METHODS OF ADMINISTERING CHIMERIC ANTIGEN RECEPTOR IMMUNOTHERAPY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 62/574,159, filed October 18, 2017, which is incorporated by reference herein in its entirety.

TECHNICAL FIELD

[0002] The present disclosure relates generally to T cell therapies and more specifically to CD19-directed genetically modified autologous T cell immunotherapies comprising chimeric antigen receptors (CARs).

BACKGROUND

[0003] Human cancers are by their nature comprised of normal cells that have undergone a genetic or epigenetic conversion to become abnormal cancer cells. In doing so, cancer cells begin to express proteins and other antigens that are distinct from those expressed by normal cells. These aberrant tumor antigens may be used by the body's innate immune system to specifically target and kill cancer cells. However, cancer cells employ various mechanisms to prevent immune cells, such as T and B lymphocytes, from successfully targeting cancer cells.

[0004] Chimeric antigen receptors (CARs), which comprise binding domains capable of interacting with a particular tumor antigen, allow T cells to target and kill cancer cells that express the particular tumor antigen.

SUMMARY

[0005] As described in detail below, the present disclosure is based, in part, on the surprising discovery that the administration methods disclosed herein identify and manage adverse side effects of CAR T-cell immunotherapy.

[0006] Any aspect or embodiment described herein may be combined with any other aspect or embodiment as disclosed herein. While the present invention has been described in

conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the present invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

[0007] In one aspect, the invention provides a method of treating relapsed or refractory diffuse large B-cell lymphoma (DLBCL) not otherwise specified, primary mediastinal large B-cell lymphoma, high grade B-cell lymphoma, or DLBCL arising from follicular lymphoma after two or more lines of systemic therapy in a patient comprising: administering to the patient in need thereof axicabtagene ciloleucel suspension by intravenous infusion at a dose between about 1×10^6 and about 2×10^6 CAR-positive viable T cells per kg body weight up to a maximum dose of about 1×10^8 CAR-positive viable T cells, wherein axicabtagene ciloleucel is a CD19-directed genetically modified autologous T cell immunotherapy, comprising the patient's own T cells harvested and genetically modified ex vivo by retroviral transduction to express a chimeric antigen receptor (CAR) comprising an anti-CD19 single chain variable fragment (scFv) linked to CD28 and CD3-zeta co-stimulatory domains.

[0008] In another aspect, the invention provides a method of treating relapsed or refractory diffuse large B-cell lymphoma (DLBCL) and primary mediastinal large B-cell lymphoma (PMBCL), after two or more lines of systemic therapy in a patient comprising: administering to the patient in need thereof axicabtagene ciloleucel suspension by intravenous infusion at a dose between about 0.4×10^8 and about 2×10^8 CAR-positive viable T cells, wherein axicabtagene ciloleucel is a CD19-directed genetically modified autologous T cell immunotherapy, comprising the patient's own T cells harvested and genetically modified ex vivo by retroviral transduction to express a chimeric antigen receptor (CAR) comprising an anti-CD19 single chain variable fragment (scFv) linked to CD28 and CD3-zeta co-stimulatory domains.

[0009] In some embodiments, the intravenous infusion time is between 15 and 120 minutes. In some embodiments, the intravenous infusion time is up to 30 minutes.

[0010] In some embodiments, the infusion volume is between 50 and 100 mL. In some embodiments, the infusion volume is about 68 mL.

[0011] In some embodiments, the immunotherapy is infused from an infusion bag. In some embodiments, the infusion bag is agitated during the infusion.

[0012] In some embodiments, the immunotherapy is administered within 3 hours after thawing.

[0013] In some embodiments, the suspension further comprises albumin. In some embodiments, albumin is present in an amount of about 2-3% (v/v). In some embodiments, albumin is present in an amount of about 2.5% (v/v). In some embodiments, albumin is human albumin.

[0014] In some embodiments, the suspension further comprises DMSO. In some embodiments, DMSO is present in an amount of about 4-6% (v/v). In some embodiments, DMSO is present in an amount of about 5% (v/v).

