg dexamethasone or equivalent thereof, per day or per 24 hours, or at or about 10 mg, 20 mg, 40 mg or 80 mg dexamethasone or equivalent thereof, per day or per 24 hours.

157. The method of any of claims 132-149, the peak level of genetically engineered cells is below the lower threshold value and the therapeutic agent is an agent that is capable of increasing expansion or proliferation of the CAR⁺ T cells, optionally CAR-specific expansion.

158. The method of claim 157, wherein the agent is an anti-idiotypic antibody or antigen-binding fragment thereof specific to the CAR, an immune checkpoint inhibitor, a modulator of a metabolic pathway, an adenosine receptor antagonist, a kinase inhibitor, an anti-TGF β antibody or an anti-TGF β R antibody or a cytokine.

159. The method of any of claims 72-158, wherein the disease or condition is a cancer.

160. The method of claim 159, wherein the cancer is a B cell malignancy.

161. The method of claim 160, wherein the cancer is selected from the group consisting of sarcomas, carcinomas, lymphomas, non-Hodgkin lymphomas (NHLs), diffuse large B cell lymphoma (DLBCL), leukemia, CLL, ALL, AML and myeloma.

162. The method of claim 161, wherein the cancer is a pancreatic cancer, bladder cancer, colorectal cancer, breast cancer, prostate cancer, renal cancer, hepatocellular cancer, lung cancer, ovarian cancer, cervical cancer, pancreatic cancer, rectal cancer, thyroid cancer, uterine cancer, gastric cancer, esophageal cancer, head and neck cancer, melanoma, neuroendocrine cancers, CNS cancers, brain tumors, bone cancer, or soft tissue sarcoma.

163. The method of any of claims 72-162, wherein the subject is a human.

164. The method of any of claims 72-163, wherein the CAR specifically binds to an antigen associated with a disease or condition and/or expressed in cells associated with the disease or condition.

165. The method of claim 164, wherein the antigen is selected from among $\alpha v \beta 6$ integrin ($\alpha v \beta 6$ integrin), B cell maturation antigen (BCMA), B7-H3, B7-H6, carbonic anhydrase 9 (CA9, also known as CAIX or G250), a cancer-testis antigen, cancer/testis antigen 1B (CTAG, also known as NY-ESO-1 and LAGE-2), carcinoembryonic antigen (CEA), a cyclin, cyclin A2, C-C Motif Chemokine Ligand 1 (CCL-1), CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD138, CD171, epidermal growth factor protein (EGFR), type III epidermal growth factor receptor mutation (EGFR vIII), epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), ephrinB2, ephrine receptor A2 (EPHa2), estrogen receptor, Fc receptor like 5 (FCRL5; also known as Fc receptor homolog 5 or FCRH5), fetal acetylcholine receptor (fetal AchR), a folate binding protein (FBP), folate receptor alpha, ganglioside GD2, O-acetylated GD2 (OGD2), ganglioside GD3, glycoprotein 100 (gp100), G Protein Coupled Receptor 5D (GPRC5D), Her2/neu (receptor tyrosine kinase erb-B2), Her3 (erb-B3), Her4 (erb-B4), erbB dimers, Human high molecular weight-melanoma-associated antigen (HMW-MAA), hepatitis B surface antigen, Human leukocyte antigen A1 (HLA-A1), Human leukocyte antigen A2 (HLA-A2), IL-22 receptor alpha (IL-22Ra), IL-13 receptor alpha 2 (IL-13Ra2), kinase insert domain receptor (kdr), kappa light chain, L1 cell adhesion molecule (L1-CAM), CE7 epitope of L1-CAM, Leucine Rich Repeat Containing 8 Family Member A (LRRC8A), Lewis Y, Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, mesothelin, c-Met, murine cytomegalovirus (CMV), mucin 1 (MUC1), MUC16, natural killer group 2 member D (NKG2D) ligands, melan A (MART-1), neural cell adhesion molecule (NCAM), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME), progesterone receptor, a prostate specific antigen, prostate stem cell antigen (PSCA), prostate specific membrane antigen (PSMA), Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1), survivin, Trophoblast glycoprotein (TPBG also known as 5T4), tumor-associated glycoprotein 72 (TAG72), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor receptor 2 (VEGFR2), Wilms Tumor 1 (WT-1), a pathogen-specific antigen, or an antigen associated with a universal tag, and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV or other pathogens.

166. The method of any of claims 72-165, wherein the chimeric antigen receptor (CAR) comprises an extracellular antigen-recognition domain that specifically binds to the antigen and an intracellular signaling domain comprising an ITAM.

167. The method of claim 166, wherein the intracellular signaling domain comprises an intracellular domain of a CD3-zeta (CD3 ζ) chain.

168. The method of claim 166 or claim 167, wherein the chimeric antigen receptor (CAR) further comprises a costimulatory signaling region.

169. The method of claim 168, wherein the costimulatory domain is a signaling domain of 4-1BB.

170. The method of any of claims 72-169, wherein the T cells are CD4⁺ or CD8⁺.

171. The method of any of claims 72-170, wherein the T cells are primary T cells obtained from a subject.

172. The method of any of claims 72-171, wherein the cells of the genetically engineered cells are autologous to the subject.

173. The method of any of claims 72-171, wherein the cells of the genetically engineered cells are allogeneic to the subject.