[0015] In one aspect, the invention provides a method of treating relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy in a patient comprising: (a) administering to the patient in need thereof CD19-directed genetically modified autologous T cell immunotherapy; and (b) monitoring the patient following infusion for signs and symptoms of an adverse reaction.

[0016] In some embodiments, the relapsed or refractory large B-cell lymphoma is diffuse large B-cell lymphoma (DLBCL) not otherwise specified, primary mediastinal large B-cell lymphoma, high grade B-cell lymphoma, or DLBCL arising from follicular lymphoma.

[0017] In some embodiments, the adverse reaction is selected from the group consisting of cytokine release syndrome (CRS), a neurologic toxicity, a hypersensitivity reaction, a serious infection, a cytopenia and hypogammaglobulinemia.

[0018] In some embodiments, the signs and symptoms of adverse reactions are selected from the group consisting of fever, hypotension, tachycardia, hypoxia, and chills, include cardiac arrhythmias (including atrial fibrillation and ventricular tachycardia), cardiac arrest, cardiac failure, renal insufficiency, capillary leak syndrome, hypotension, hypoxia, organ toxicity, hemophagocytic lymphohistiocytosis/macrophage activation syndrome (HLH/MAS), seizure, encephalopathy, headache, tremor, dizziness, aphasia, delirium, insomnia anxiety, anaphylaxis, febrile neutropenia, thrombocytopenia, neutropenia, and anemia.

[0019] In some embodiments, the method further comprises administering an IL-6 receptor inhibitor.

[0020] In some embodiments, the method further comprises administering an effective amount of tocilizumab to treat a symptom of an adverse reaction.

[0021] In some embodiments, tocilizumab is administered at a dose of about 8 mg/kg intravenously. In some embodiments, tocilizumab is administered intravenously over about 1 hour. In some embodiments, tocilizumab is administered about every 8 hours. In some embodiments, tocilizumab is administered for no more than about 24 hours.

[0022] In some embodiments, the method further comprises administering a corticosteroid to treat a symptom of an adverse reaction.

[0023] In some embodiments, the corticosteroid is at least one of methylprednisone or dexamethasone.

[0024] In some embodiments, methylprednisone is administered at a dose of about 1 mg/kg intravenously. In some embodiments, methylprednisone is administered twice daily. In some embodiments, methylprednisone is administered at a dose of about 1,000 mg per day intravenously. In some embodiments, methylprednisone is administered intravenously for about 3 days.

[0025] In some embodiments, dexamethasone is administered at a dose of about 10 mg. In some embodiments, dexamethasone is administered intravenously about every 6 hours.

[0026] In some embodiments, the adverse reaction is cytokine release syndrome (CRS). In some embodiments, the monitoring for signs and symptoms of cytokine release syndrome (CRS) is at least daily for about 7 days following infusion. In some embodiments, the monitoring for signs and symptoms of cytokine release syndrome (CRS) is at least daily for about 8 days, about 9 days, or about 10 days following infusion. In some embodiments, the monitoring for signs and symptoms of cytokine release syndrome (CRS) is at least daily for about 10 days following infusion. In some embodiments, the monitoring for signs and symptoms of cytokine release syndrome (CRS) is for about 4 weeks following infusion.

[0027] In some embodiments, the adverse reaction is neurologic toxicity.

[0028] In some embodiments, the monitoring for signs and symptoms of neurologic toxicity up to about 8 weeks following infusion.

[0029] In some embodiments, the method further comprises administering a non-sedating, anti-seizure medicine for seizure prophylaxis.

[0030] In some embodiments, the non-sedating, anti-seizure medicine is levetiracetam.

[0031] In some embodiments, the adverse reaction is a cytopenia. In some embodiments, the cytopenia is thrombocytopenia, neutropenia, and/or anemia.

[0032] In some embodiments, the method further comprises administering at least one of erythropoietin, darbepoetin alfa, platelet transfusion, colony-stimulating factor (CSF), granulocyte colony-stimulating factor, filgrastim, pegfilgrastim, or granulocyte-macrophage colony-stimulating factor.

[0033] In some embodiments, the method further comprises measuring cytokine and chemokine levels. In some embodiments, the level of at least one of IL-6, IL-8, IL-10, IL-15, TNF-α, IFN-γ, and sIL2Rα is measured.

[0034] In one aspect, the invention provides a container comprising a suspension of CD19-directed genetically modified autologous T cells, about 5% dimethylsulfoxide (DMSO) and about 2.5% human albumin (v/v). In another aspect, the container comprises a suspension of between about $0.4 \times 10^8 - 2 \times 10^8$ CD19-directed genetically modified autologous T cells (CAR-positive viable T cells).

[0035] In some embodiments, the container is a sterile infusion bag. In some embodiments, the infusion bag volume is about 100 mL, 250 mL, 500 mL, 750 mL, 1000 mL, 1500 mL, 2000 mL or 3000 mL.

[0036] In one aspect, the invention provides a method of treating relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy in a human comprising administering to the human in need thereof CD19-directed genetically modified autologous T cell immunotherapy comprising: (a) administering to the patient a composition comprising CD19-directed chimeric antigen receptor (CAR) positive viable T cells; (b) monitoring the patient following administration for signs and symptoms of an adverse reaction; and (c) if cytokine release syndrome (CRS) greater than Grade 2 is observed in (b), administering tocilizumab at a dose of about 8 mg/kg IV over 1 hour, repeating tocilizumab every 8 hours as needed if not responsive to IV fluids or increasing supplemental oxygen; (d) if CRS symptoms observed in (b) do not improve after 24 hours of (c), administering methylprednisolone about 1 mg/kg IV twice daily or administering equivalent dexamethasone dose and continuing corticosteroids use until the event is Grade 1 or less, then tapering over 3 days; (e) if CRS Grade 3 is observed in (b), administering tocilizumab at a dose of 8 mg/kg

IV over 1 hour, repeating tocilizumab every 8 hours as needed if not responsive to IV fluids or increasing supplemental oxygen and administering methylprednisolone 1 mg/kg IV twice daily or administering equivalent dexamethasone dose and continuing corticosteroids use until the event is Grade 1 or less, then tapering over 3 days; and (f) if CRS Grade 4 is observed in (b), administering tocilizumab at a dose of about 8 mg/kg IV over 1 hour, repeating tocilizumab every 8 hours as needed if not responsive to IV fluids or increasing supplemental oxygen and administering about 1,000 mg IV methylprednisolone per day for 3 days.

[0037] In one aspect, the invention provide a method of treating relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy in a patient comprising administering to the patient in need thereof CD19-directed genetically modified autologous T cell immunotherapy comprising: (a) administering to the patient a composition comprising CD19-directed chimeric antigen receptor (CAR) positive viable T cells; (b) monitoring the patient following administration for signs and symptoms of an adverse reaction; and (c) if cytokine release syndrome (CRS) and/or neurologic toxicity is observed, managing cytokine release syndrome (CRS) and/or neurologic toxicity according to Table 1 and/or Table 2.

[0038] Other features and advantages of the disclosure will be apparent from the following Detailed Description, including the Examples, and the claims.

DETAILED DESCRIPTION

[0039] The present disclosure relates to engineered cells (e.g., T cells) comprising a CD19 CAR genetically modified autologous T cell immunotherapy indicated for the treatment of adult patients with relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy, including diffuse large B-cell lymphoma (DLBCL) not otherwise specified, primary mediastinal large B-cell lymphoma, high grade B-cell lymphoma, and DLBCL arising from follicular lymphoma. In some embodiments, the present disclosure provides methods of treatment using the engineered T cells for the treatment of a patient suffering from a cancer.