FIG. 1

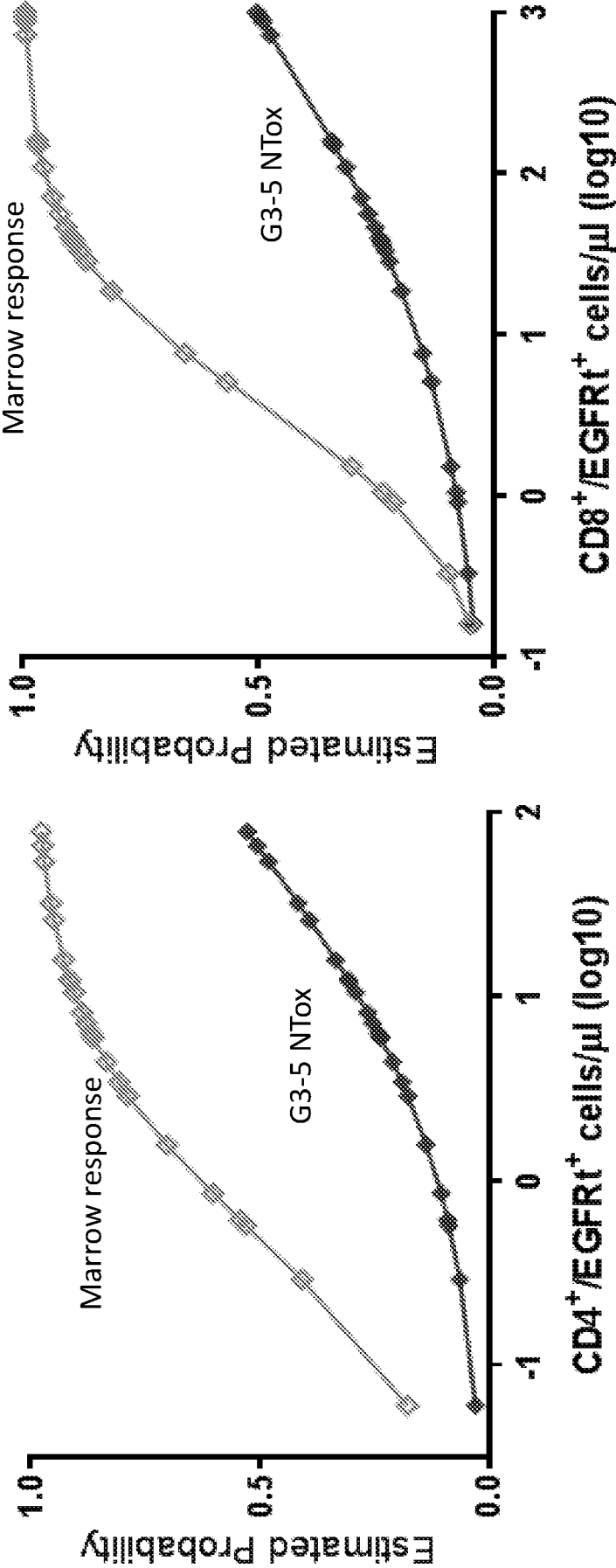


FIG. 2A

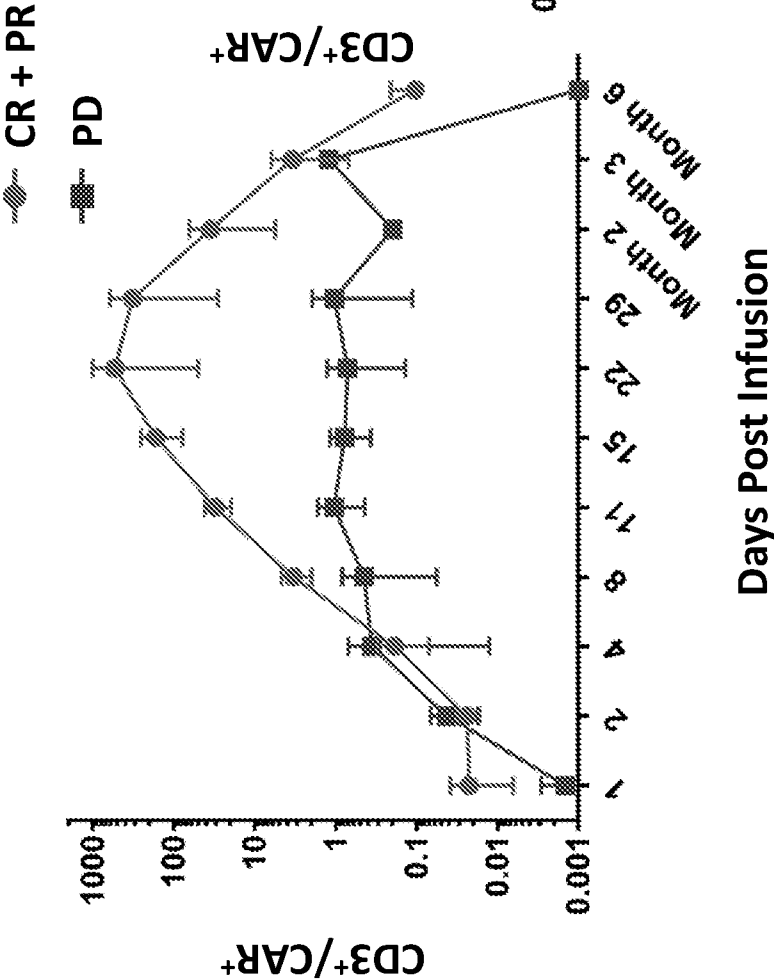


FIG. 2B

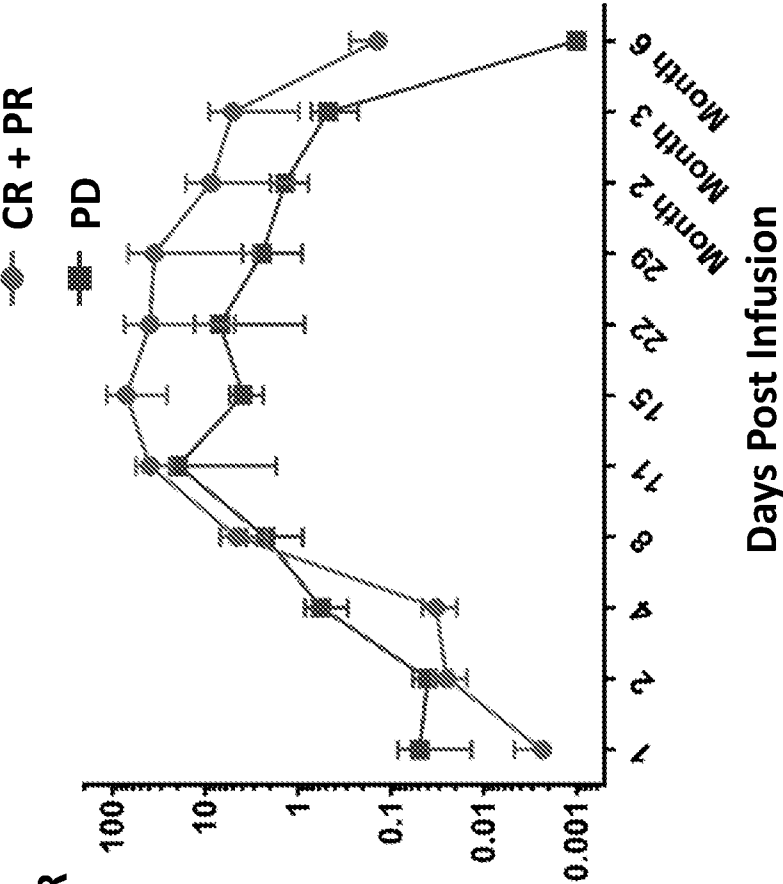


FIG. 2C

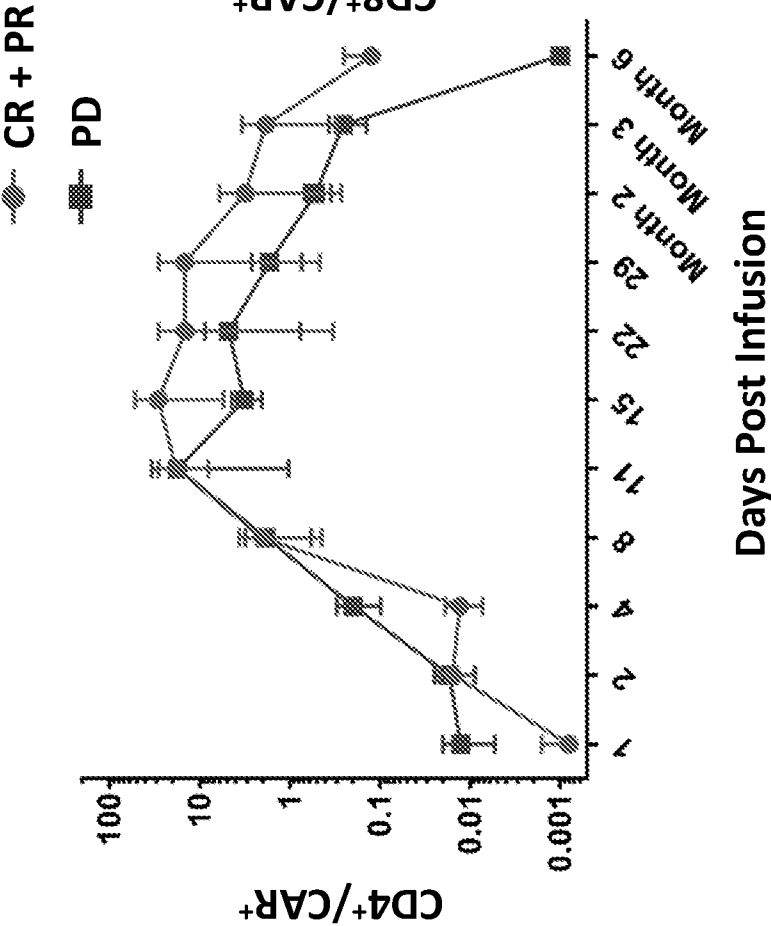


FIG. 2D

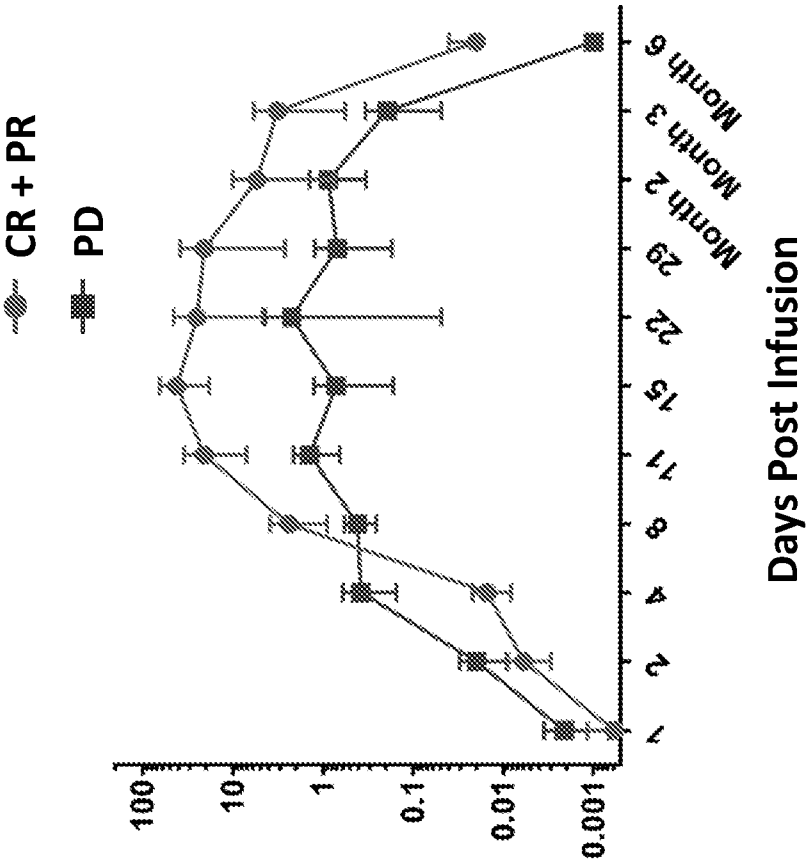


FIG. 3

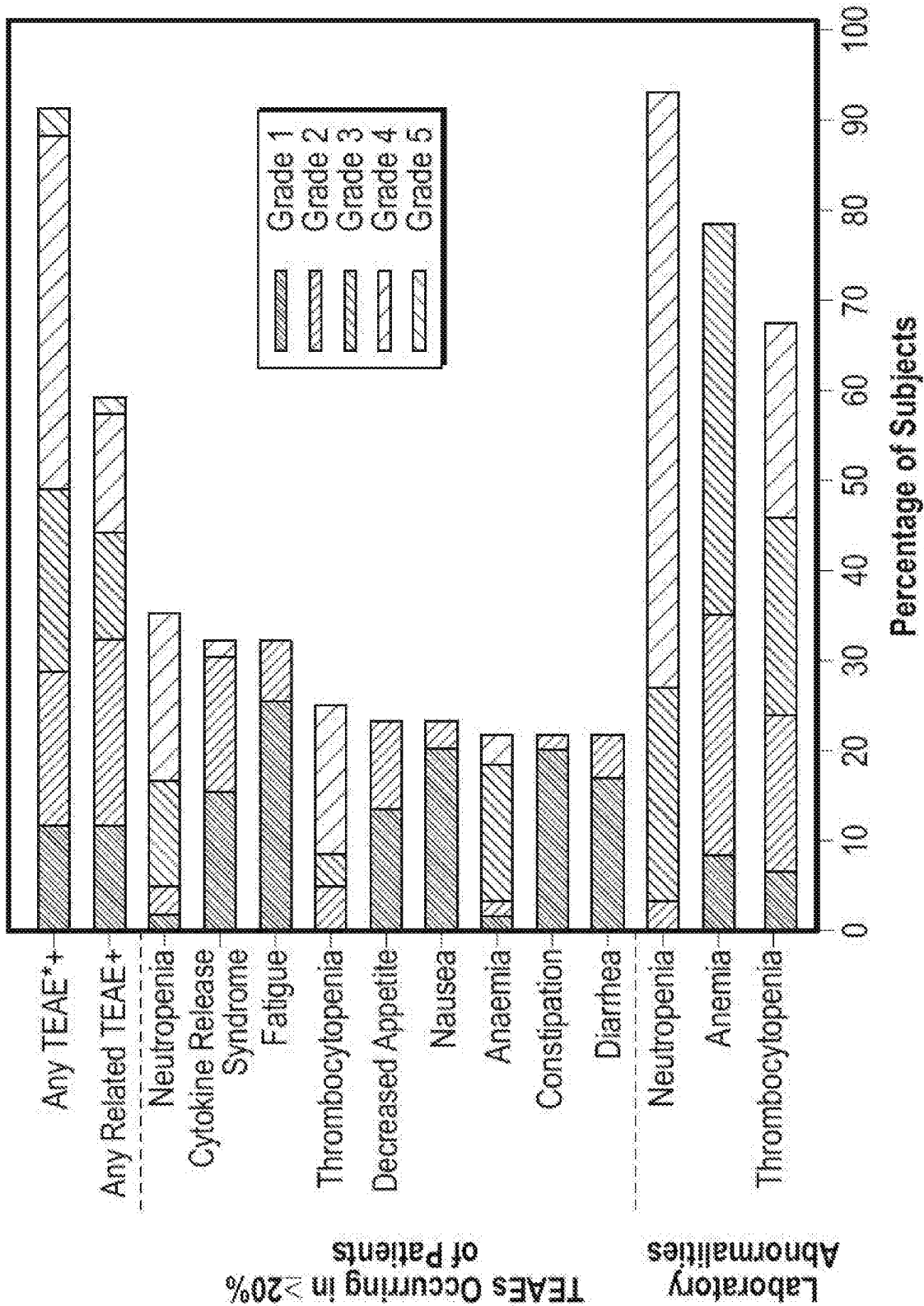


FIG. 4

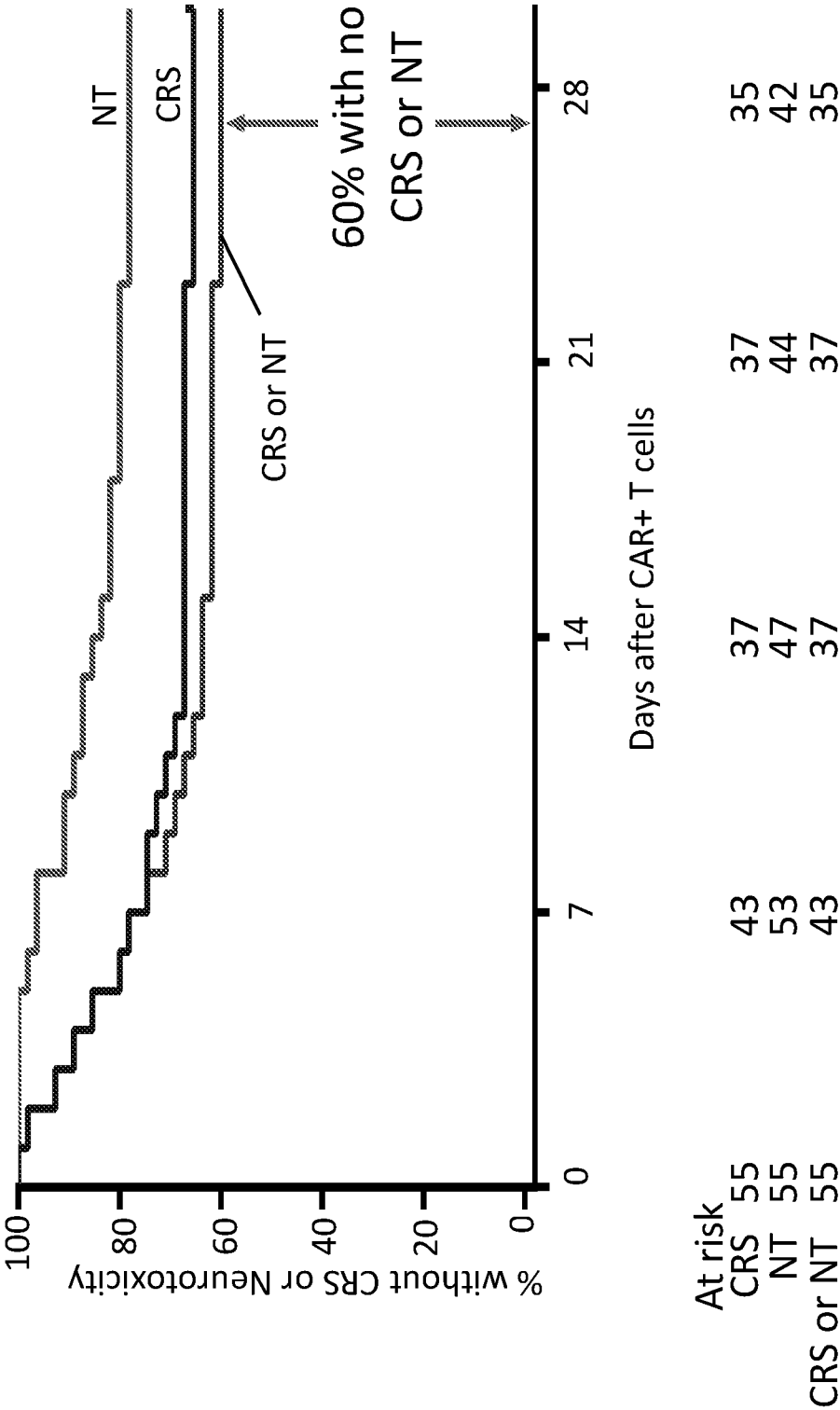


FIG. 5A

FULL