[0040] To prepare CD19-directed genetically modified autologous T cell immunotherapy, a patient's own T cells may be harvested and genetically modified ex vivo by retroviral transduction to express a chimeric antigen receptor (CAR) comprising a murine anti-CD19 single chain variable fragment (scFv) linked to CD28 and CD3-zeta co-

stimulatory domains. In some embodiments, the CAR comprises a murine anti-CD19 single chain variable fragment (scFv) linked to 4-1BB and CD3-zeta co-stimulatory domain. The anti-CD19 CAR T cells may be expanded and infused back into the patient, where they may recognize and eliminate CD19-expressing target cells. YESCARTA® (Axi-cel™; axicabtagene ciloleucel) is an example of such CD19-directed genetically modified autologous T cell immunotherapy. *See* Kochenderfer, *et al.*, (J Immunother 2009;32:689 702). Additional CD19 directed CAR therapies include JCAR017, JCAR015, JCAR014, Kymriah (tisagenlecleucel). See Sadelain et al. Nature Rev. Cancer Vol. 3 (2003), Ruella et al., Curr Hematol Malig Rep., Springer, NY (2016) and Sadelain et al. Cancer Discovery (Apr 2013).

[0041] CD19-directed genetically modified autologous T cell immunotherapy may be prepared from the patient's peripheral blood mononuclear cells, which are typically obtained via a standard leukapheresis procedure. The mononuclear cells may be enriched for T cells and activated with anti-CD3 antibody in the presence of IL-2, then transduced with the replication incompetent retroviral vector containing the anti-CD19 CAR transgene. The transduced T cells may be expanded in cell culture, washed, formulated into a suspension, and/or cryopreserved. Typically, the product comprising genetically modified autologous T cells must pass a sterility test before release for shipping as a frozen suspension in a patient-specific infusion container such as an infusion bag. Typically, the product is thawed prior to infusion.

[0042] In addition to T cells, CD19-directed genetically modified autologous T cell immunotherapy may contain NK and NK-T cells. In some embodiments, the CD19-directed genetically modified autologous T cell immunotherapy formulation contains about 5% dimethylsulfoxide (DMSO) and about 2.5% albumin (human) (v/v).

[0043] CD19-directed genetically modified autologous T cells bind to CD19-expressing cancer cells and normal B cells. Studies have demonstrated that, following anti-CD19 CAR T cell engagement with CD19-expressing target cells, the CD28 and CD3-zeta co-stimulatory domains activate downstream signaling cascades that lead to T-cell activation, proliferation, acquisition of effector functions and secretion of inflammatory cytokines and chemokines. This sequence of events leads to killing of CD19-expressing cells.

[0044] In one aspect, the invention provides a method of treating relapsed or refractory diffuse large B-cell lymphoma (DLBCL) not otherwise specified, primary

mediastinal large B-cell lymphoma, high grade B-cell lymphoma, or DLBCL arising from follicular lymphoma after two or more lines of systemic therapy in a patient comprising: administering to the patient in need thereof a CD19-directed genetically modified autologous T cell suspension by intravenous infusion at a dose between about 1×10^6 and about 2×10^6 CAR-positive viable T cells per kg body weight up to a maximum dose of about 1×10^8 CAR-positive viable T cells.

DEFINITIONS

[0045] In order for the present invention to be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms are set forth throughout the Specification.

[0046] As used in this Specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

[0047] Unless specifically stated or obvious from context, as used herein, the term "or" is understood to be inclusive and covers both "or" and "and".

[0048] The term "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include A and B; A or B; A (alone); and B (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0049] The terms "e.g.," and "i.e." as used herein, are used merely by way of example, without limitation intended, and should not be construed as referring only those items explicitly enumerated in the specification.

[0050] The terms "or more", "at least", "more than", and the like, e.g., "at least one" are understood to include but not be limited to at least 1, 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, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149 or 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000,

5000 or more than the stated value. Also included is any greater number or fraction in between.

[0051] Conversely, the term "no more than" includes each value less than the stated value. For example, "no more than 100 nucleotides" includes 100, 99, 98, 97, 96, 95, 94, 93, 92, 91, 90, 89, 88, 87, 86, 85, 84, 83, 82, 81, 80, 79, 78, 77, 76, 75, 74, 73, 72, 71, 70, 69, 68, 67, 66, 65, 64, 63, 62, 61, 60, 59, 58, 57, 56, 55, 54, 53, 52, 51, 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, and 0 nucleotides. Also included is any lesser number or fraction in between.

[0052] The terms "plurality", "at least two", "two or more", "at least second", and the like, are understood to include but not limited to at least 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, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149 or 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000 or more. Also included is any greater number or fraction in between.

[0053] Throughout the specification the word "comprising," or variations such as "comprises" or "comprising," will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps. It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

[0054] Unless specifically stated or evident from context, as used herein, the term "about" refers to a value or composition that is within an acceptable error range for the particular value or composition as determined by one of ordinary skill in the art, which will depend in part on how the value or composition is measured or determined, i.e., the limitations of the measurement system. For example, "about" or "approximately" may mean within one or more than one standard deviation per the practice in the art. "About" or

"approximately" may mean a range of up to 10% (i.e., ±10%). Thus, "about" may be understood to be within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, 0.01%, or 0.001% greater or less than the stated value. For example, about 5 mg may include any amount between 4.5 mg and 5.5 mg. Furthermore, particularly with respect to biological systems or processes, the terms may mean up to an order of magnitude or up to 5-fold of a value. When particular values or compositions are provided in the instant disclosure, unless otherwise stated, the meaning of "about" or "approximately" should be assumed to be within an acceptable error range for that particular value or composition.

[0055] As described herein, any concentration range, percentage range, ratio range or integer range is to be understood to be inclusive of the value of any integer within the recited range and, when appropriate, fractions thereof (such as one-tenth and one-hundredth of an integer), unless otherwise indicated.

[0056] Units, prefixes, and symbols used herein are provided using their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range.

[0057] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, Juo, "The Concise Dictionary of Biomedicine and Molecular Biology", 2nd ed., (2001), CRC Press; "The Dictionary of Cell & Molecular Biology", 5th ed., (2013), Academic Press; and "The Oxford Dictionary Of Biochemistry And Molecular Biology", Cammack et al. eds., 2nd ed, (2006), Oxford University Press, provide those of skill in the art with a general dictionary for many of the terms used in this disclosure.

[0058] "Administering" refers to the physical introduction of an agent to a subject, using any of the various methods and delivery systems known to those skilled in the art. Exemplary routes of administration for the formulations disclosed herein include intravenous, intramuscular, subcutaneous, intraperitoneal, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intralymphatic, intralesional, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular,

subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion, as well as in vivo electroporation. In some embodiments, the formulation is administered via a non-parenteral route, e.g., orally. Other non-parenteral routes include a topical, epidermal or mucosal route of administration, for example, intranasally, vaginally, rectally, sublingually or topically. Administering may also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

[0059] The term "antibody" (Ab) includes, without limitation, a glycoprotein immunoglobulin which binds specifically to an antigen. In general, and antibody may comprise at least two heavy (H) chains and two light (L) chains interconnected by disulfide bonds, or an antigen-binding molecule thereof. Each H chain comprises a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region comprises three constant domains, CH1, CH2 and CH3. Each light chain comprises a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region is comprises one constant domain, CL. The VH and VL regions may be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL comprises three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the Abs may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system.

[0060] Antibodies may include, for example, monoclonal antibodies, recombinantly produced antibodies, monospecific antibodies, multispecific antibodies (including bispecific antibodies), human antibodies, engineered antibodies, humanized antibodies, chimeric antibodies, immunoglobulins, synthetic antibodies, tetrameric antibodies comprising two heavy chain and two light chain molecules, an antibody light chain monomer, an antibody heavy chain monomer, an antibody light chain dimer, an antibody heavy chain dimer, an antibody light chain- antibody heavy chain pair, intrabodies, antibody fusions (sometimes referred to herein as "antibody conjugates"), heteroconjugate antibodies, single domain antibodies, monovalent antibodies, single chain antibodies or single-chain Fvs (scFv), camelized antibodies, affybodies, Fab fragments, F(ab')2 fragments, disulfide-linked Fvs

(sdFv), anti-idiotypic (anti-Id) antibodies (including, e.g., anti-anti-Id antibodies), minibodies, domain antibodies, synthetic antibodies (sometimes referred to herein as "antibody mimetics"), and antigen-binding fragments of any of the above. In some embodiments, antibodies described herein refer to polyclonal antibody populations.