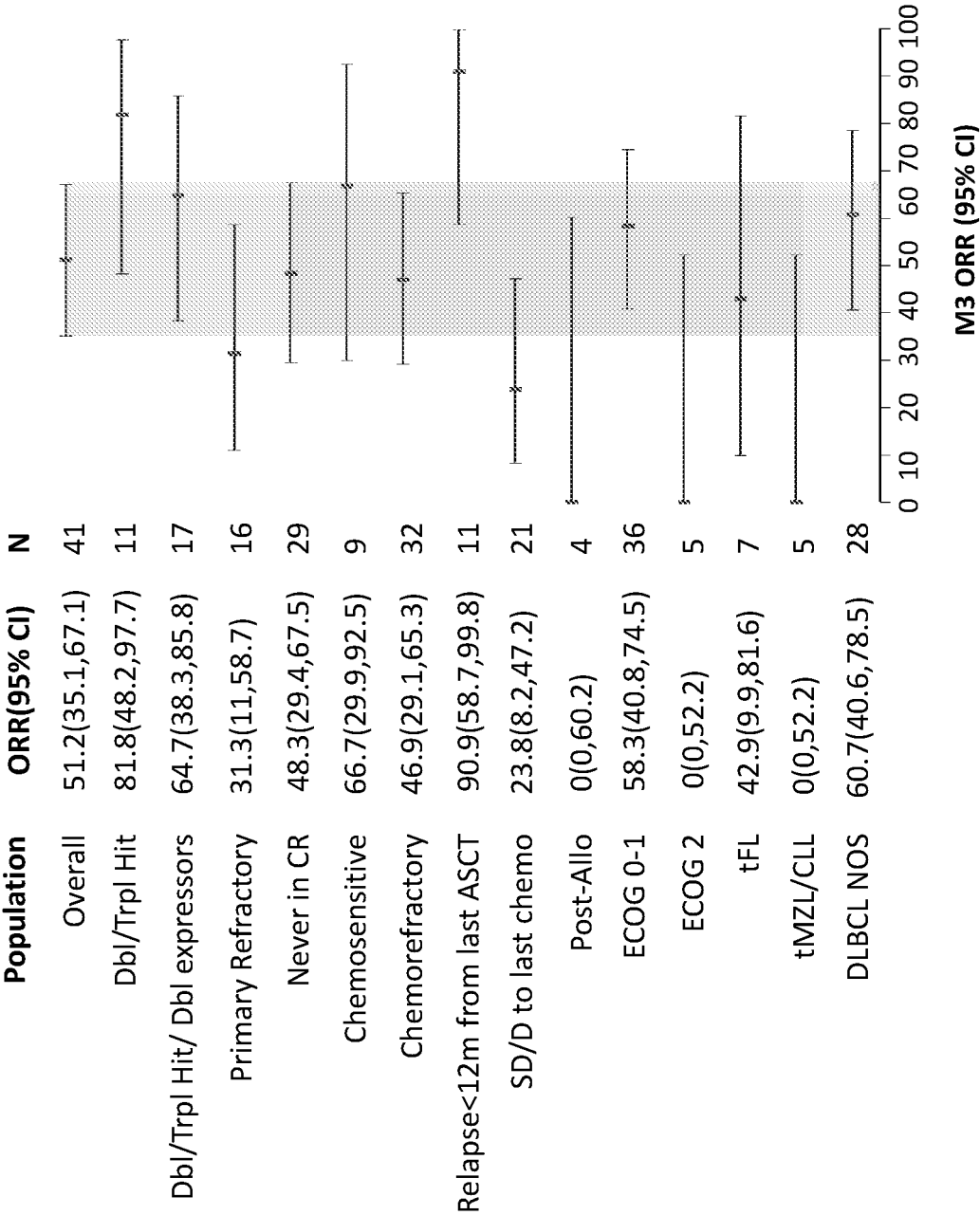


FIG. 5B

CORE

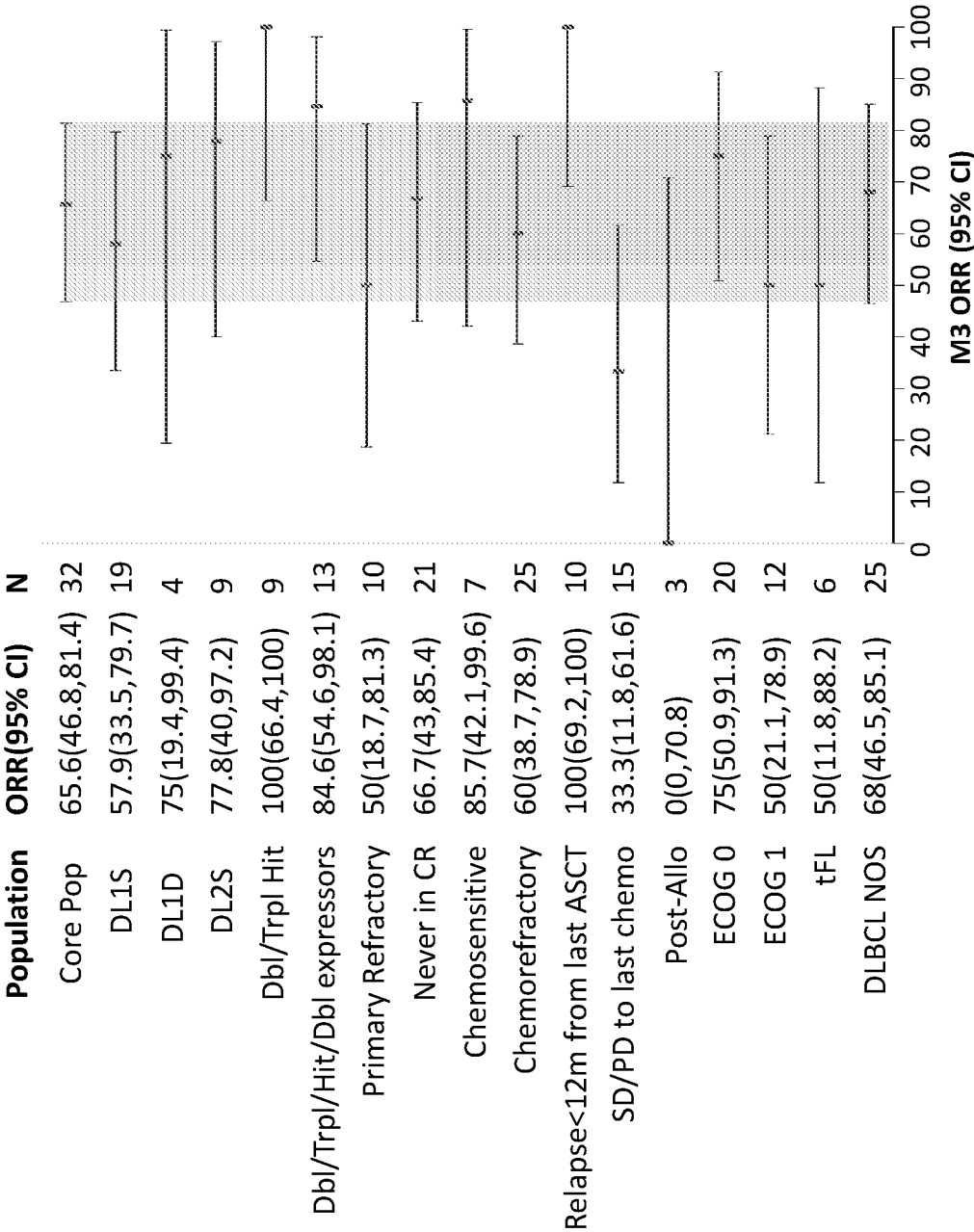


FIG. 6A

FULL

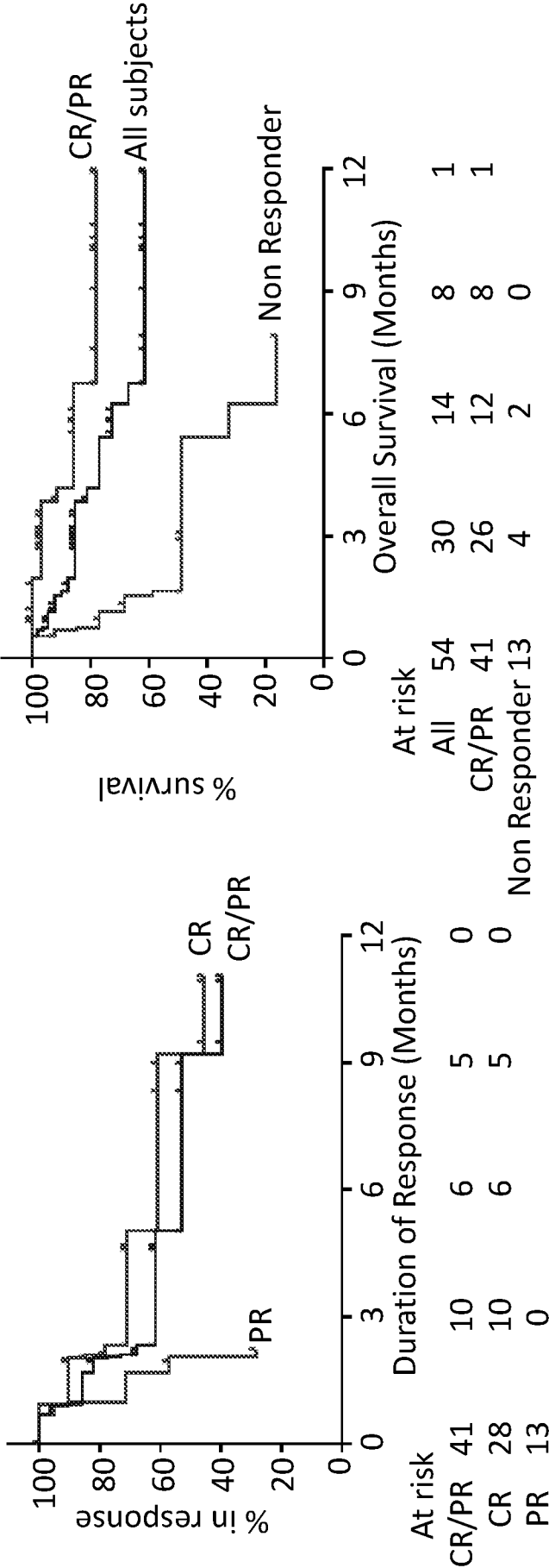


FIG. 6B
CORE

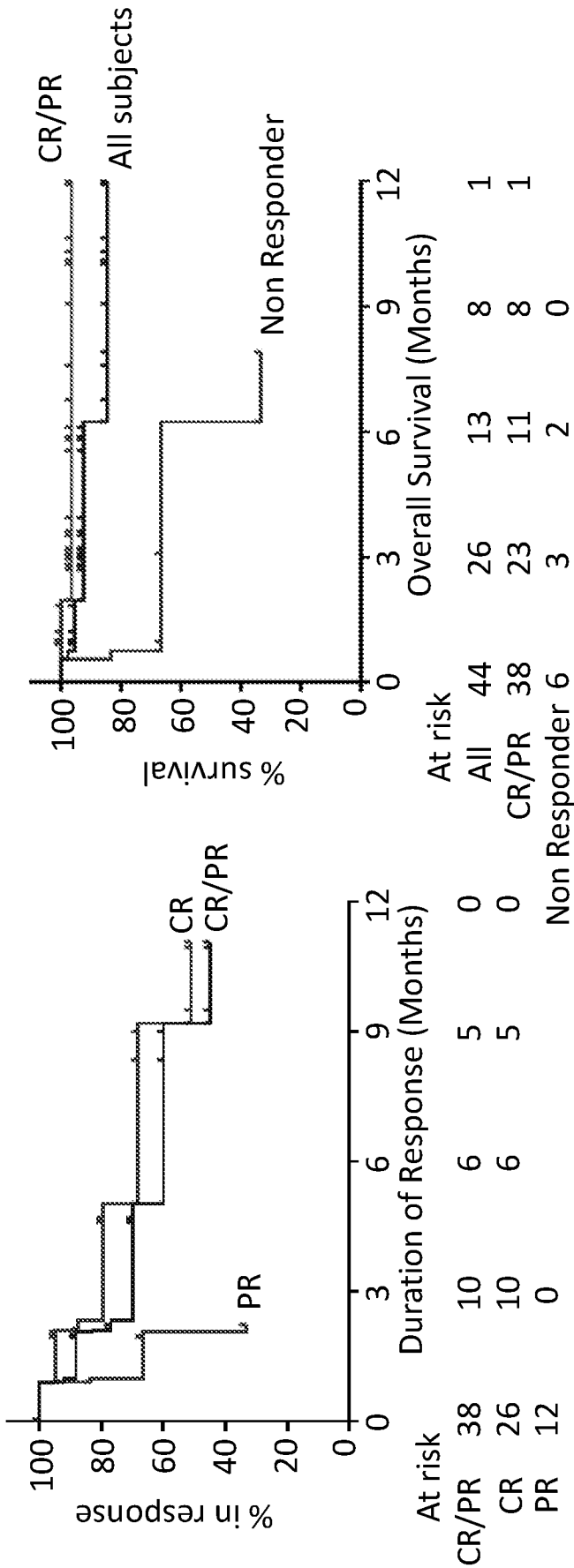


FIG. 7A

Dose Level

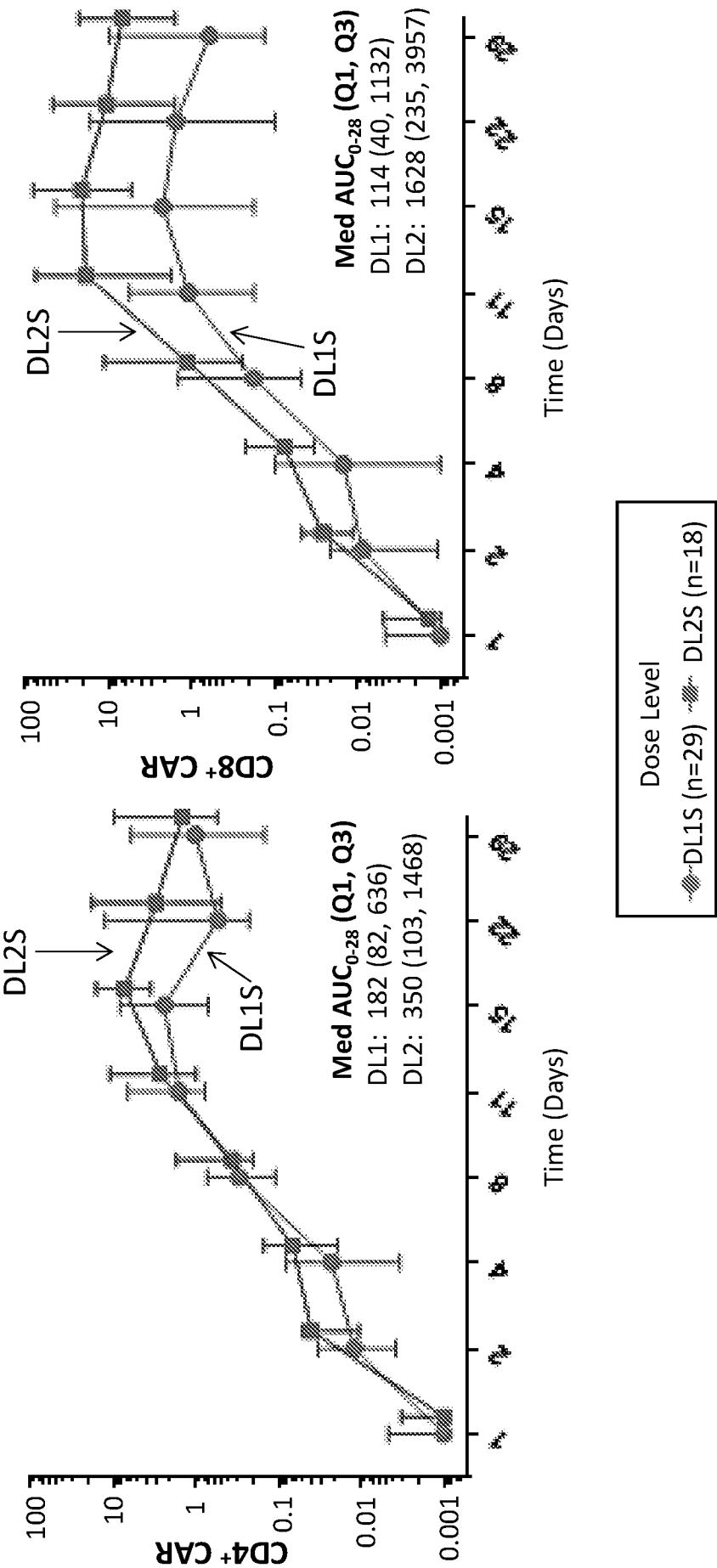


FIG. 7B
Month 3 Response

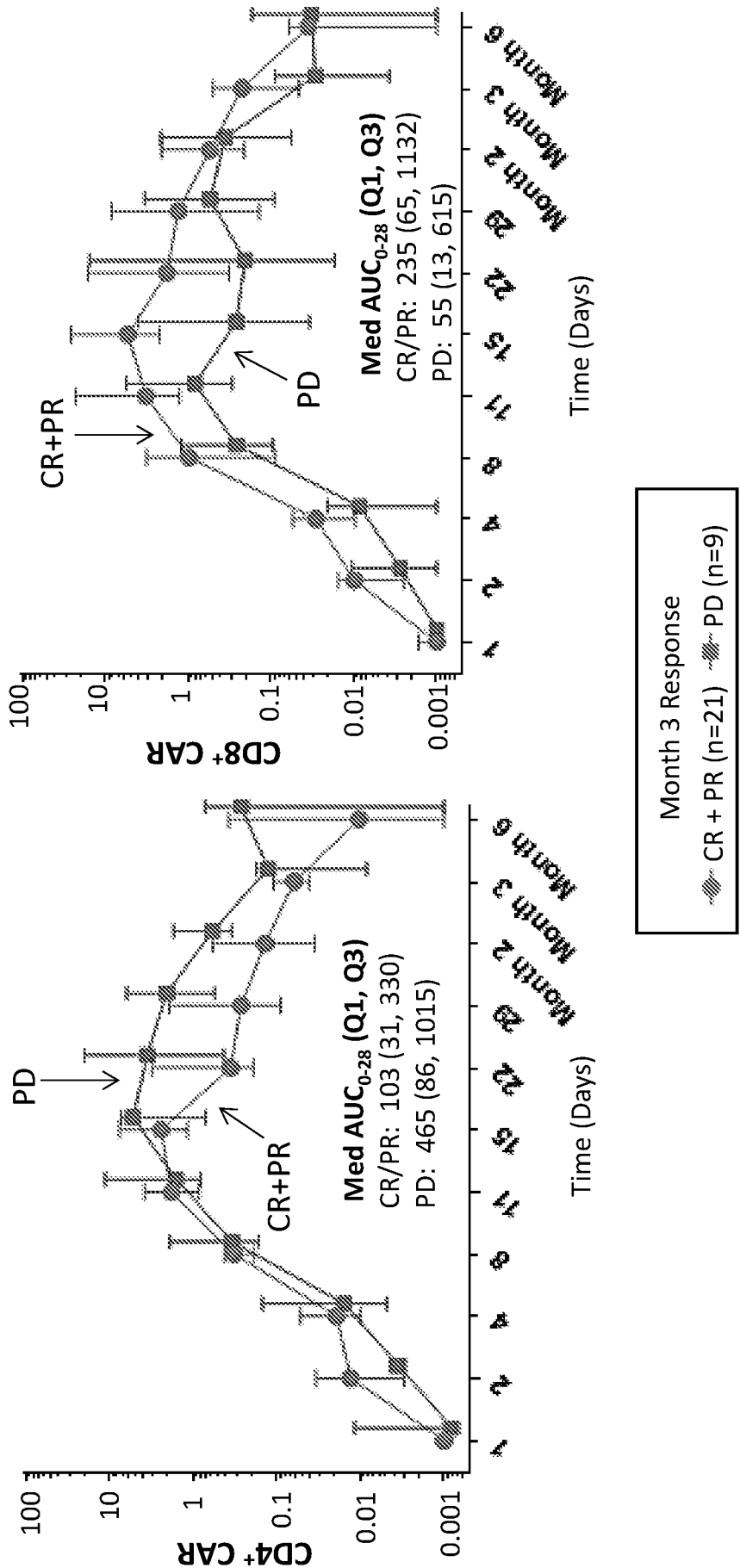
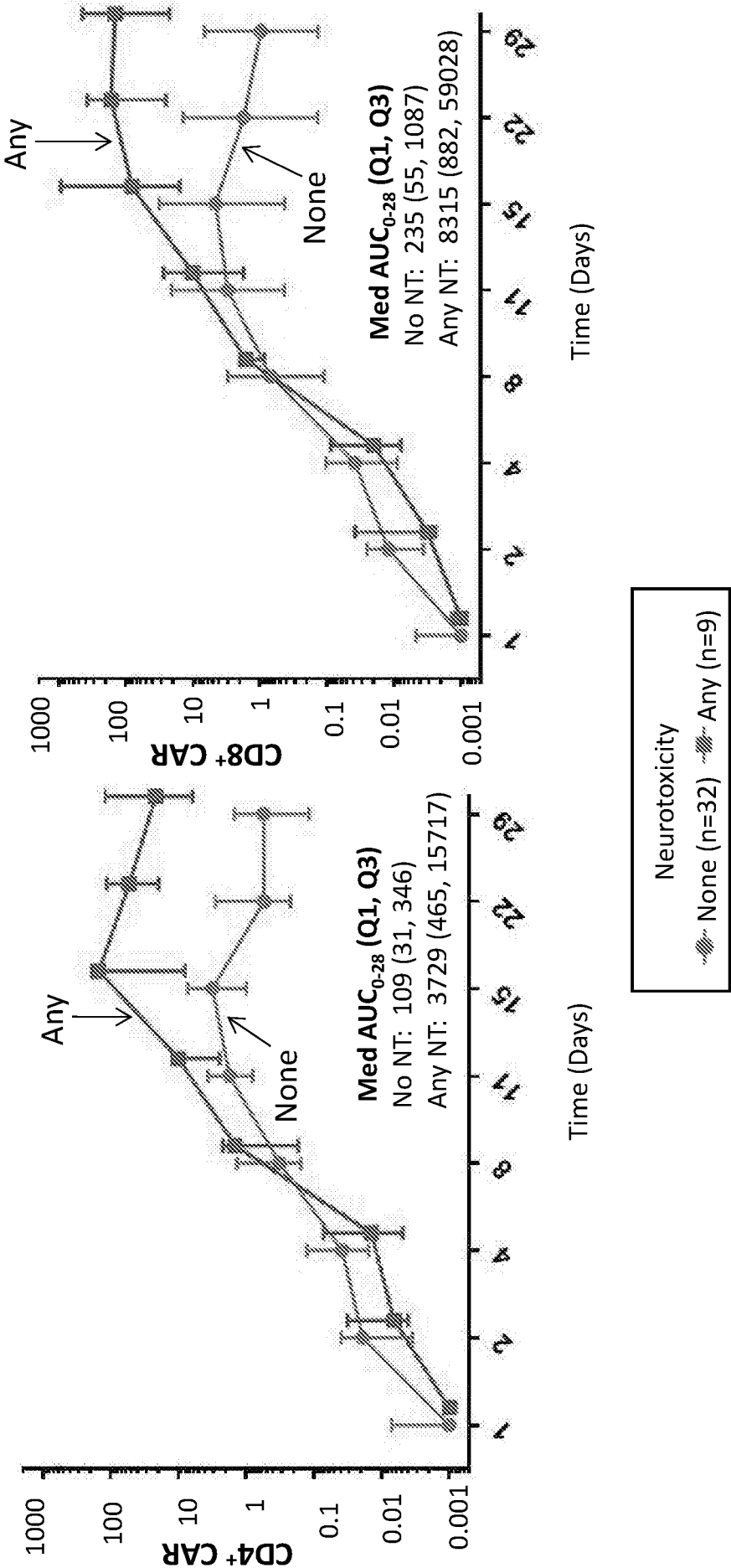


FIG. 7C

Neurotoxicity



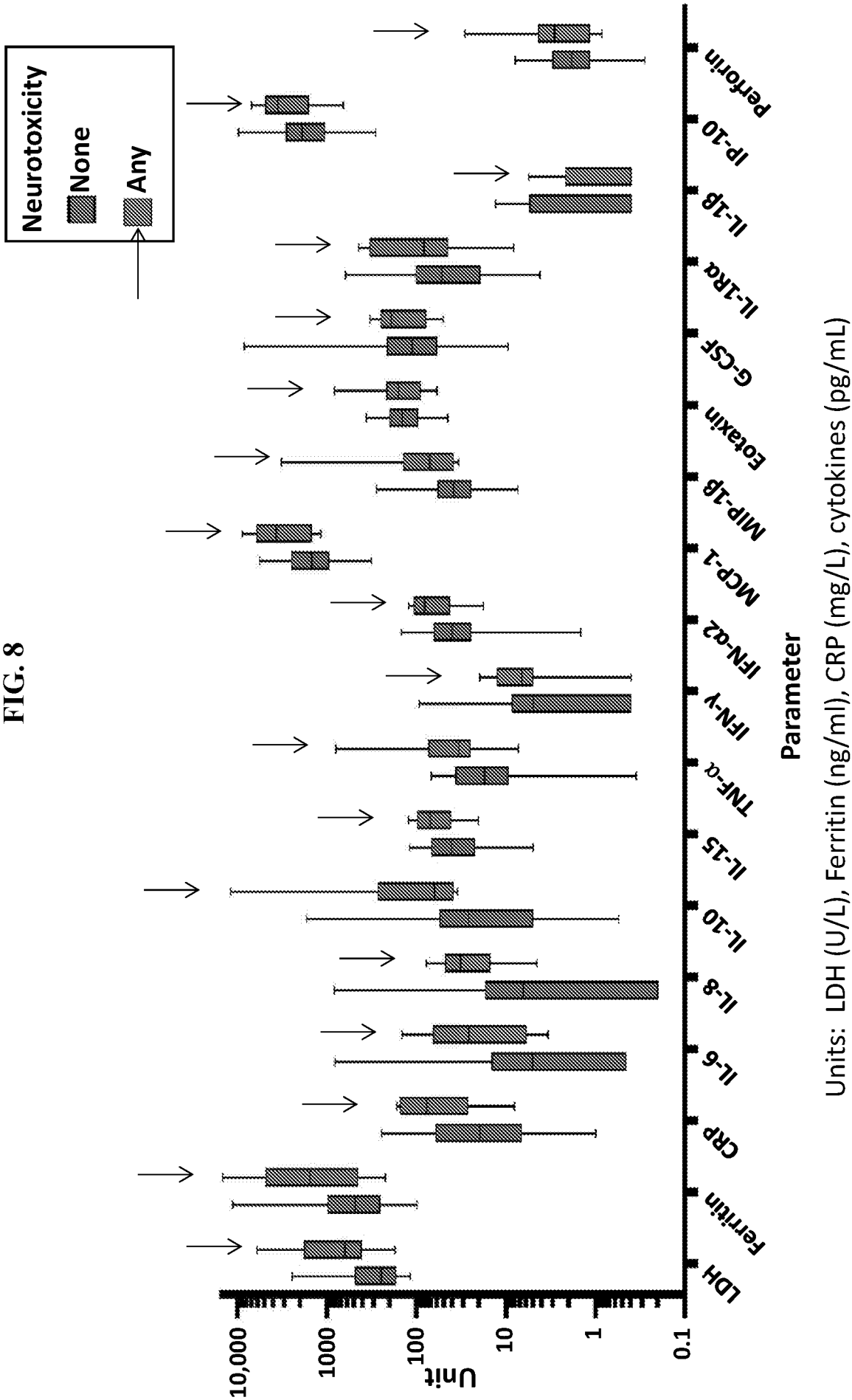


FIG. 9

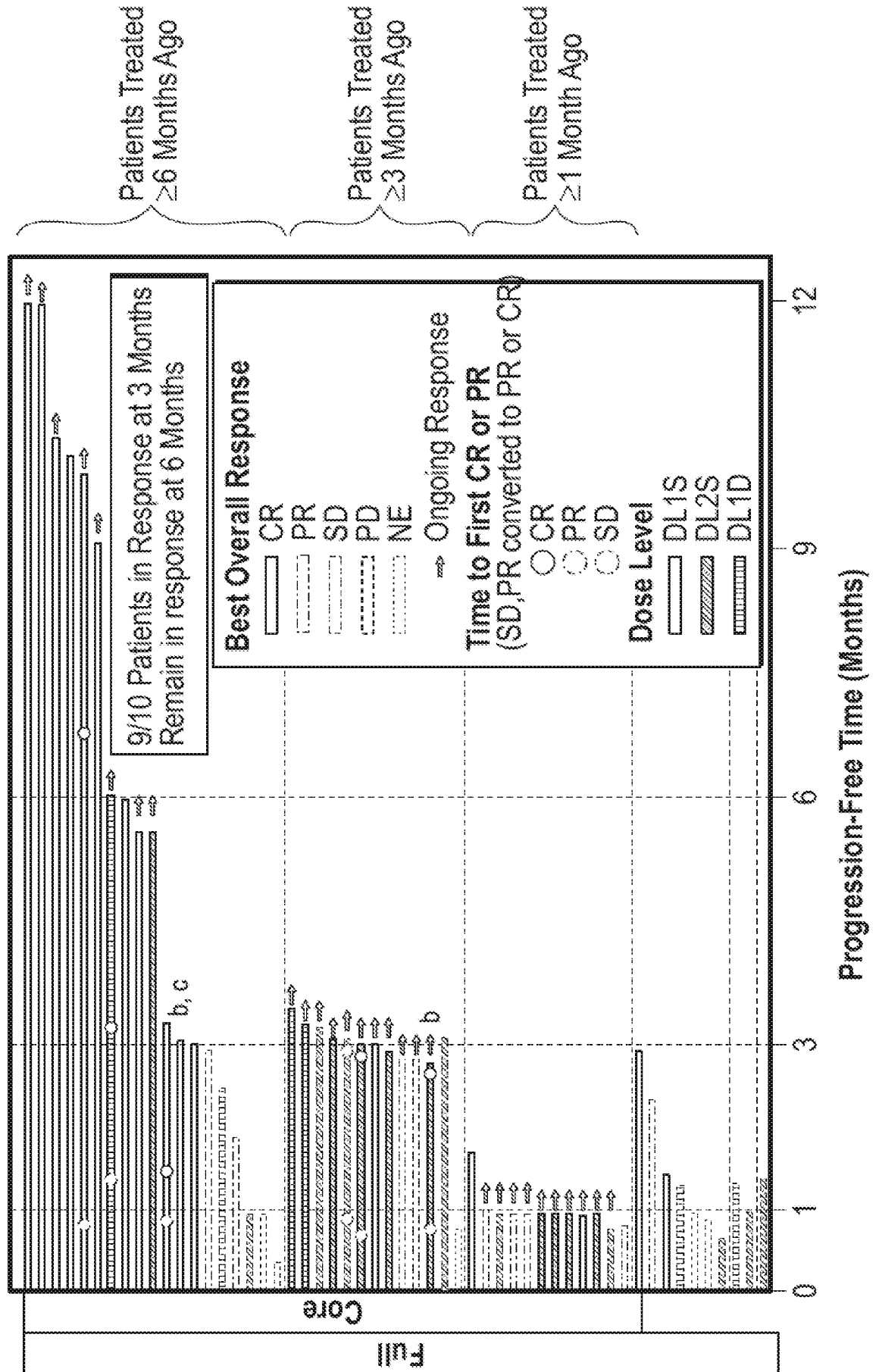


FIG. 10B

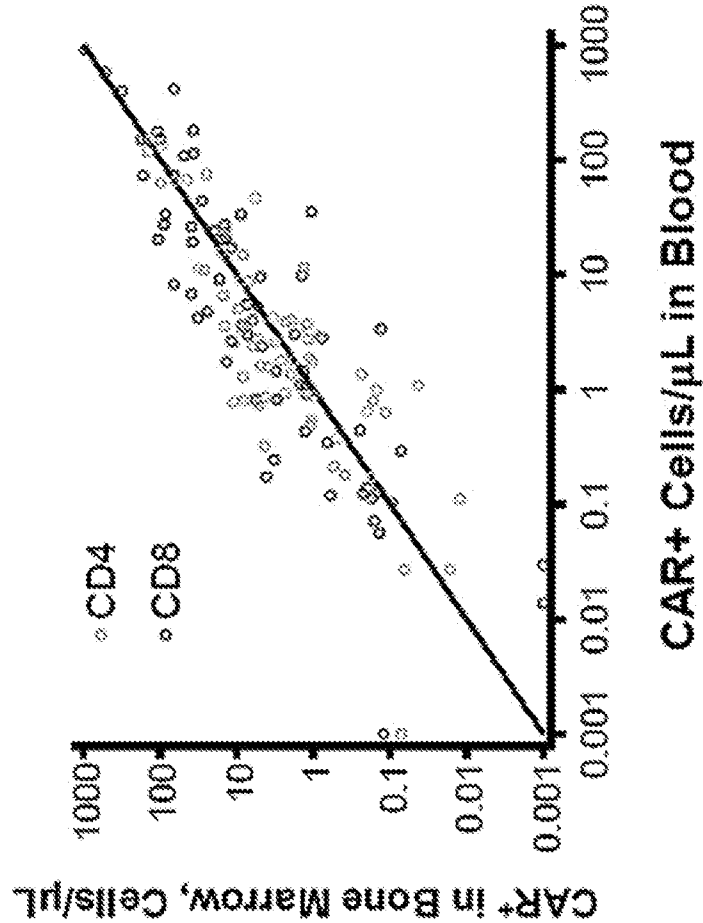


FIG. 10A

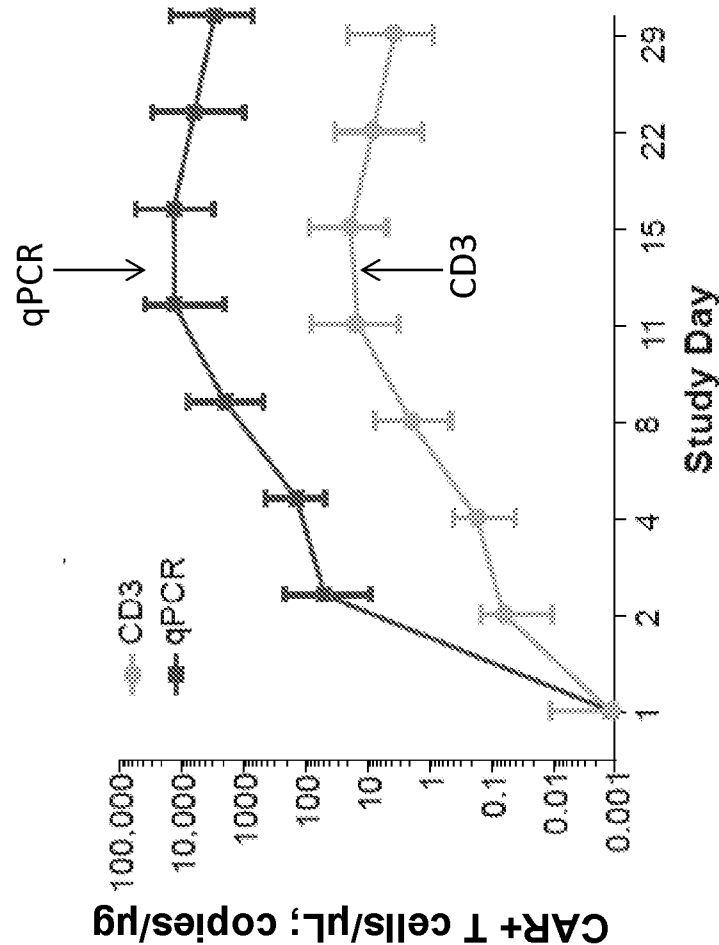


FIG. 11B

CD4⁺ & CD8⁺ C_{max}

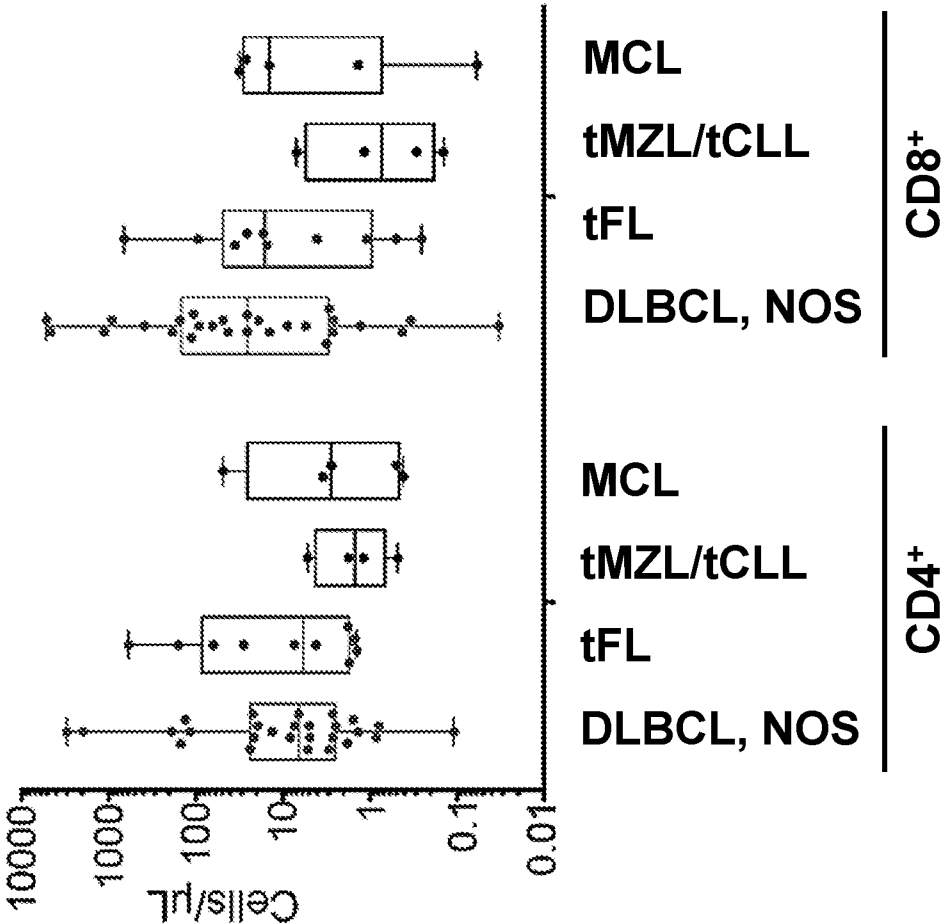


FIG. 11A

CD4⁺ & CD8⁺ AUC₀₋₂₈

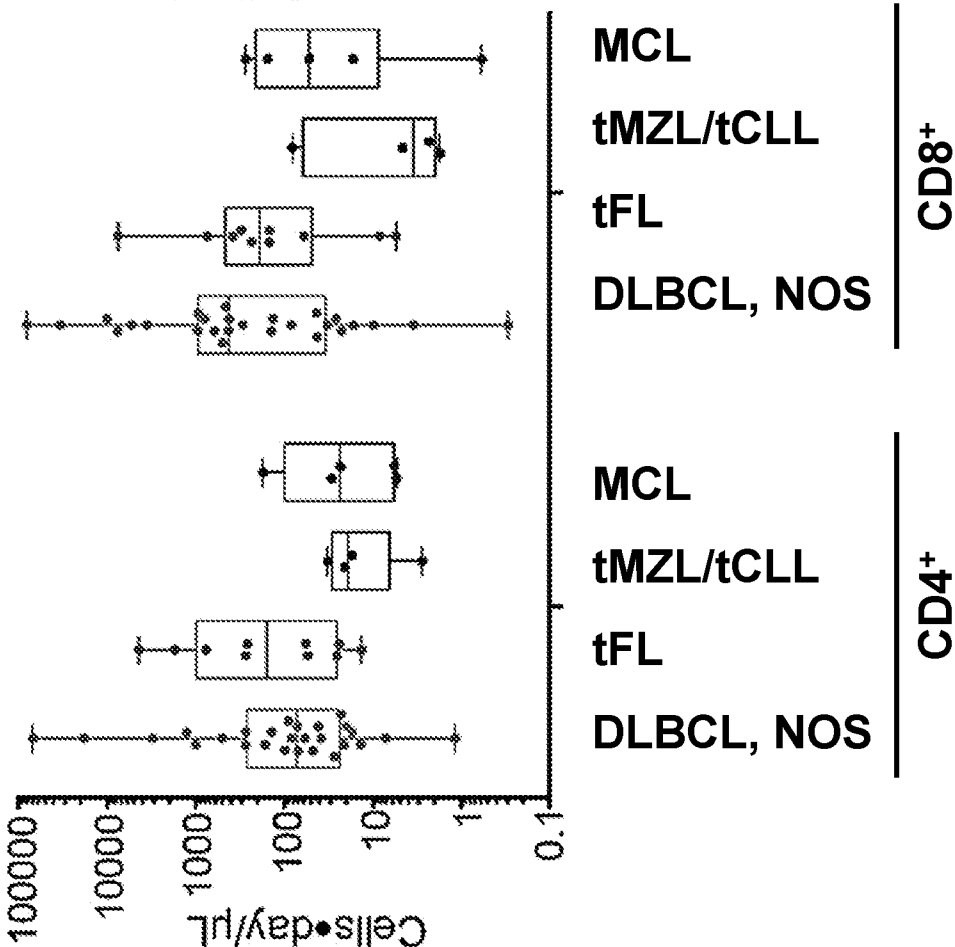


FIG. 12A

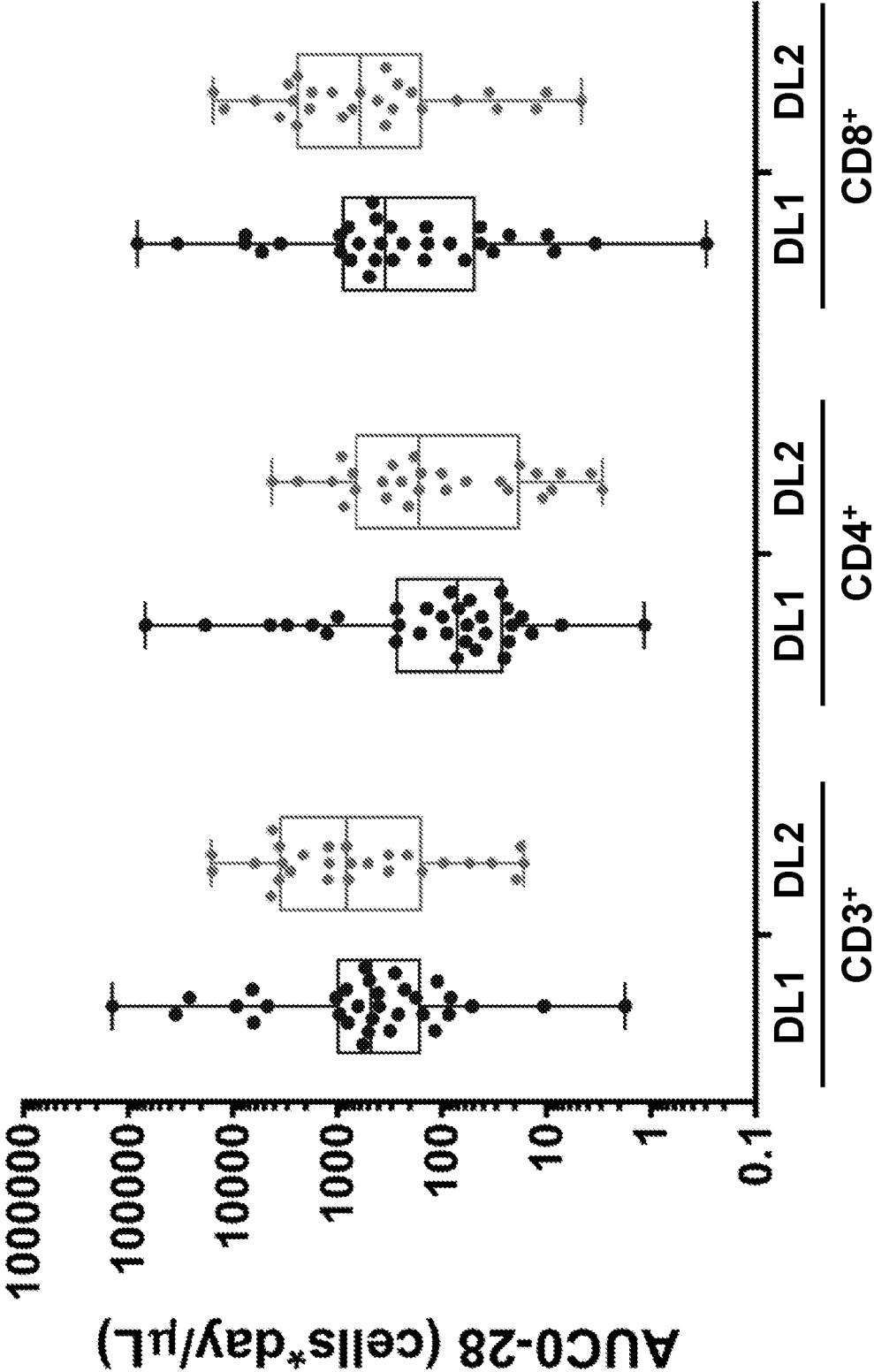


FIG. 12B

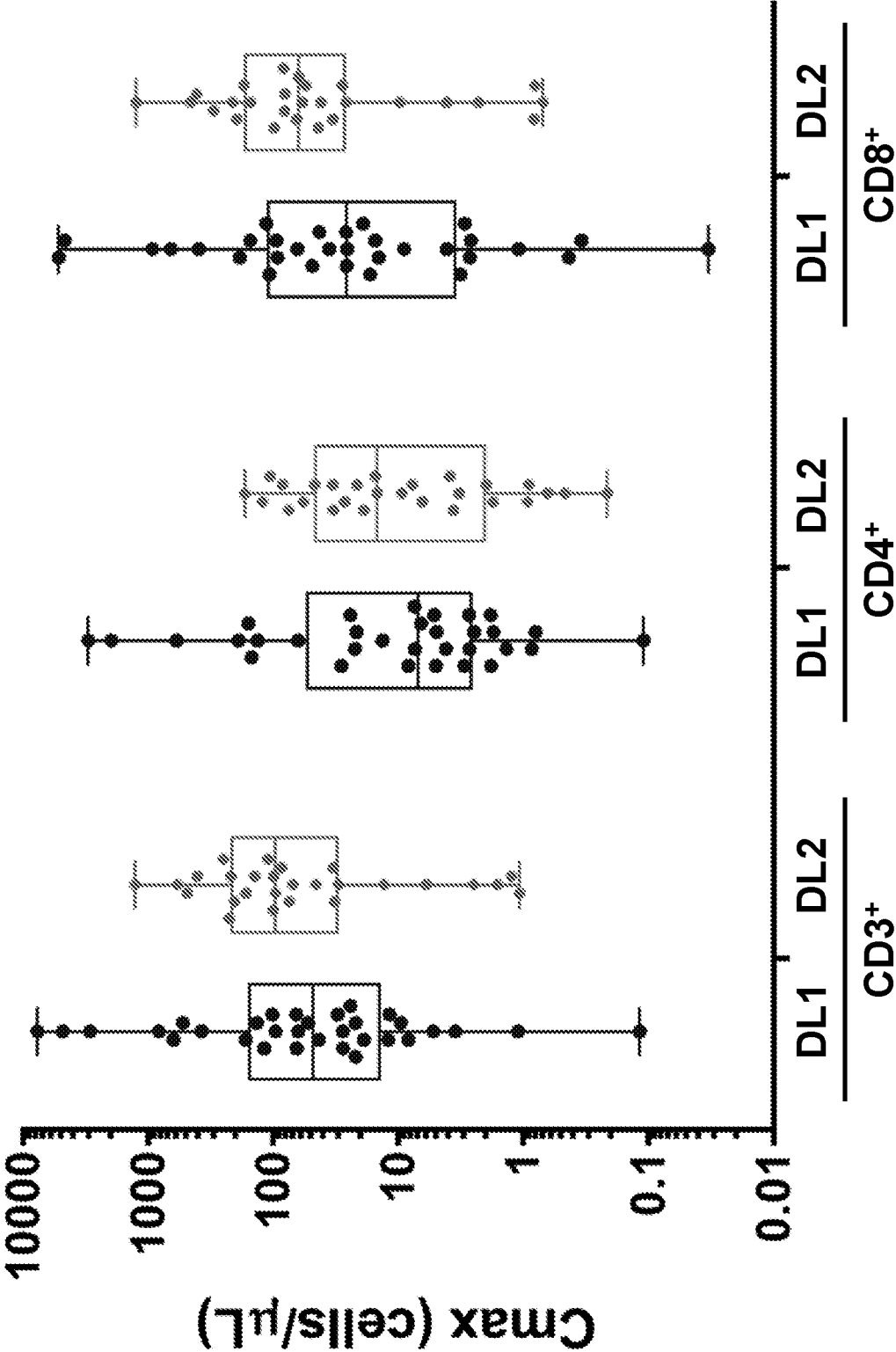


FIG. 13A

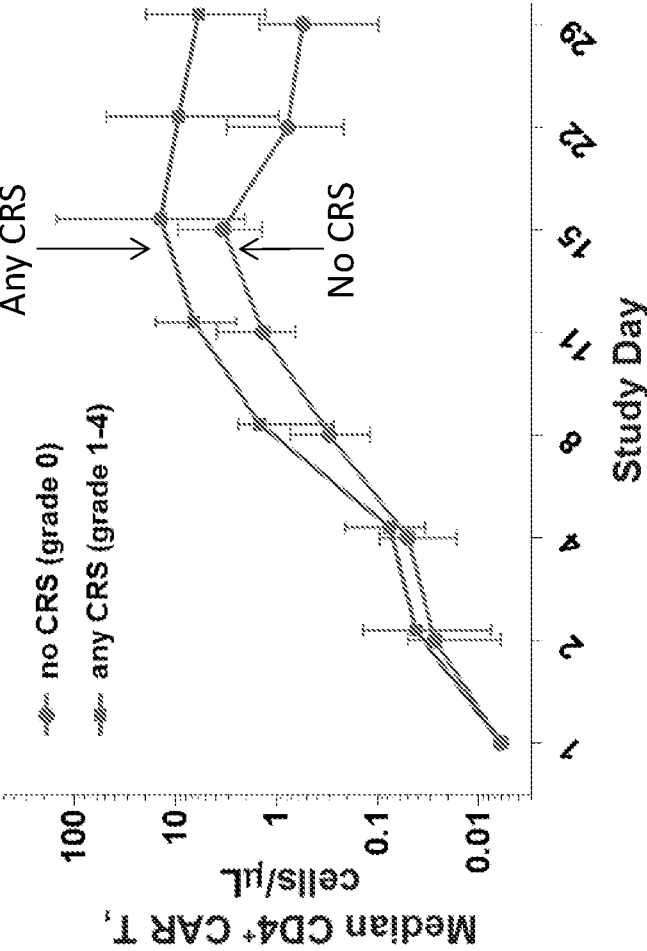


FIG. 13B

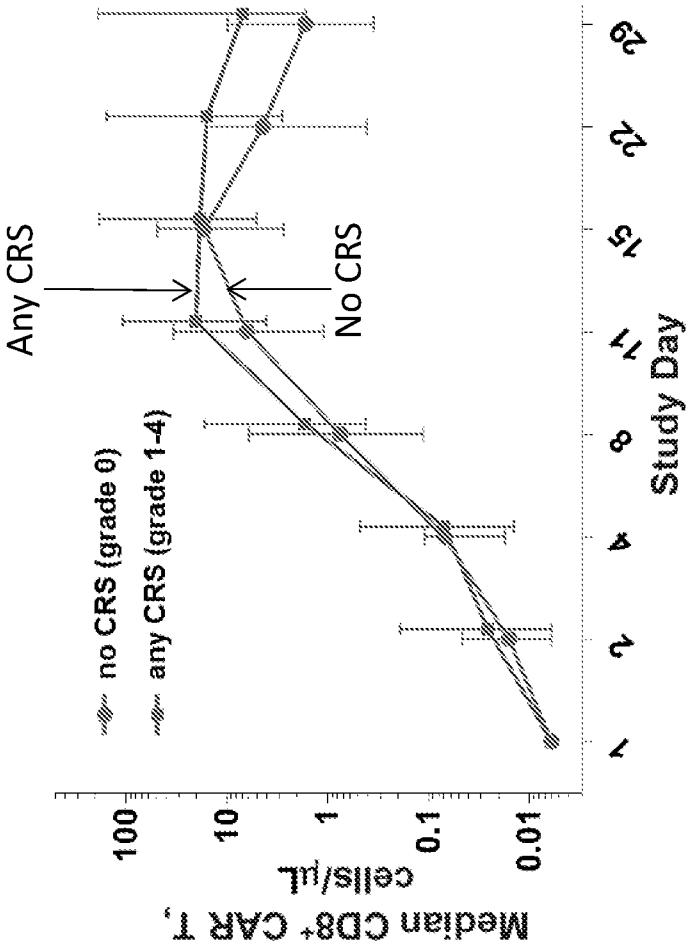


FIG. 13C

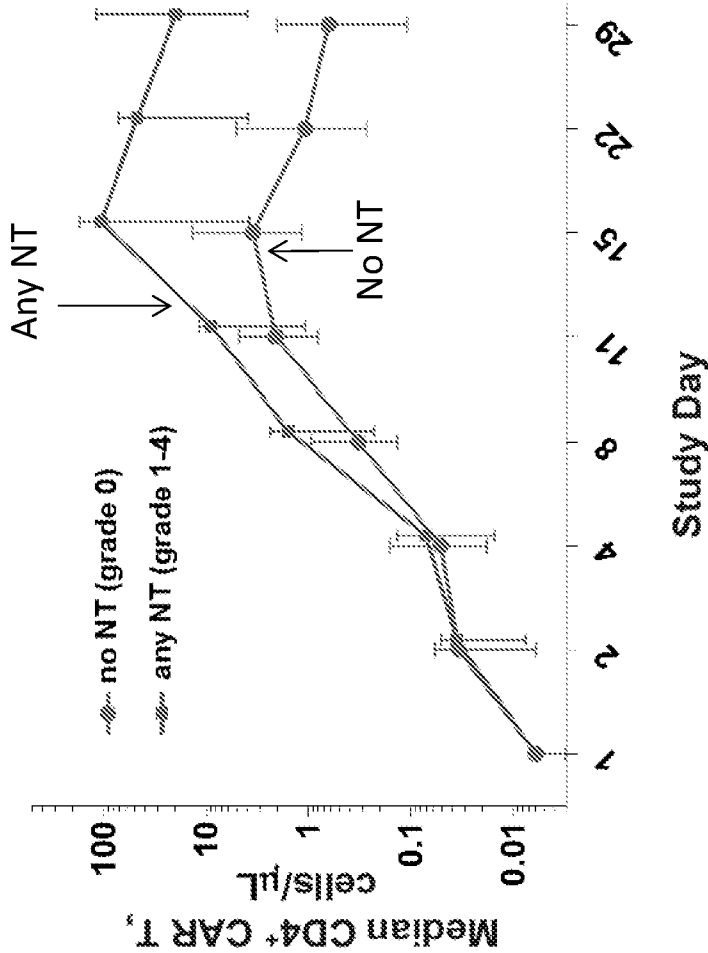
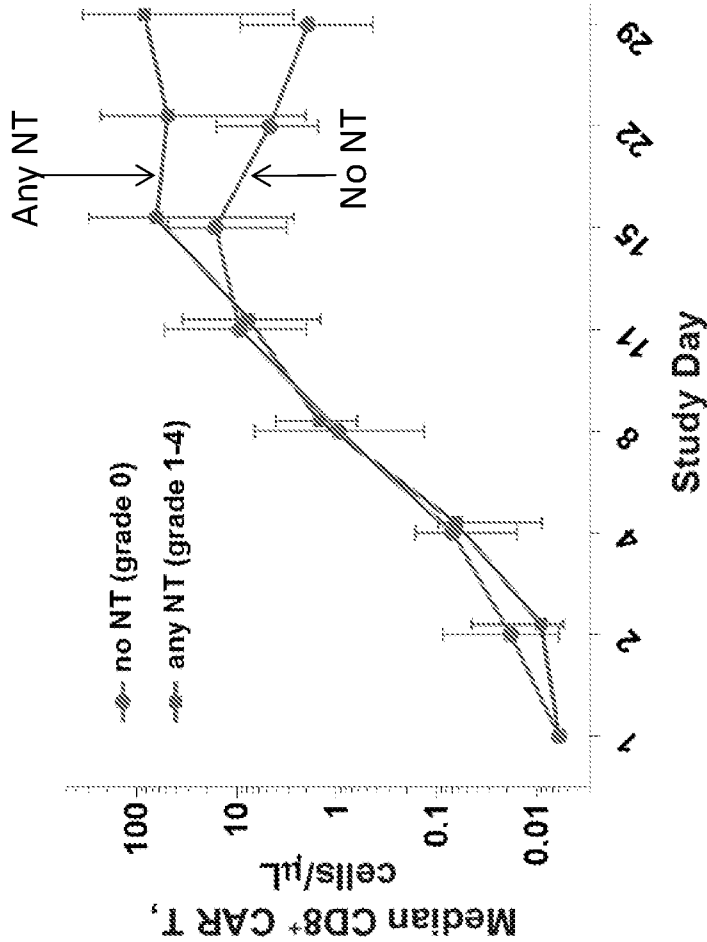


FIG. 13D



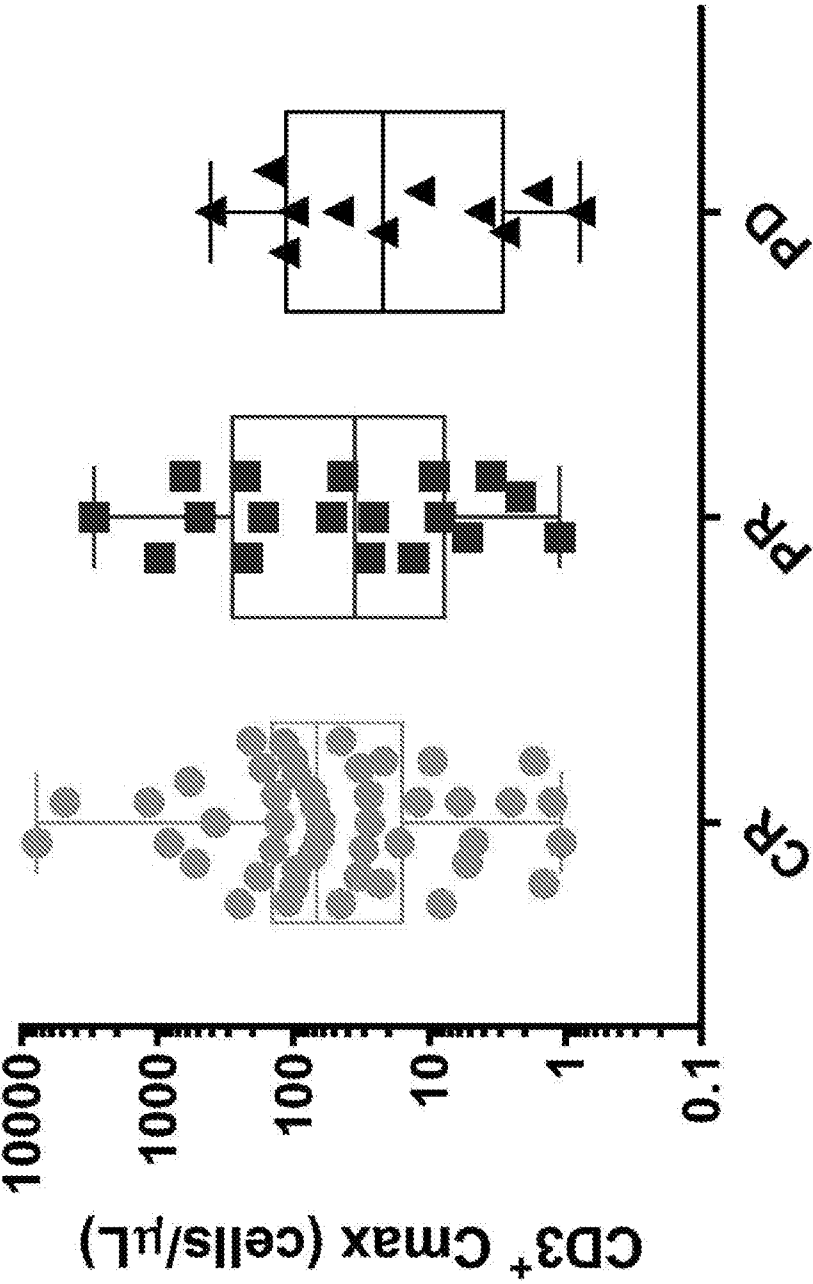


FIG. 14A

FIG. 14B

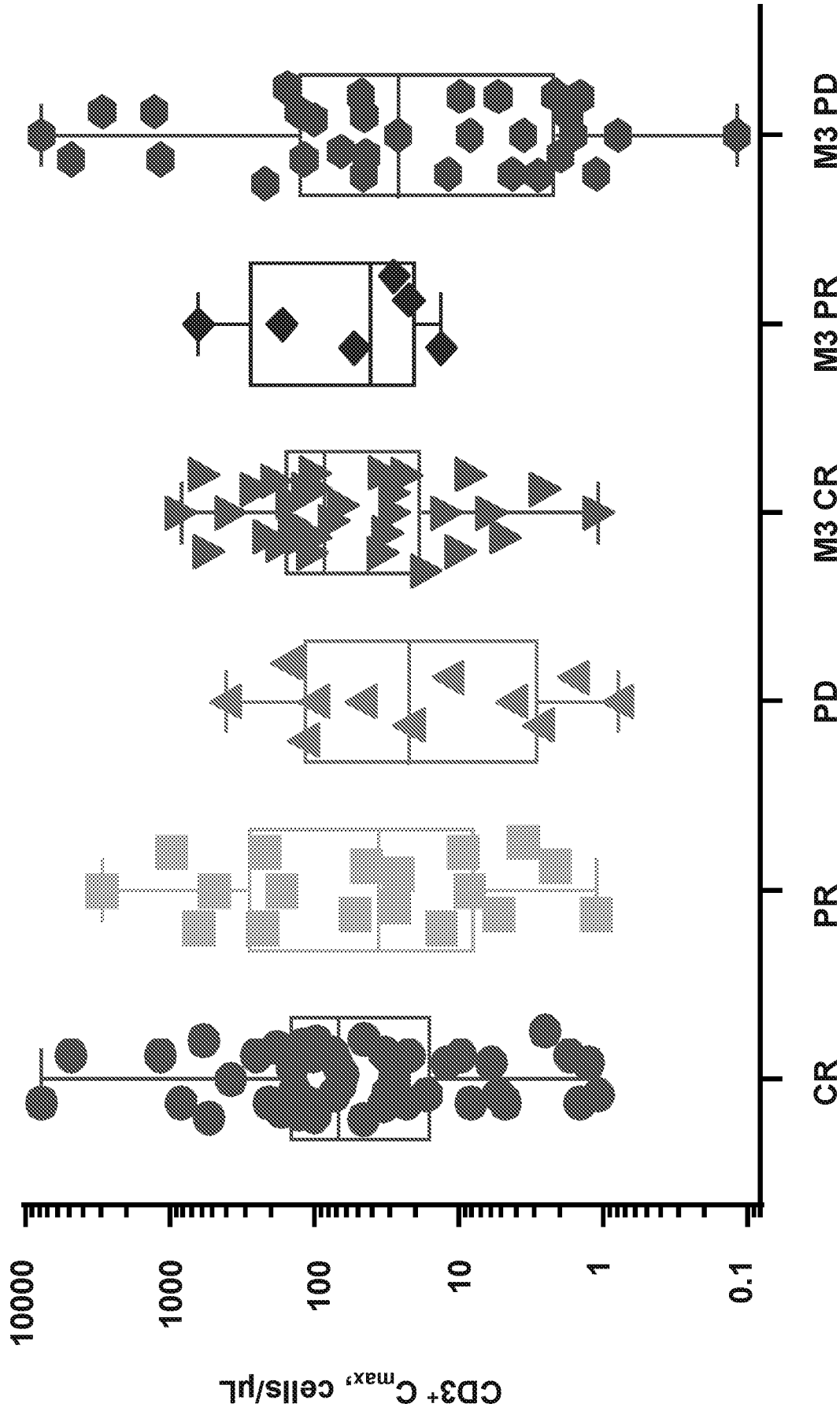


FIG. 15A

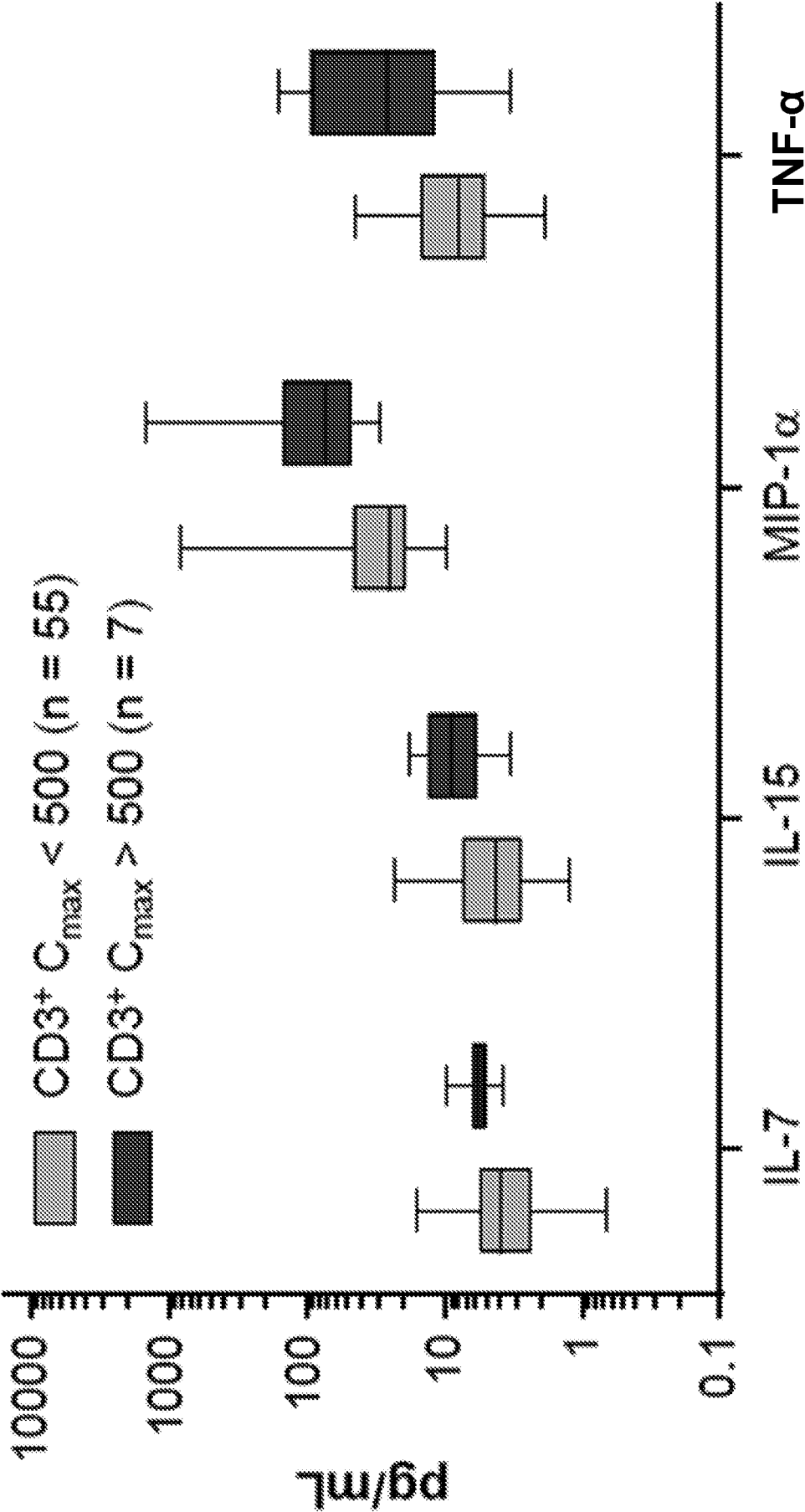


FIG. 15B

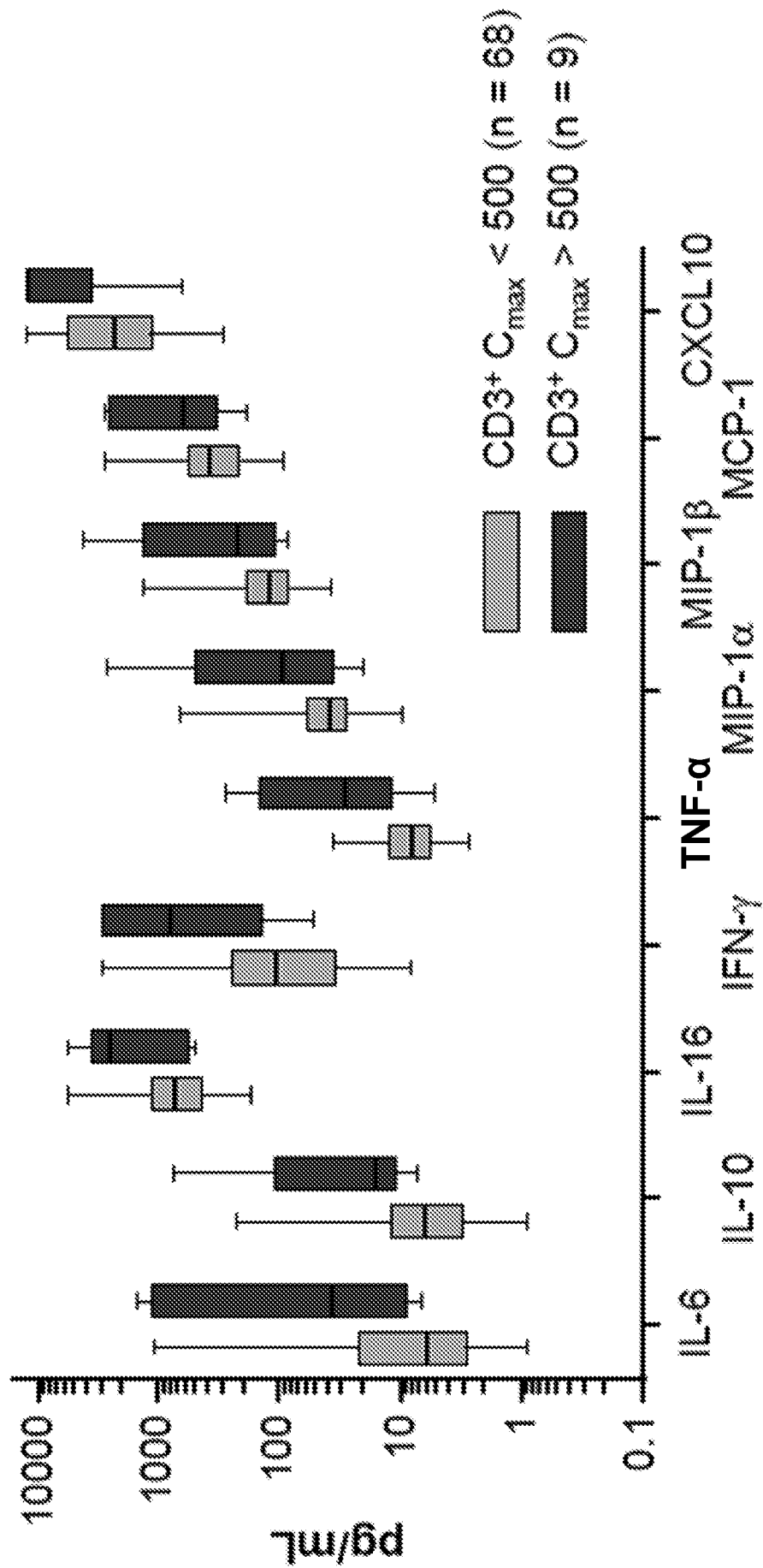


FIG. 16

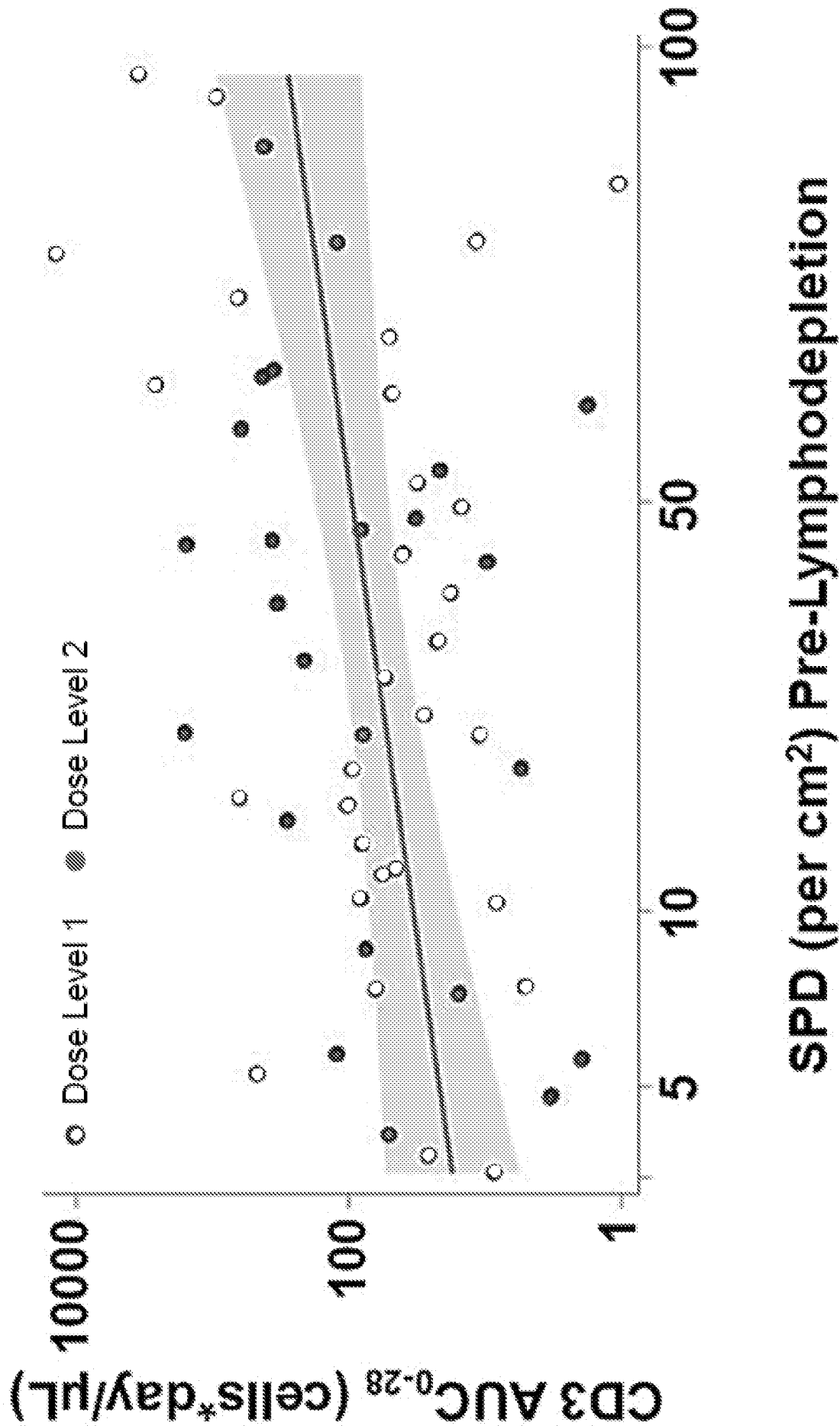


FIG. 17A

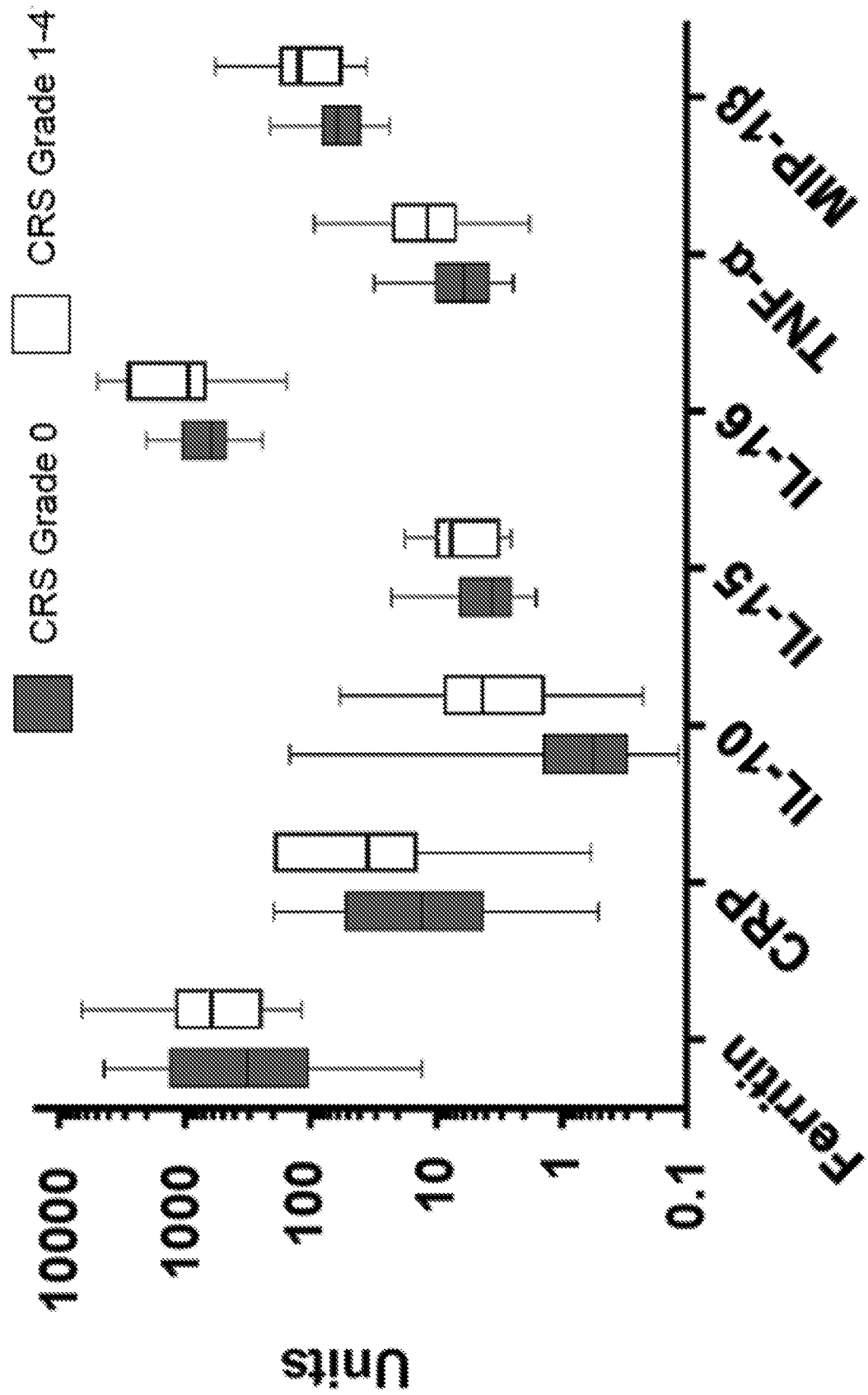


FIG. 17B

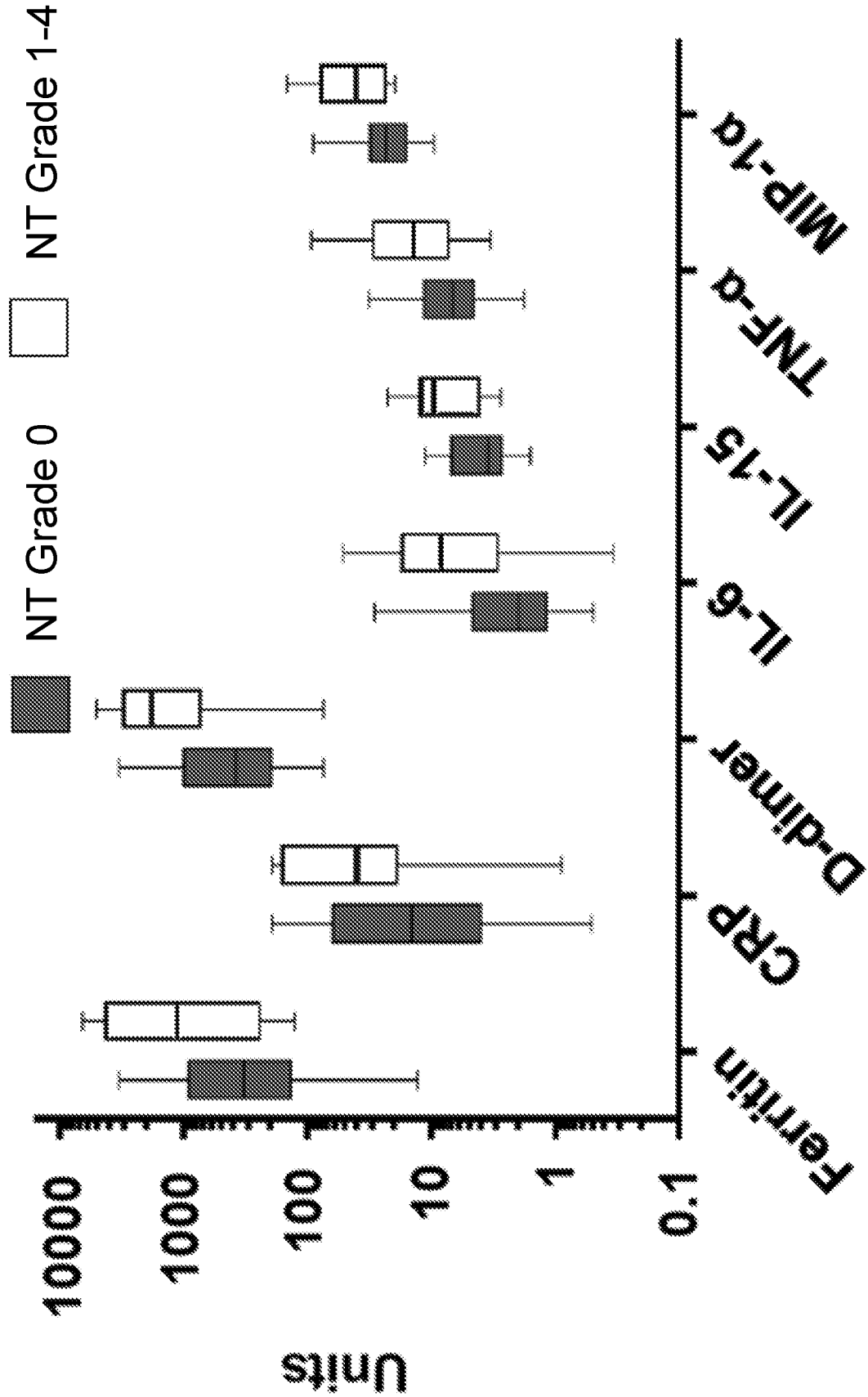


FIG. 18

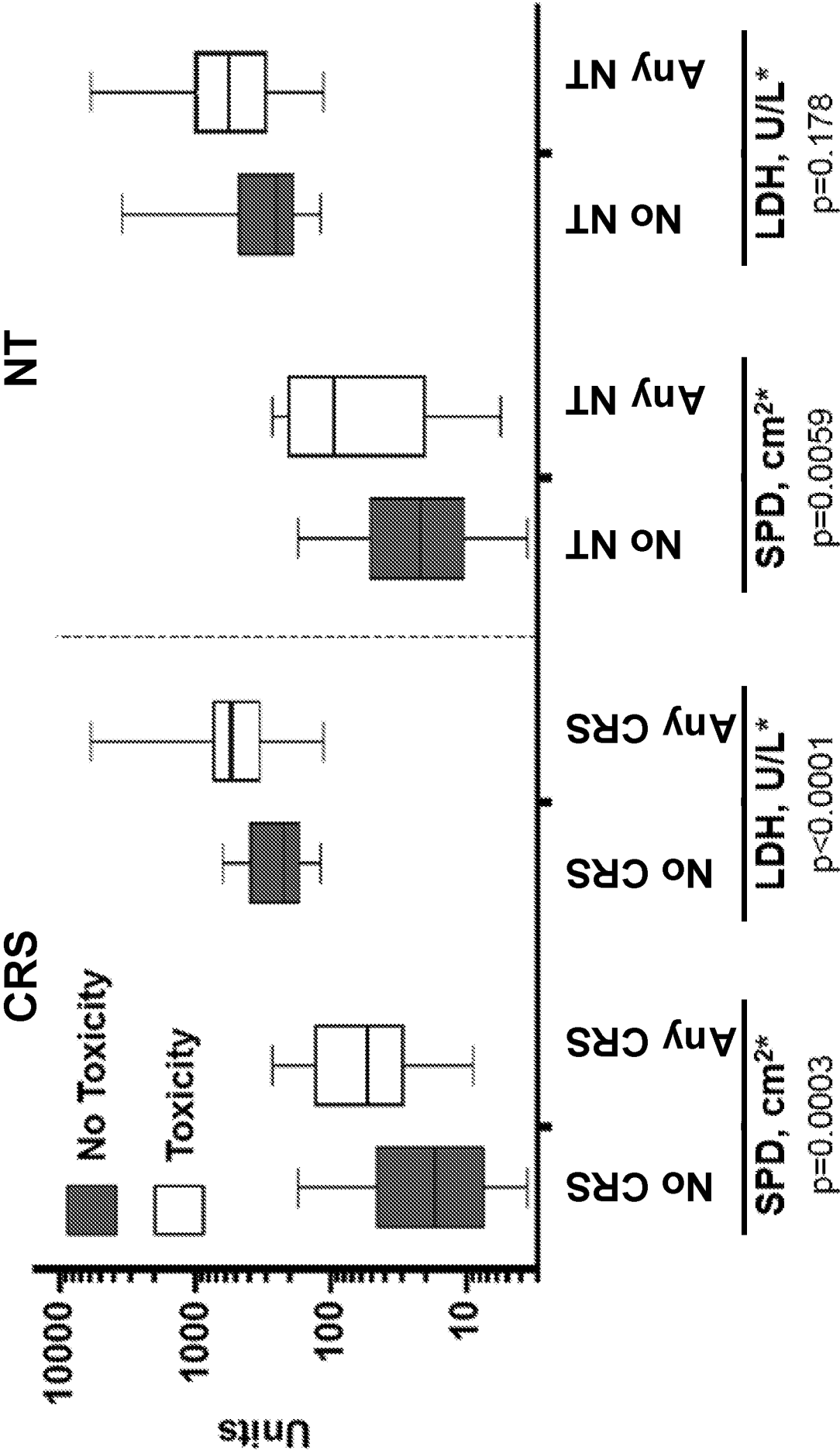
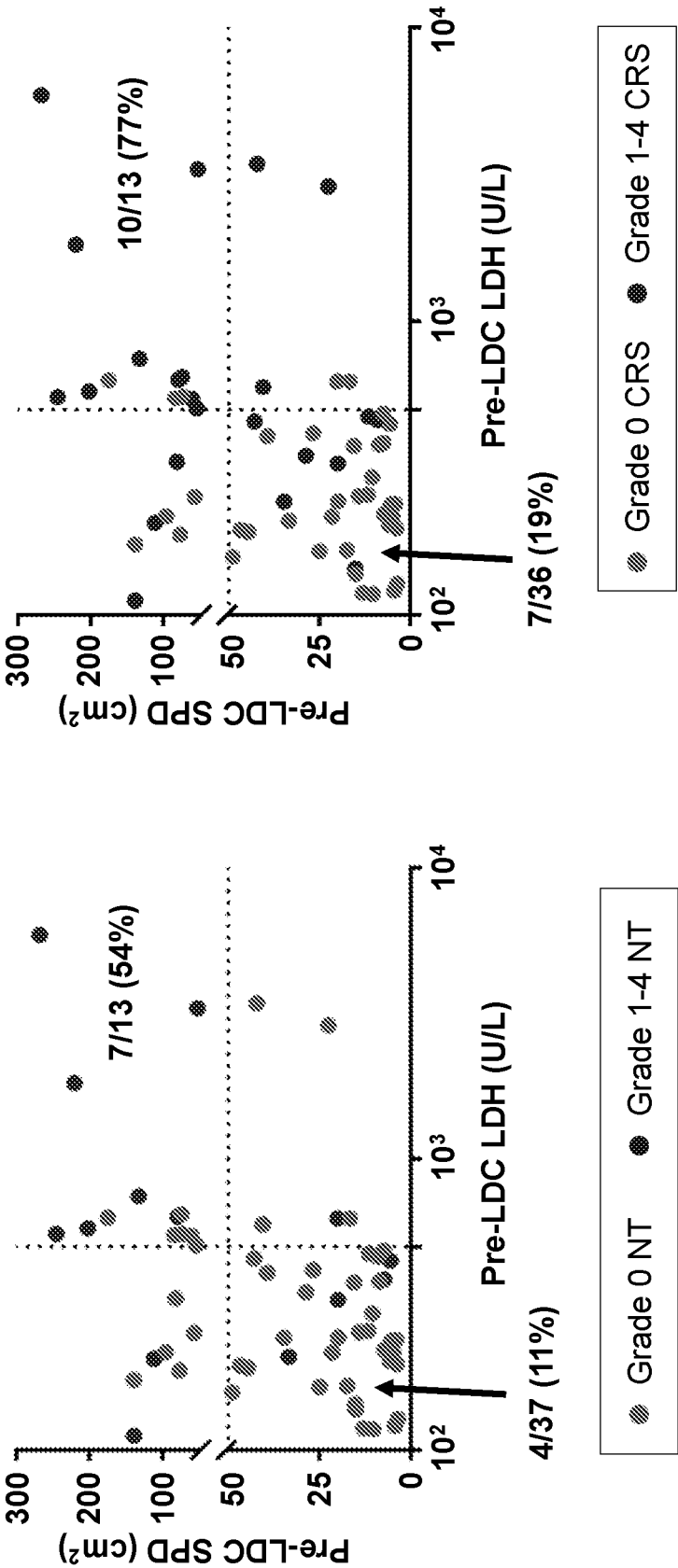


FIG. 19A



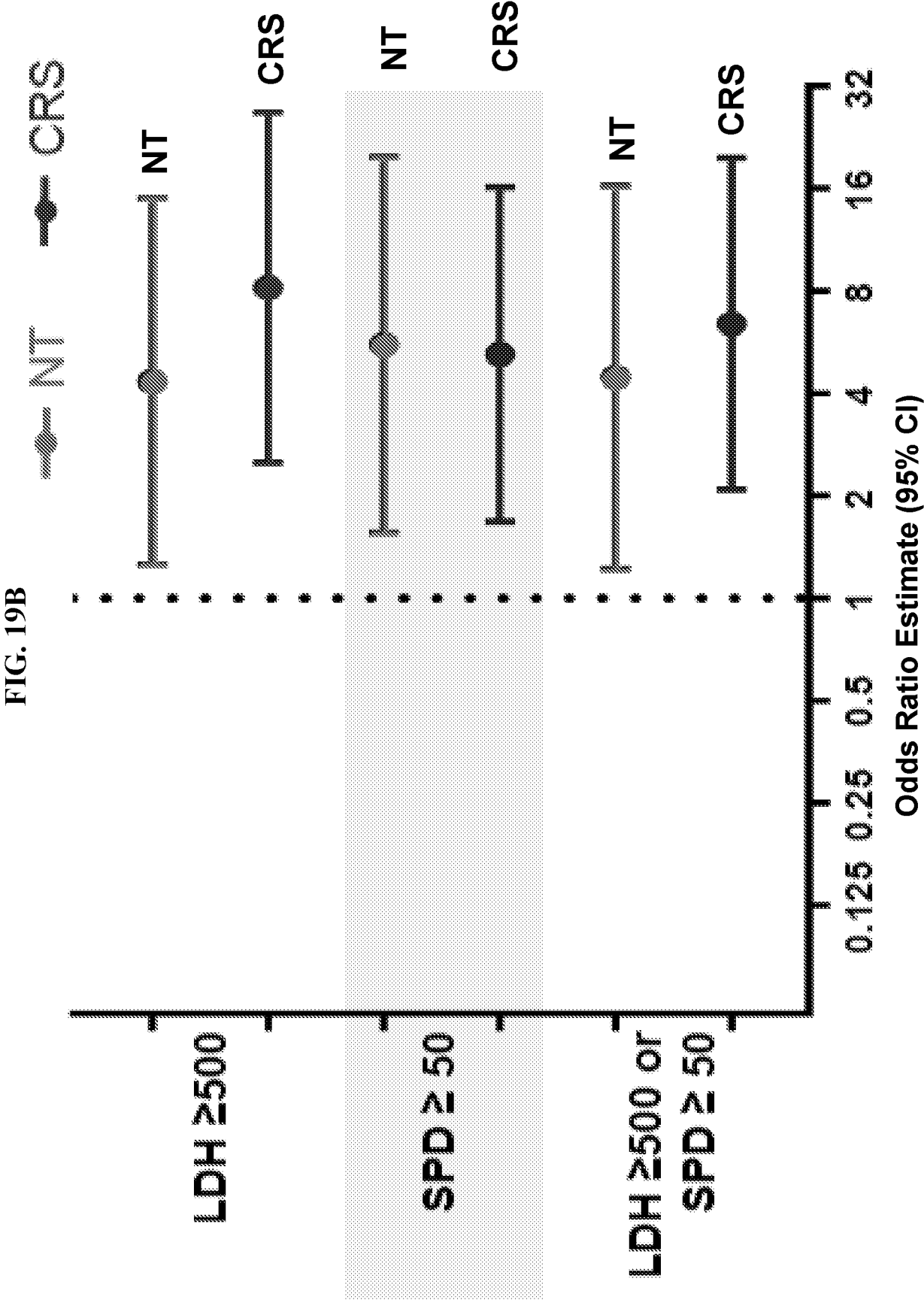


FIG. 19C

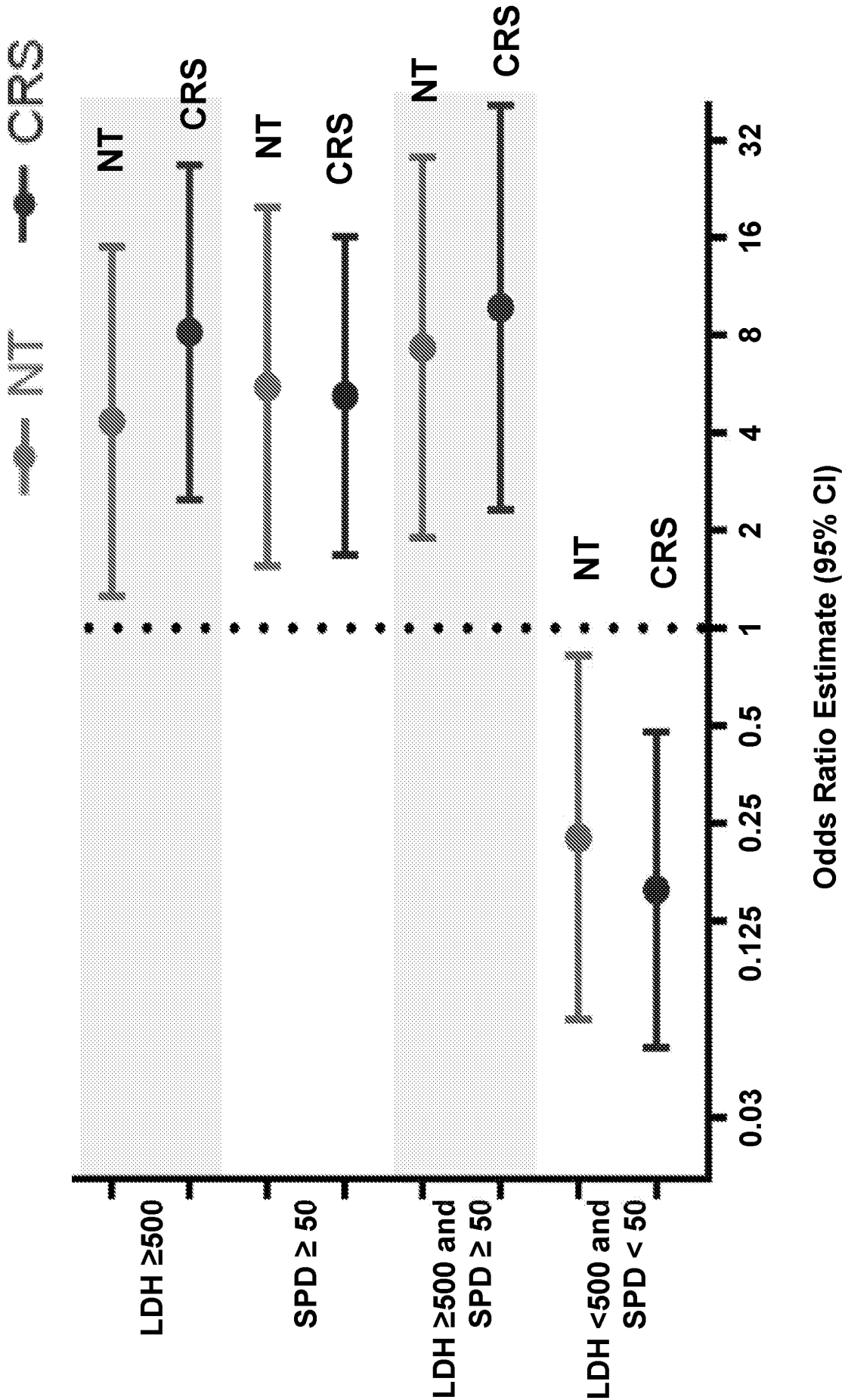


FIG. 20

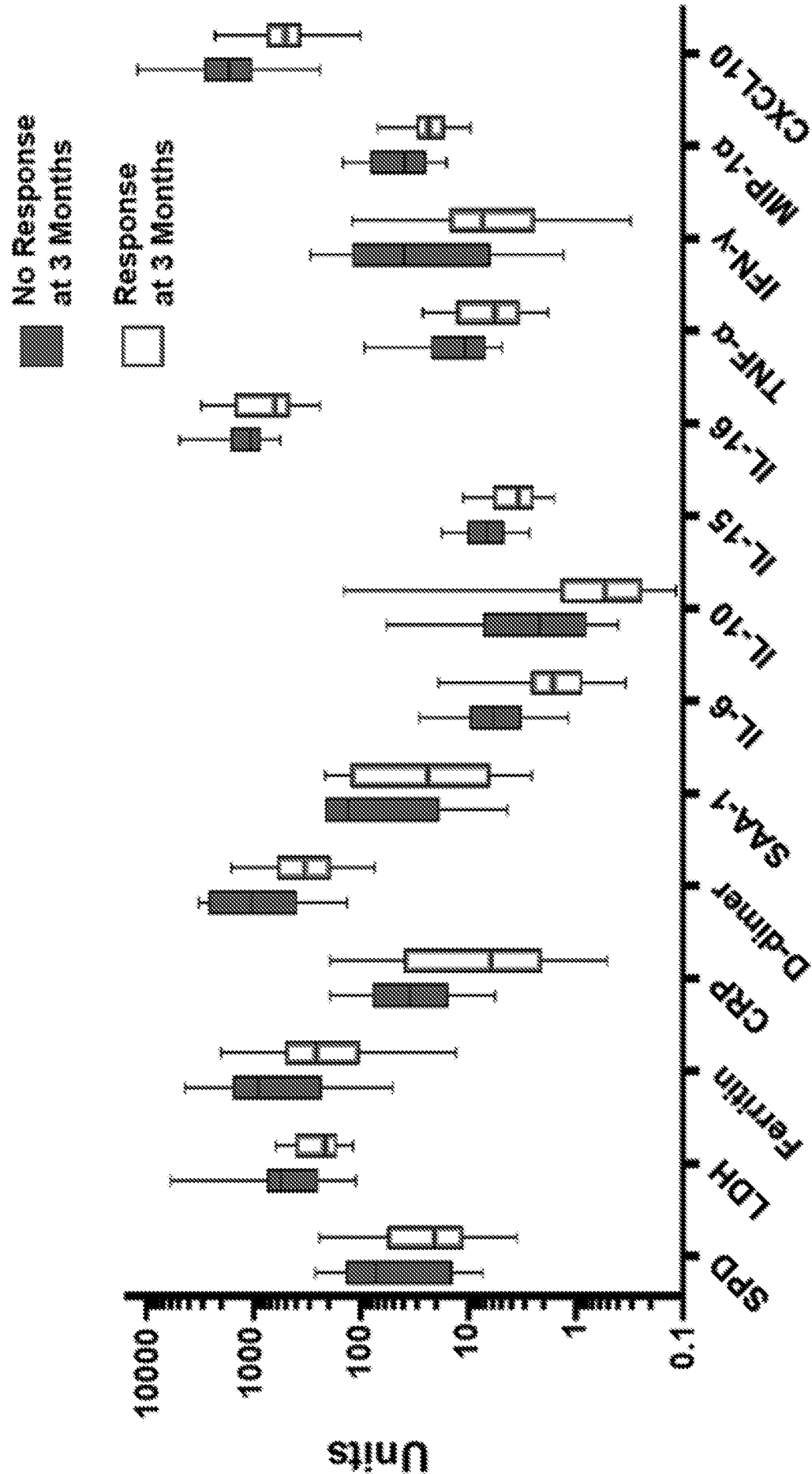


FIG. 21A

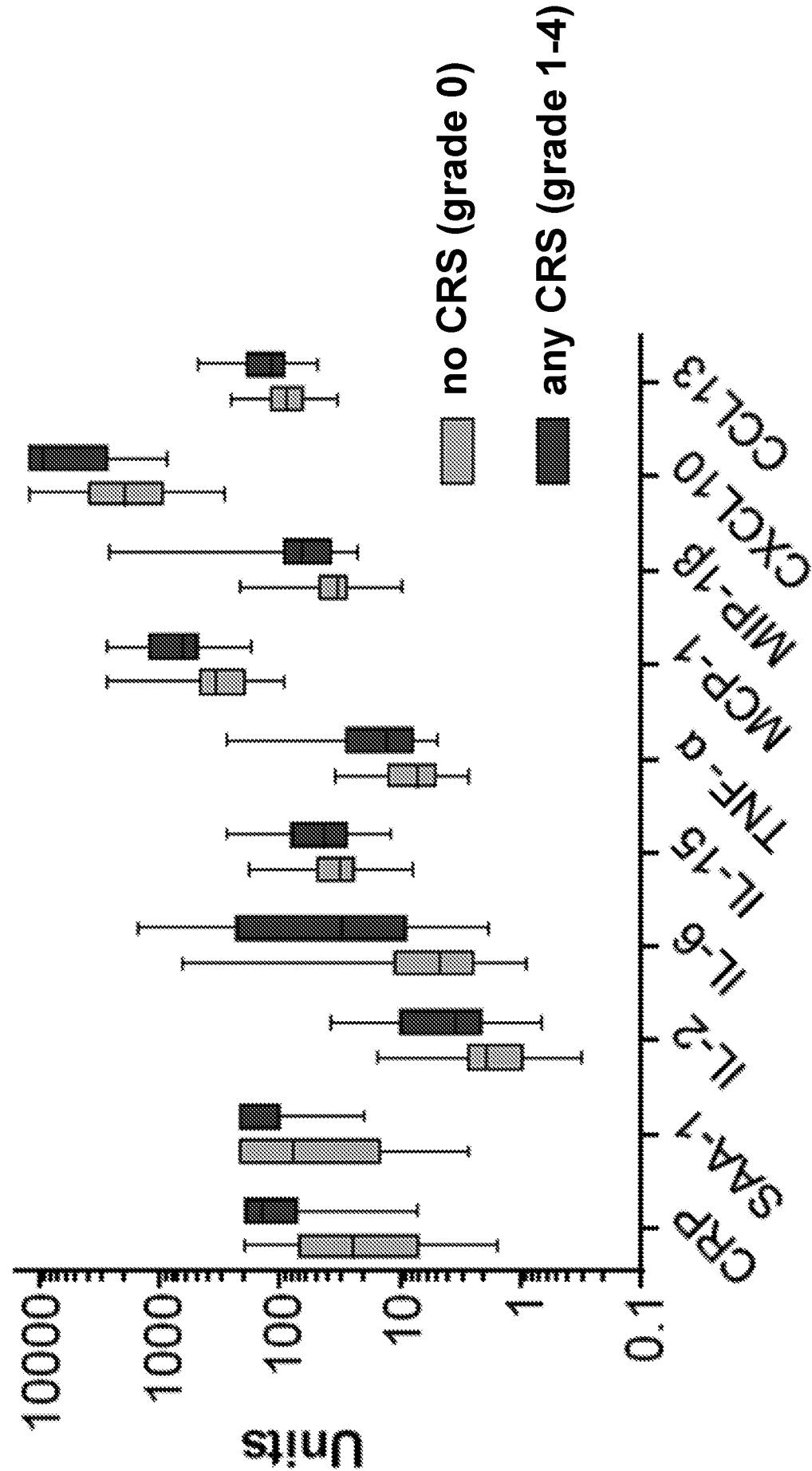


FIG. 21B

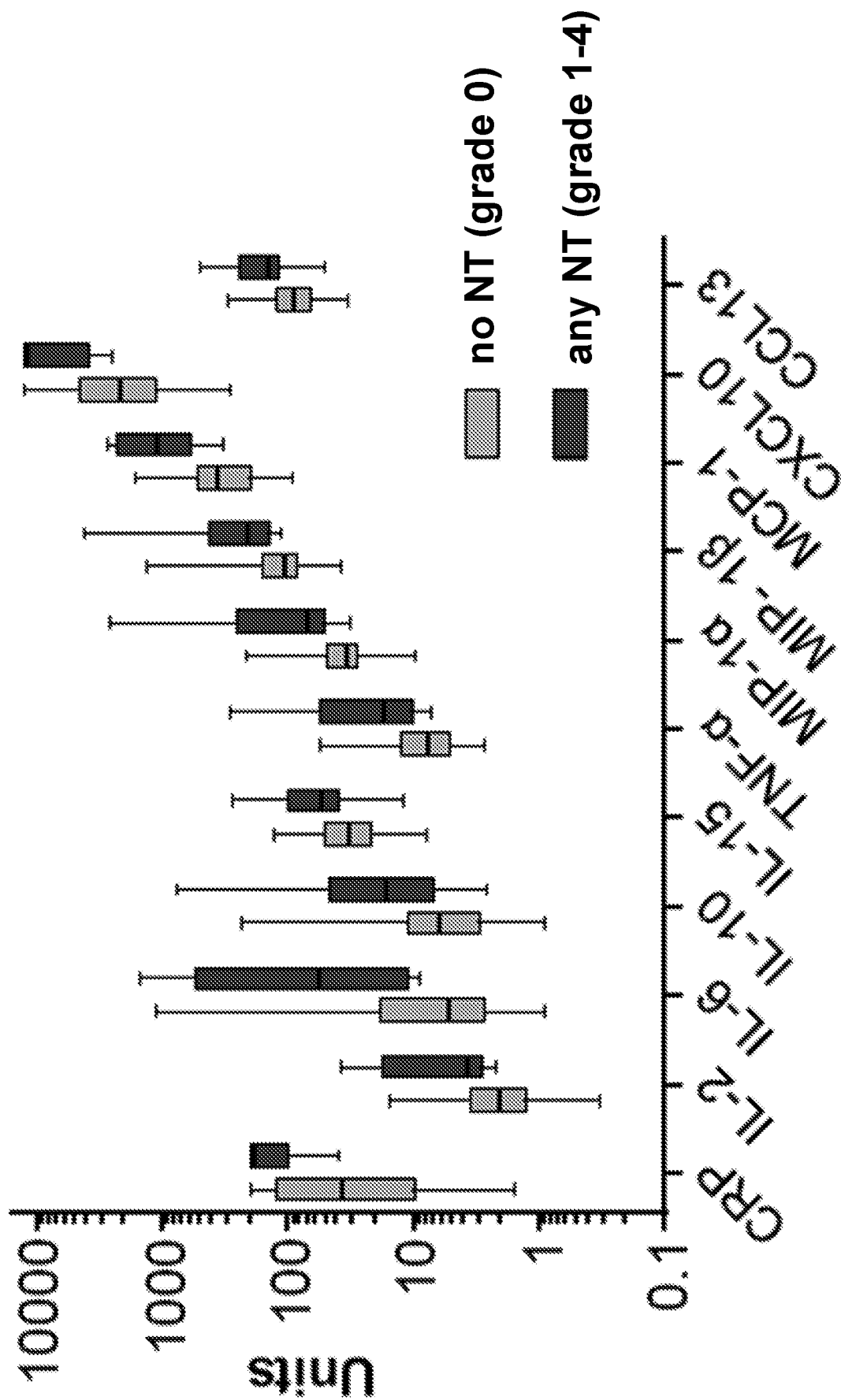


FIG. 22A

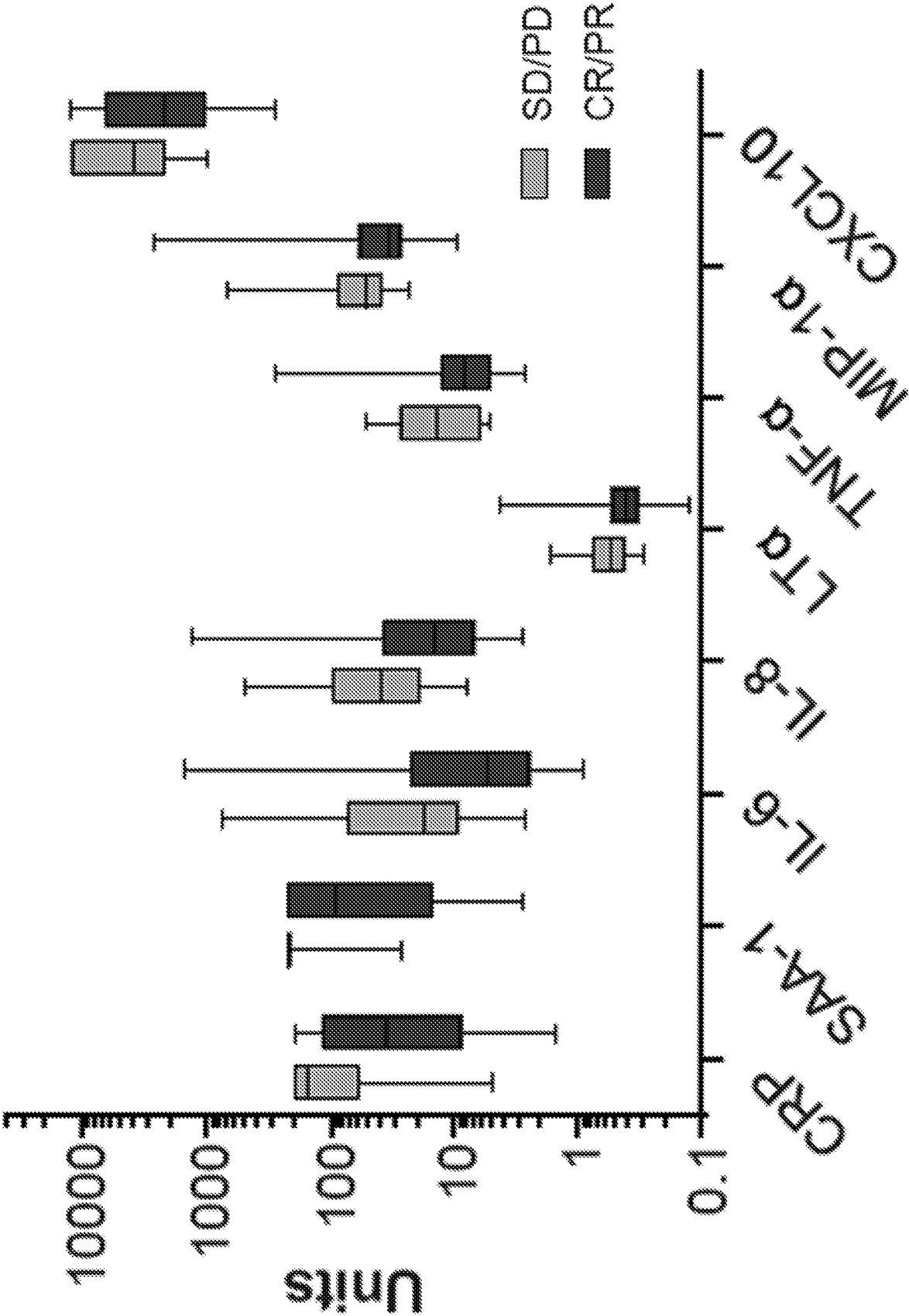


FIG. 23A

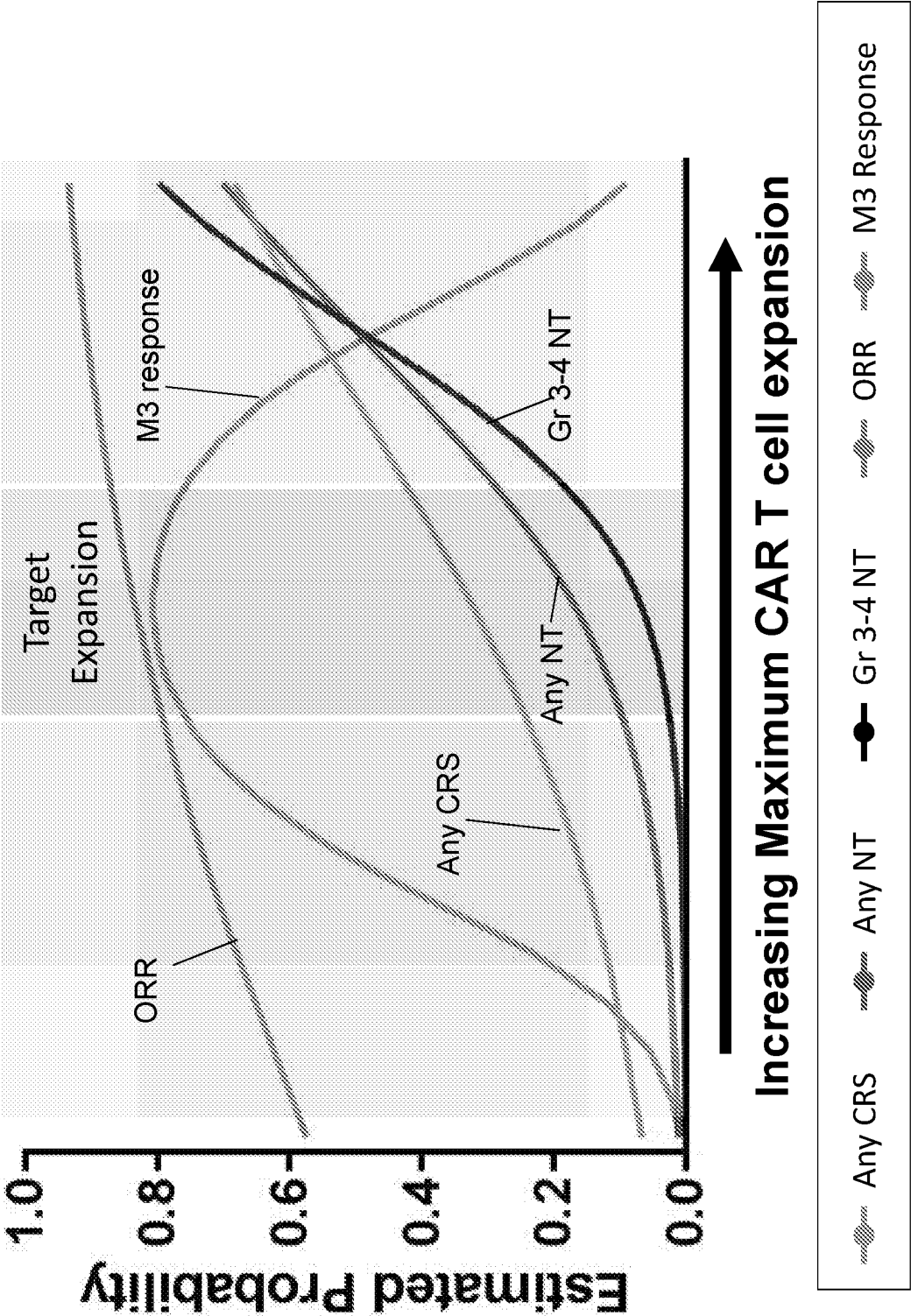


FIG. 23B

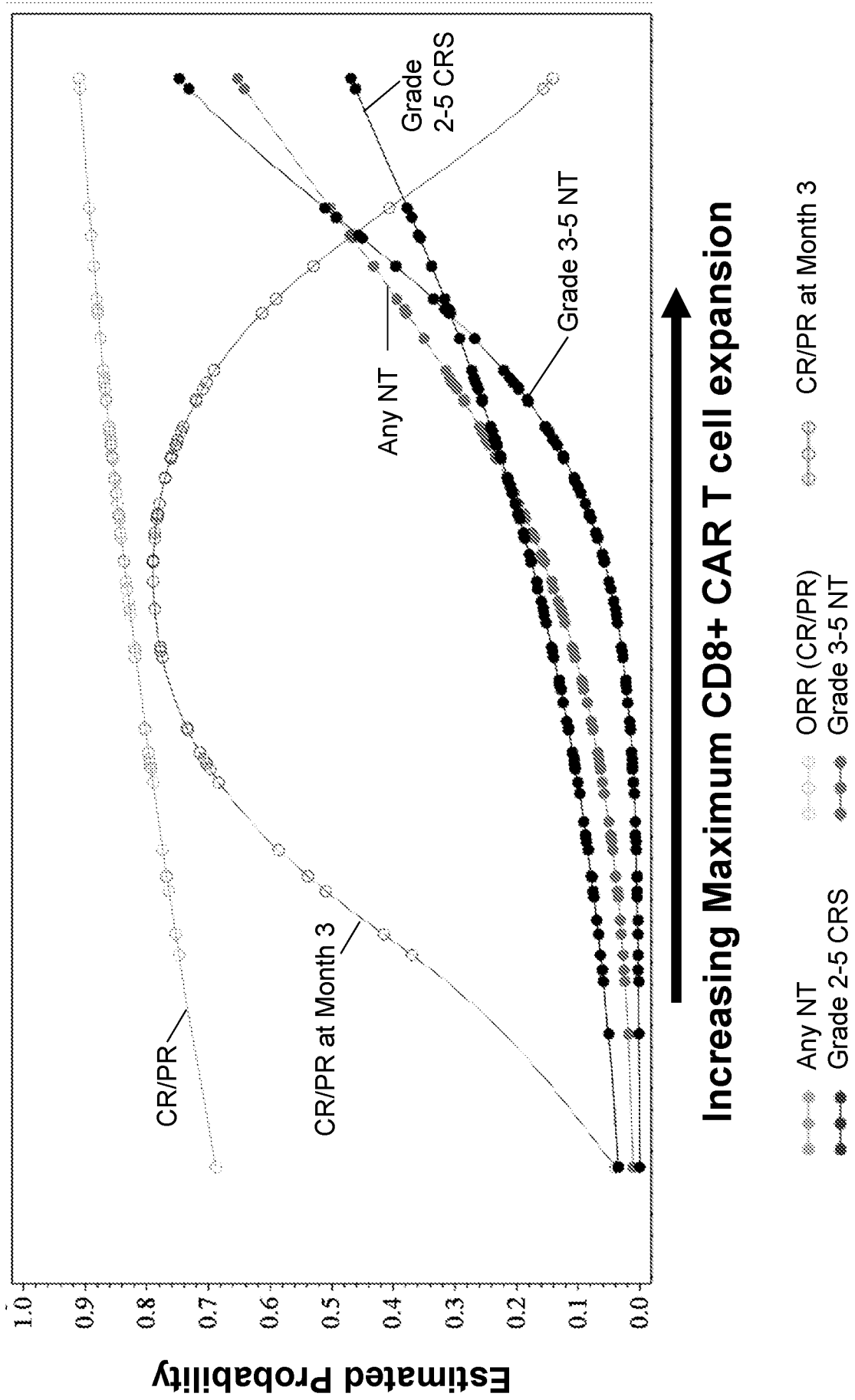


FIG. 23C

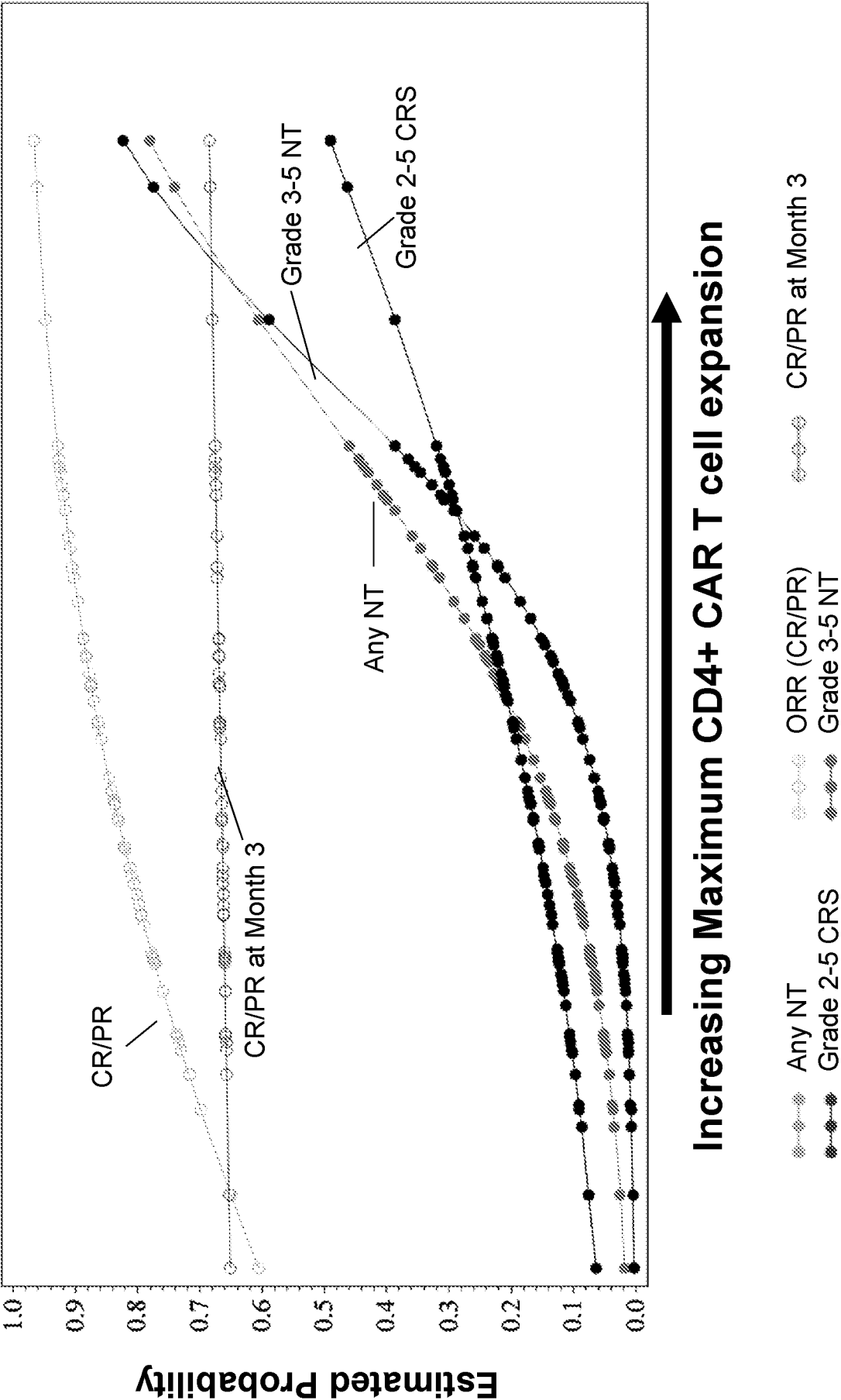


FIG. 24

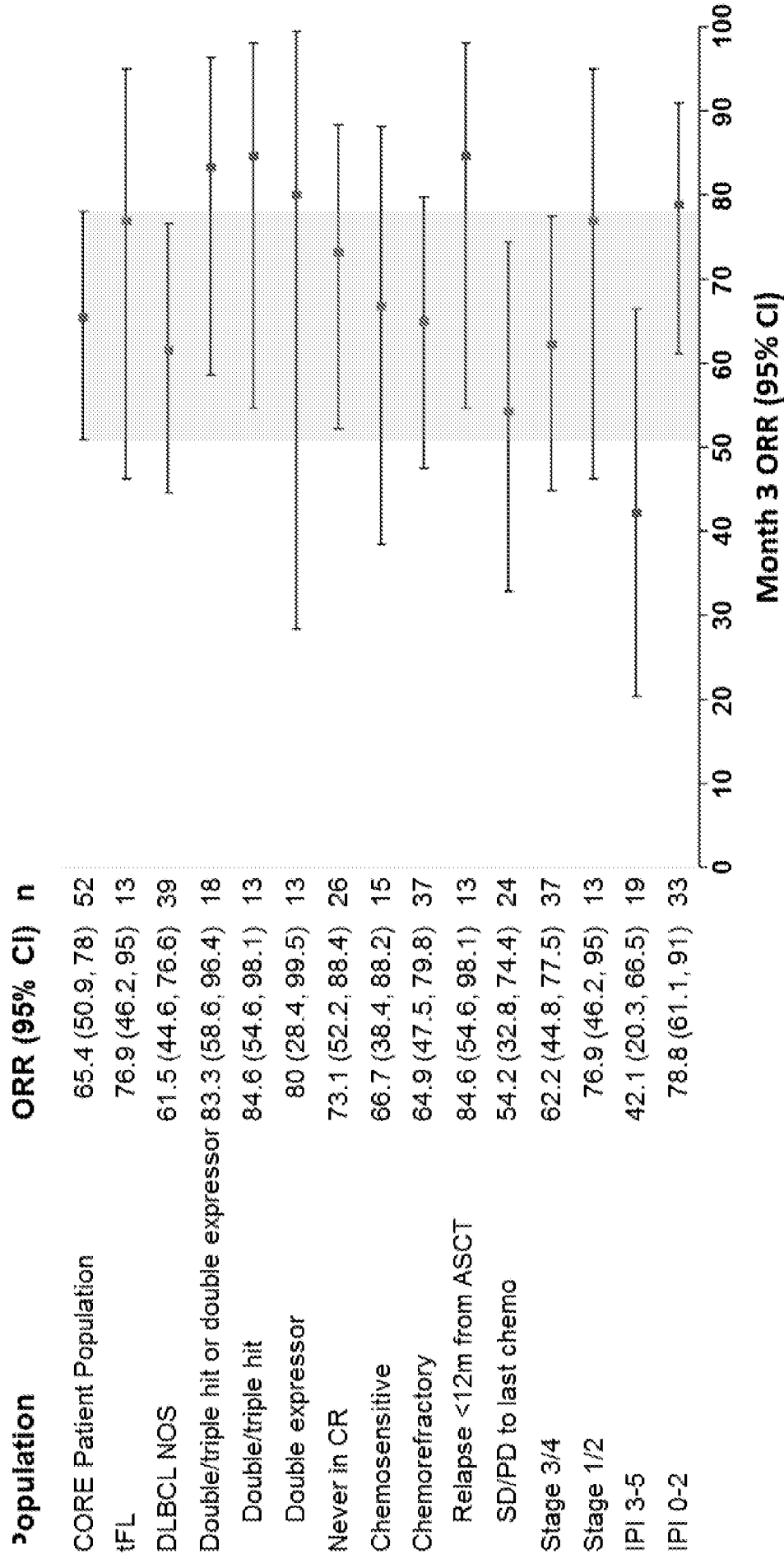


FIG. 25A

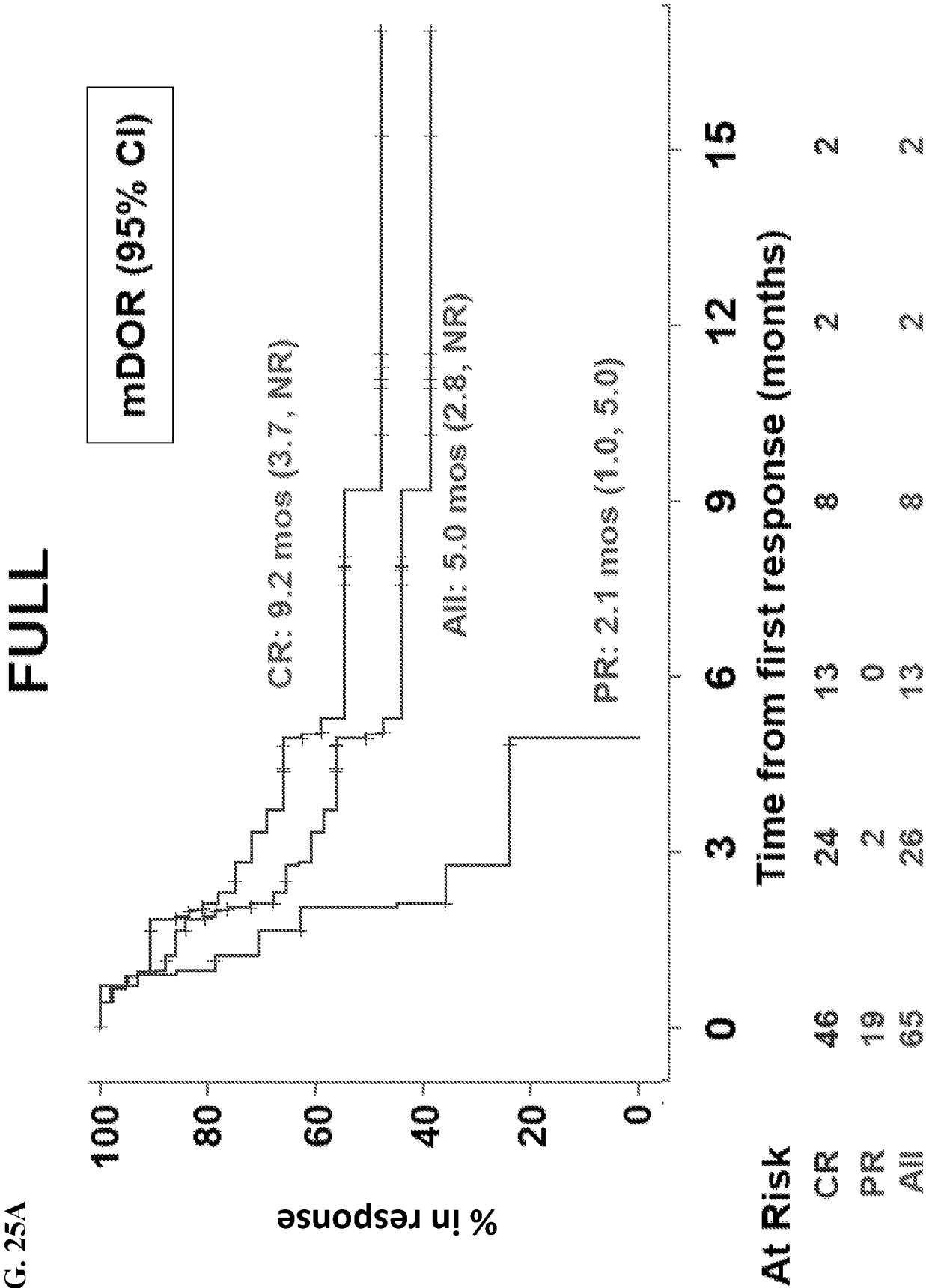


FIG. 25B

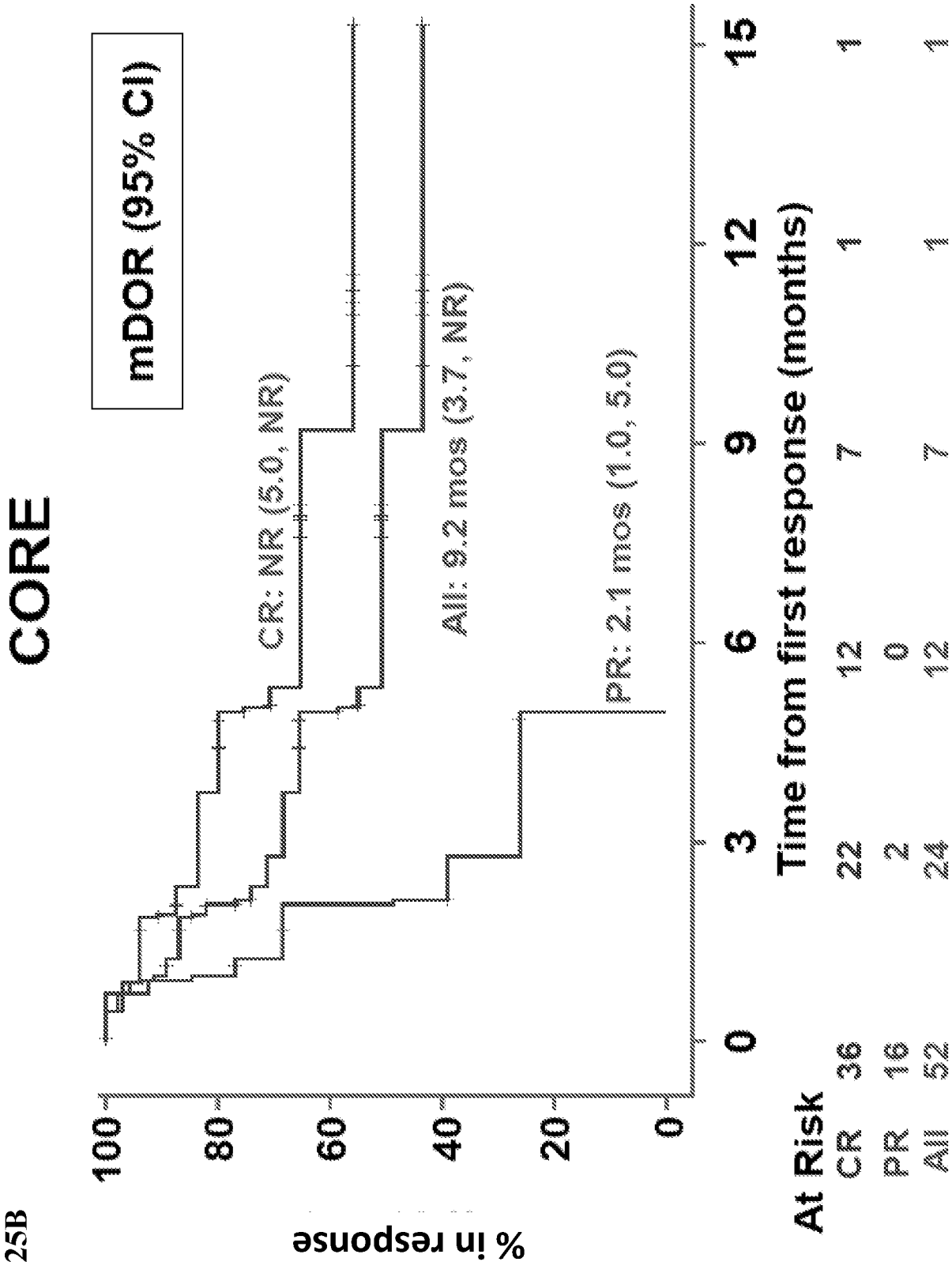


FIG. 25C

FULL

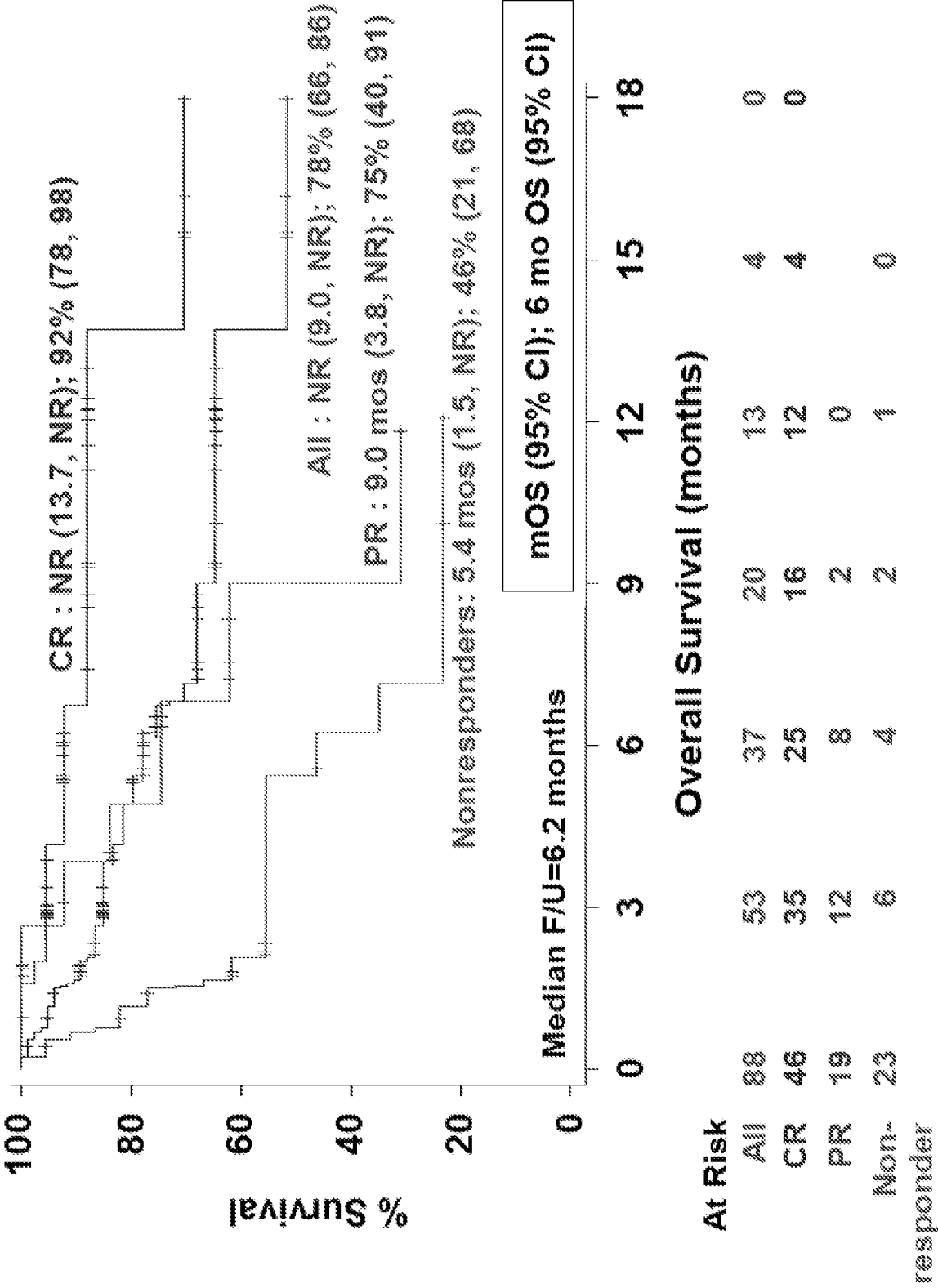


FIG. 25D

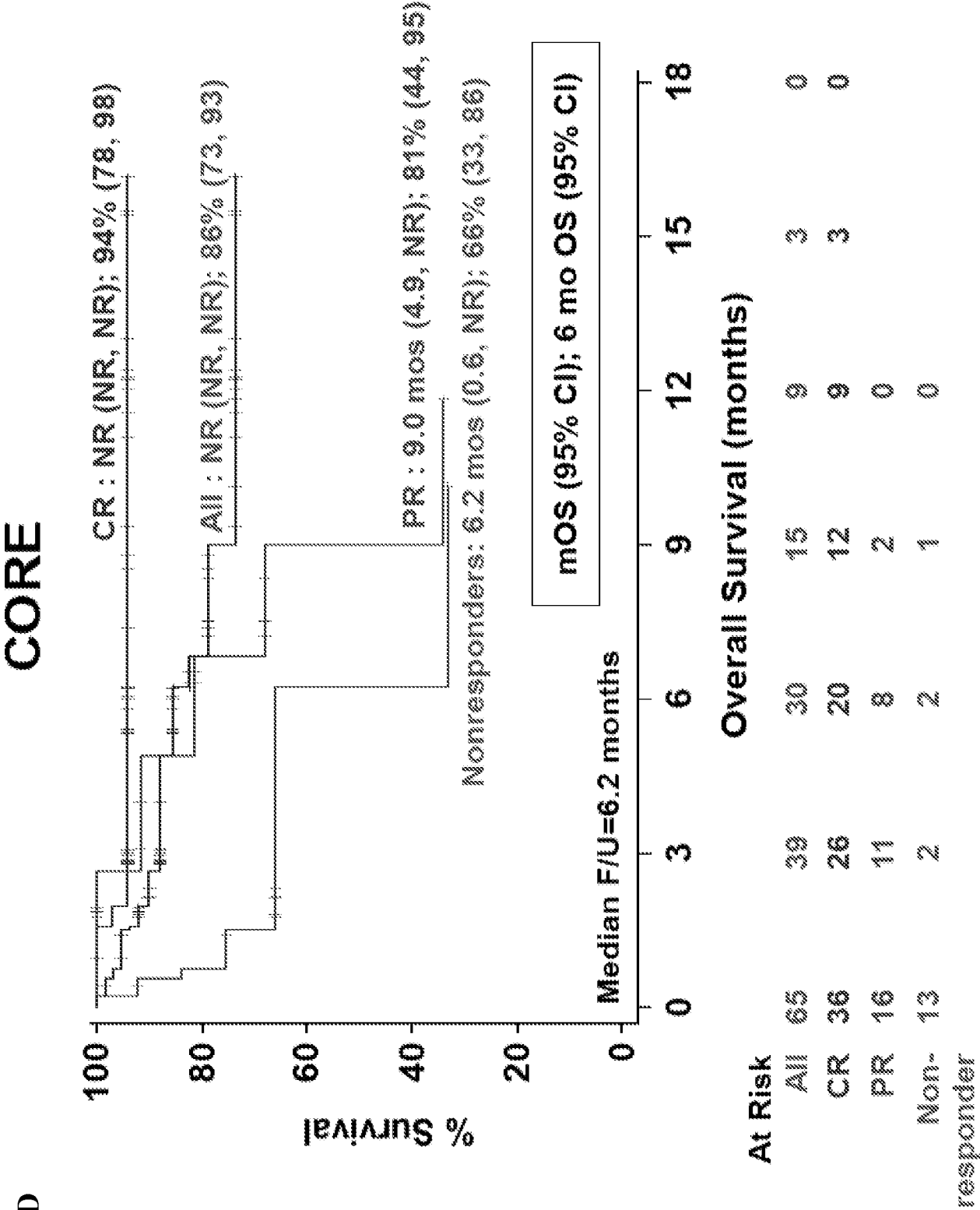


FIG. 26

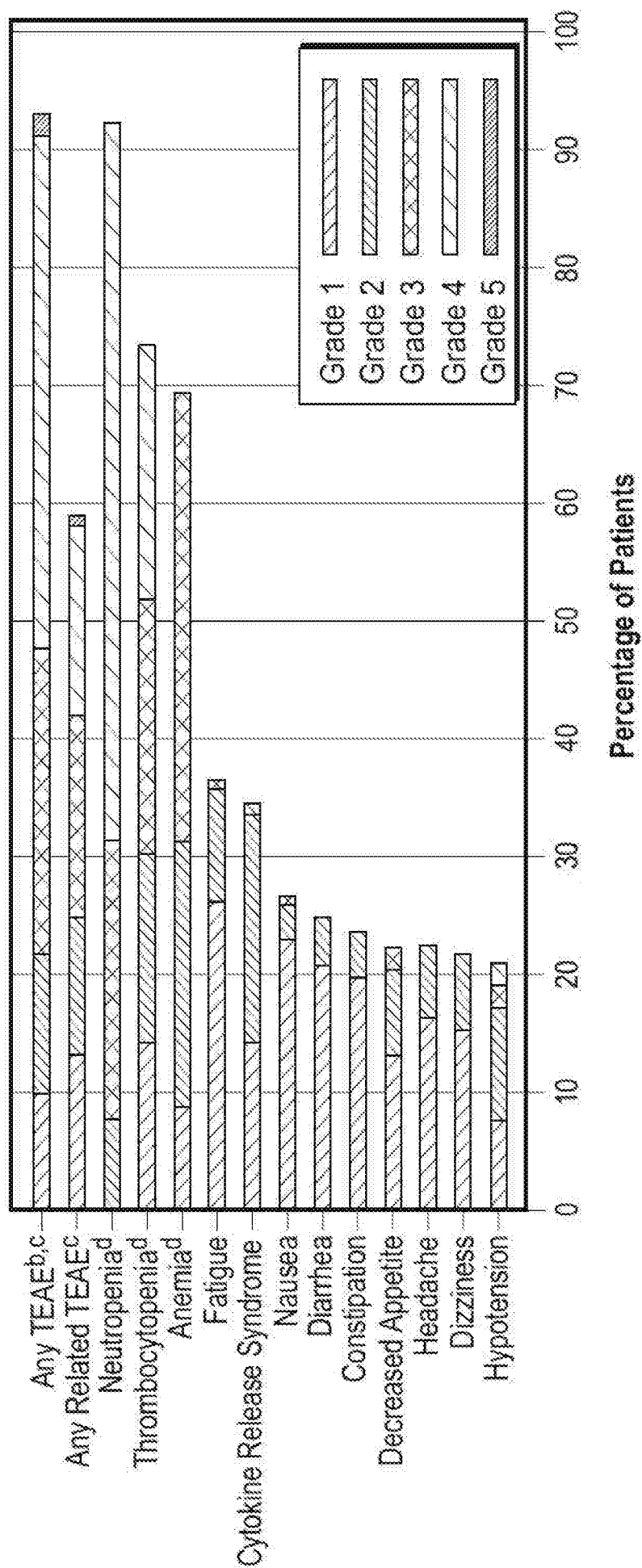


FIG. 27

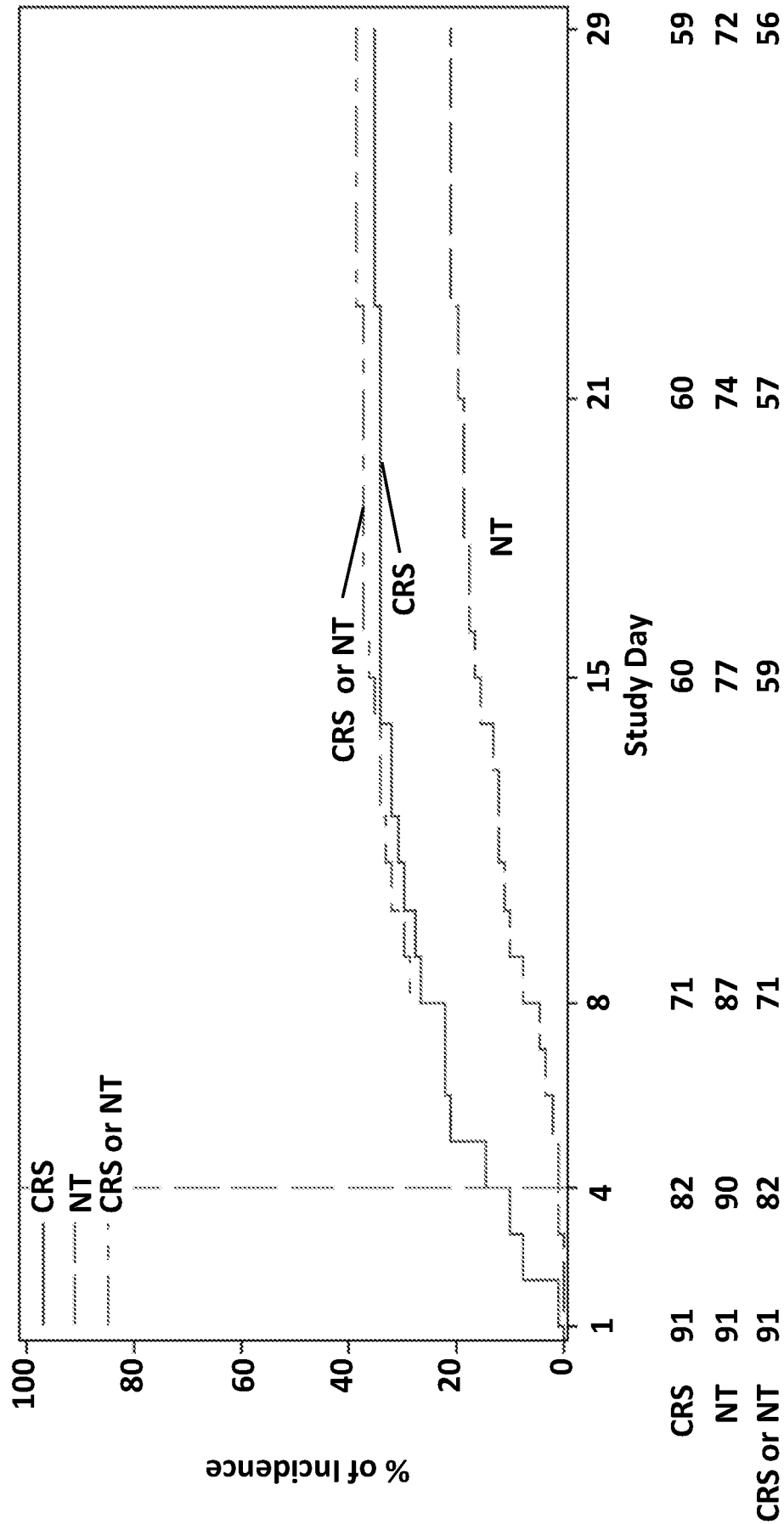


FIG. 28

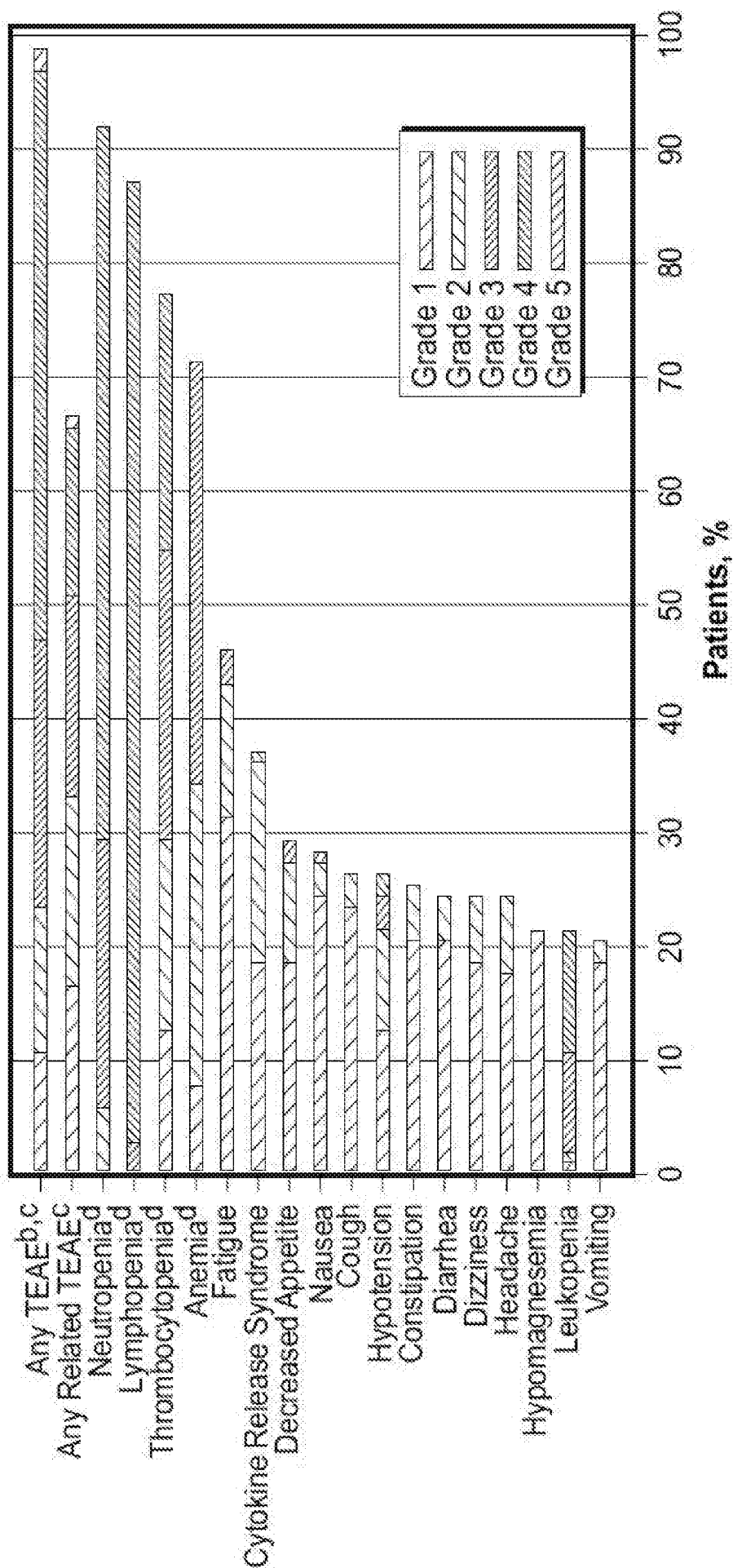


FIG. 29

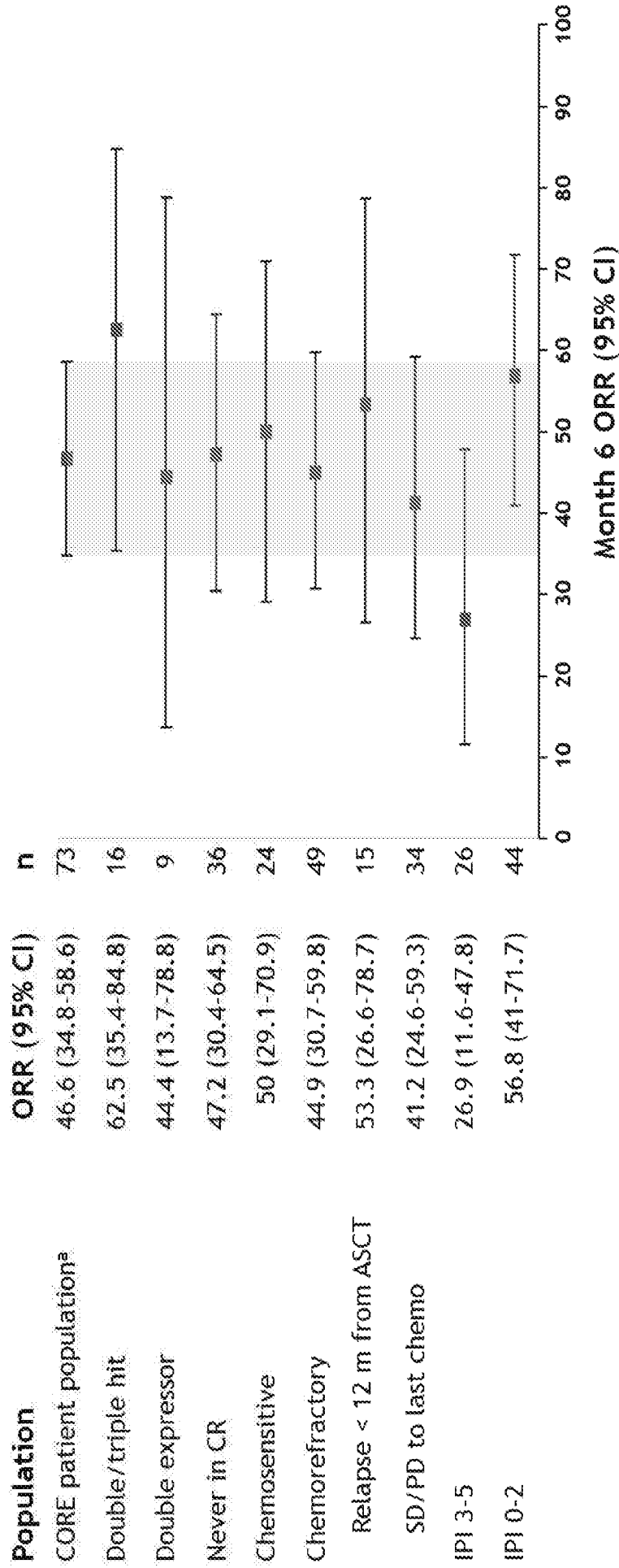


FIG. 30A

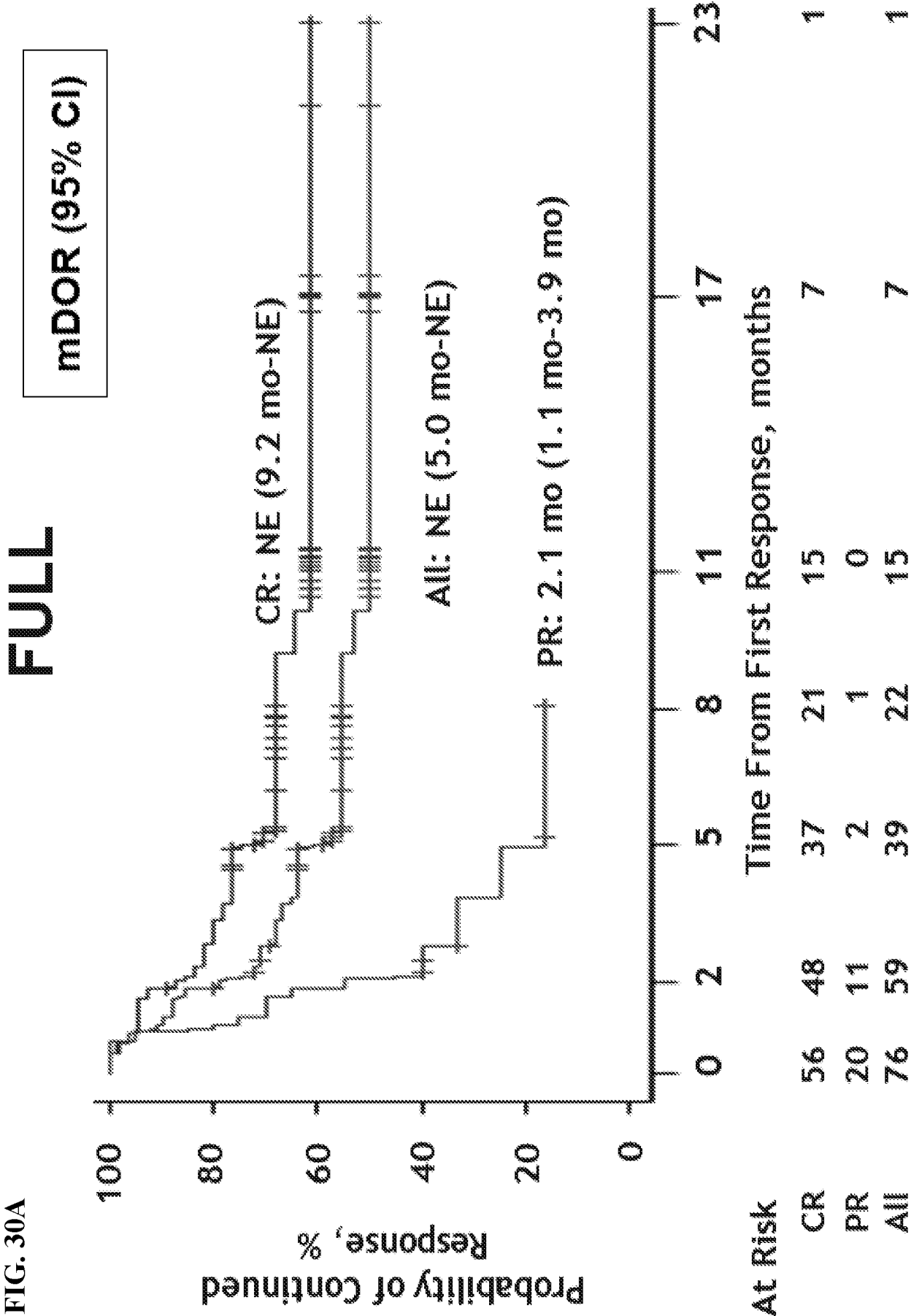


FIG. 30B

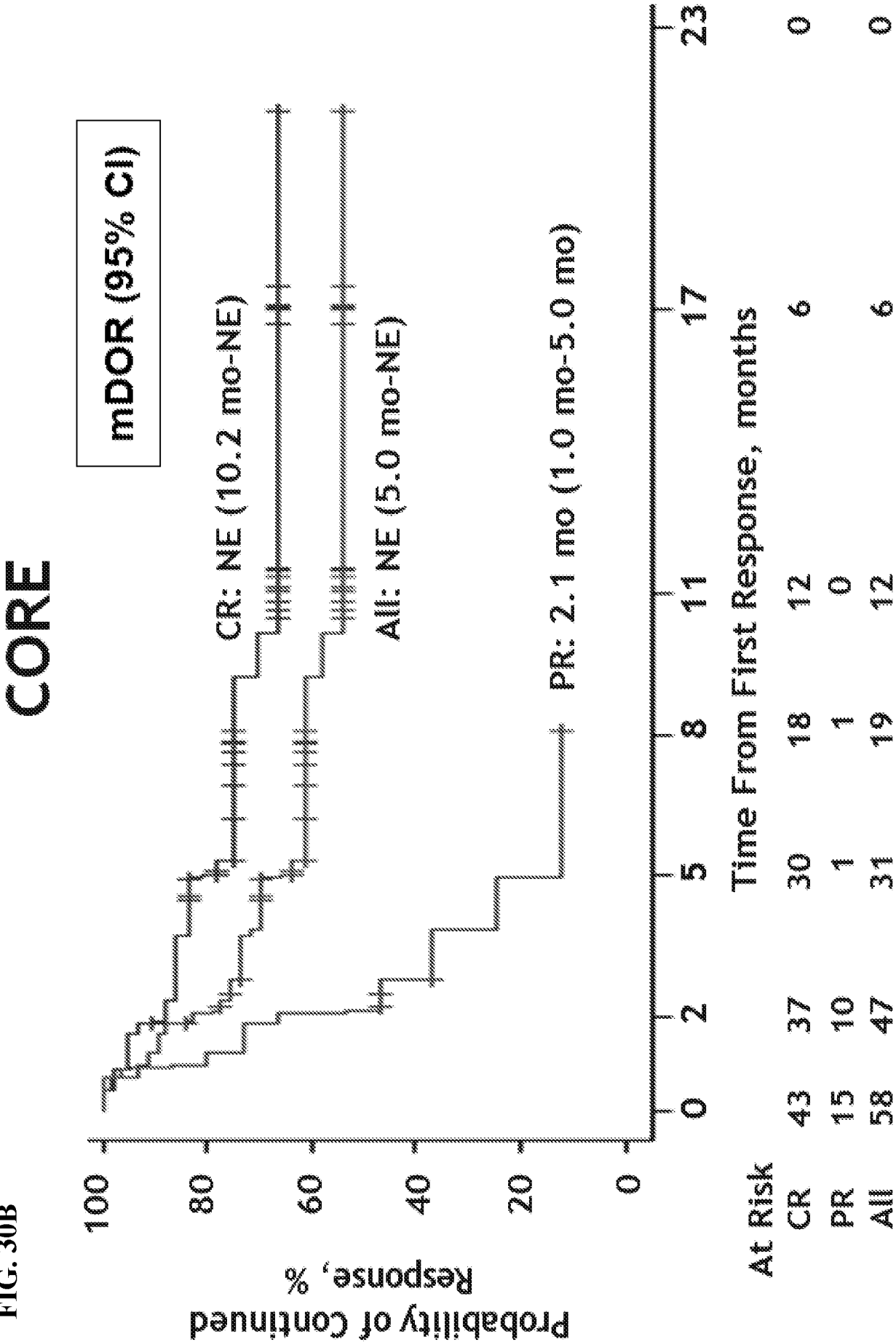


FIG. 30C

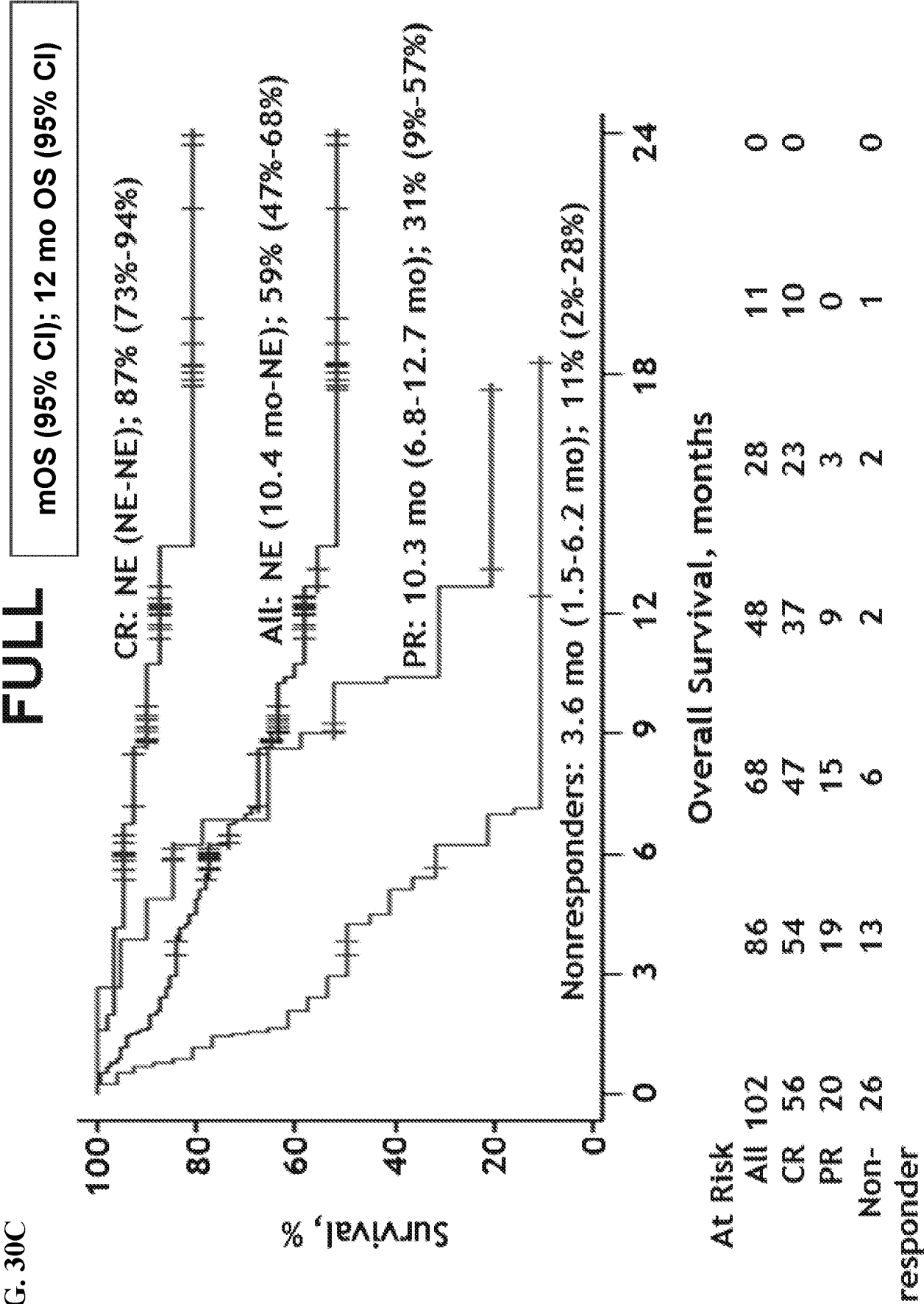


FIG. 30D

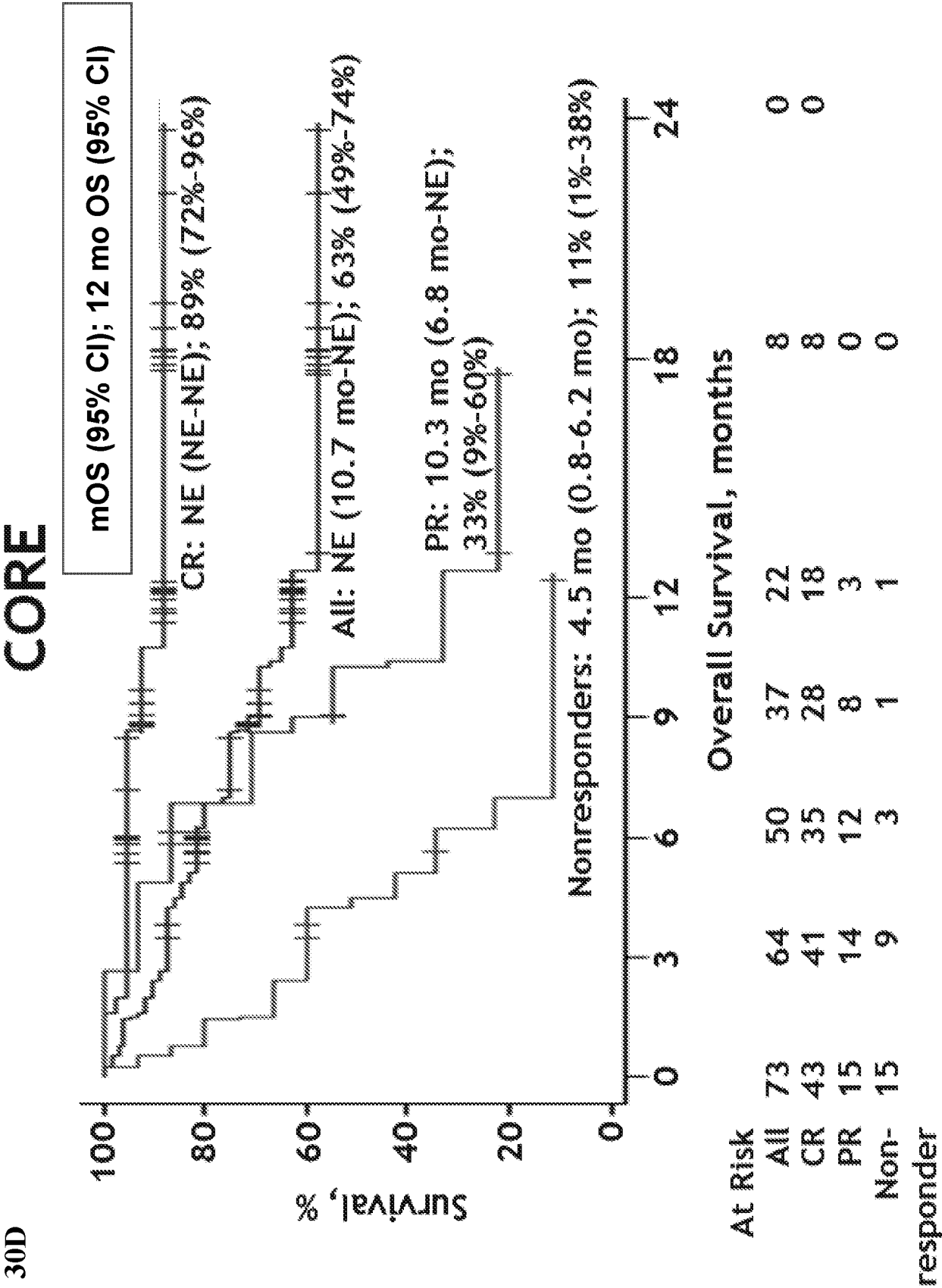
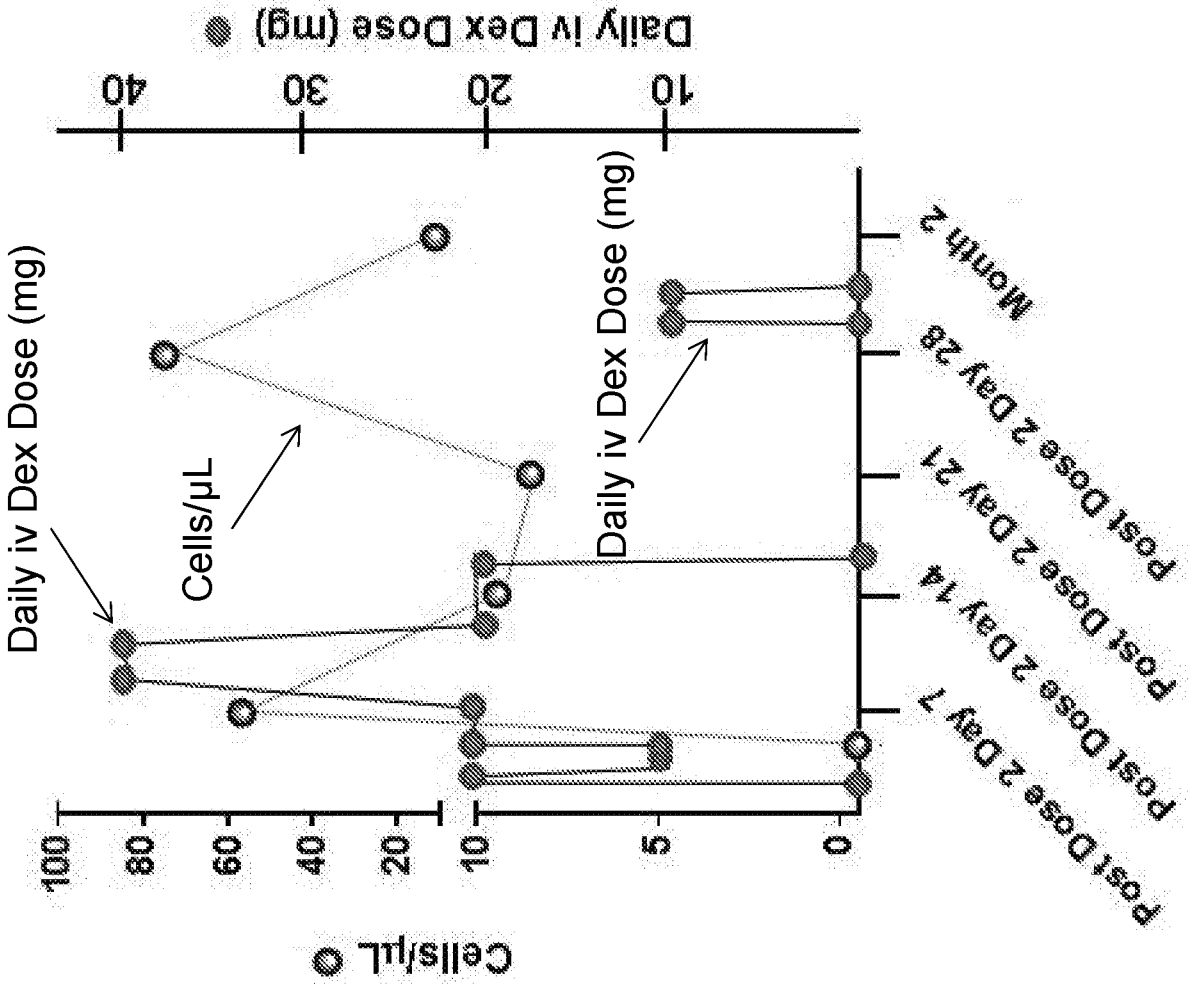


FIG. 31



INTERNATIONAL SEARCH REPORT

International application No

PCT/US2018/063502

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/573 C07K16/28 A61P43/00 G01N33/68
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K C07K A61P G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2017/165571 A1 (SEATTLE CHILDREN'S HOSPITAL [US]) 28 September 2017 (2017-09-28)	1-173
Y	claims 1-123; examples 1,2 paragraph [0287]	1-173
X	----- BATLEVI C L ET AL: "Novel immunotherapies in lymphoid malignancies", NATURE REVIEWS CLINICAL ONCOLOGY, NATURE, NY, US, vol. 13, no. 1, 1 January 2016 (2016-01-01), pages 25-40, XP009503771, ISSN: 1759-4774, DOI: 10.1038/NRCLINONC.2015.187	1-173
Y	page 34, left-hand column, paragraph 2 ----- -/-	1-173



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

13 February 2019

Date of mailing of the international search report

06/03/2019

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Authorized officer

Hörtner, Michael

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2018/063502

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JENNIFER N. BRUDNO ET AL: "Toxicities of chimeric antigen receptor T cells: recognition and management", BLOOD, vol. 127, no. 26, 20 May 2016 (2016-05-20) , pages 3321-3330, XP055547499, US ISSN: 0006-4971, DOI: 10.1182/blood-2016-04-703751	1-173
Y	Section "Management of neurologic toxicities"; page 3328, left-hand column -----	1-173
X	US 2015/283178 A1 (JUNE CARL H [US] ET AL) 8 October 2015 (2015-10-08) cited in the application	1-173
Y	pages 868-869 -----	1-173
X	WO 2017/096331 A1 (JUNO THERAPEUTICS INC [US]; HUTCHINSON FRED CANCER RES [US]) 8 June 2017 (2017-06-08)	1-173
Y	claims 1-151 paragraphs [0375], [0388], [0389] -----	1-173
A	BRANDON R. SHANK ET AL: "Chimeric Antigen Receptor T Cells in Hematologic Malignancies", PHARMACOTHERAPY : THE JOURNAL OF HUMAN PHARMACOLOGY AND DRUG THERAPY, vol. 37, no. 3, 1 March 2017 (2017-03-01), pages 334-345, XP055471941, US ISSN: 0277-0008, DOI: 10.1002/phar.1900 abstract -----	1-173
X,P	WO 2018/102787 A1 (JUNO THERAPEUTICS INC [US]) 7 June 2018 (2018-06-07)	1-173
Y,P	claims 1-126 -----	1-173
E	WO 2018/223101 A1 (JUNO THERAPEUTICS INC [US]) 6 December 2018 (2018-12-06) claims 1-158 -----	1-173
E	WO 2018/223098 A1 (JUNO THERAPEUTICS INC [US]) 6 December 2018 (2018-12-06) the whole document -----	1-173

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2018/063502

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2018/063502

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2017165571 A1	28-09-2017	AU 2017238218 A1 CA 3018588 A1 EP 3433276 A1 WO 2017165571 A1	11-10-2018 28-09-2017 30-01-2019 28-09-2017
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WO 2018102787 A1	07-06-2018	NONE	
WO 2018223101 A1	06-12-2018	NONE	
WO 2018223098 A1	06-12-2018	NONE	