[0061] An "antigen binding molecule," "antigen binding portion," or "antibody fragment" refers to any molecule that comprises the antigen binding parts (e.g., CDRs) of the antibody from which the molecule is derived. An antigen binding molecule may include the antigenic complementarity determining regions (CDRs). Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')2, and Fv fragments, dAb, linear antibodies, scFv antibodies, and multispecific antibodies formed from antigen binding molecules. Peptibodies (i.e., Fc fusion molecules comprising peptide binding domains) are another example of suitable antigen binding molecules. In some embodiments, the antigen binding molecule binds to an antigen on a tumor cell. In some embodiments, the antigen binding molecule binds to an antigen on a cell involved in a hyperproliferative disease or to a viral or bacterial antigen. In some embodiments, the antigen binding molecule binds to CD19. In further embodiments, the antigen binding molecule is an antibody fragment that specifically binds to the antigen, including one or more of the complementarity determining regions (CDRs) thereof. In further embodiments, the antigen binding molecule is a single chain variable fragment (scFv). In some embodiments, the antigen binding molecule comprises or consists of avimers.

[0062] An "antigen" refers to any molecule that provokes an immune response or is capable of being bound by an antibody or an antigen binding molecule. The immune response may involve either antibody production, or the activation of specific immunologically-competent cells, or both. A person of skill in the art would readily understand that any macromolecule, including virtually all proteins or peptides, may serve as an antigen. An antigen may be endogenously expressed, i.e. expressed by genomic DNA, or may be recombinantly expressed. An antigen may be specific to a certain tissue, such as a cancer cell, or it may be broadly expressed. In addition, fragments of larger molecules may act as antigens. In some embodiments, antigens are tumor antigens.

[0063] "CD19-directed genetically modified autologous T cell immunotherapy" refers to a suspension of chimeric antigen receptor (CAR)-positive T cells. An example of such immunotherapy is axicabtagene ciloleucel (also known as Axi-cel™, YESCARTA®), developed by Kite Pharmaceuticals, Inc.

[0064] The term "neutralizing" refers to an antigen binding molecule, scFv, antibody, or a fragment thereof, that binds to a ligand and prevents or reduces the biological effect of that ligand. In some embodiments, the antigen binding molecule, scFv, antibody, or a fragment thereof, directly blocking a binding site on the ligand or otherwise alters the ligand's ability to bind through indirect means (such as structural or energetic alterations in the ligand). In some embodiments, the antigen binding molecule, scFv, antibody, or a fragment thereof prevents the protein to which it is bound from performing a biological function.

[0065] The term "autologous" refers to any material derived from the same individual to which it is later to be re-introduced. For example, the engineered autologous cell therapy (eACT™) method described herein involves collection of lymphocytes from a patient, which are then engineered to express, e.g., a CAR construct, and then administered back to the same patient.

[0066] The term "allogeneic" refers to any material derived from one individual which is then introduced to another individual of the same species, e.g., allogeneic T cell transplantation.

[0067] The terms "transduction" and "transduced" refer to the process whereby foreign DNA is introduced into a cell via viral vector (see Jones et al., "Genetics: principles and analysis," Boston: Jones & Bartlett Publ. (1998)). In some embodiments, the vector is a retroviral vector, a DNA vector, a RNA vector, an adenoviral vector, a baculoviral vector, an Epstein Barr viral vector, a papovaviral vector, a vaccinia viral vector, a herpes simplex viral vector, an adenovirus associated vector, a lentiviral vector, or any combination thereof.

[0068] A "cancer" refers to a broad group of various diseases characterized by the uncontrolled growth of abnormal cells in the body. Unregulated cell division and growth results in the formation of malignant tumors that invade neighboring tissues and may also metastasize to distant parts of the body through the lymphatic system or bloodstream. A "cancer" or "cancer tissue" may include a tumor. Examples of cancers that may be treated by the methods disclosed herein include, but are not limited to, cancers of the immune system including lymphoma, leukemia, myeloma, and other leukocyte malignancies. In some embodiments, the methods disclosed herein may be used to reduce the tumor size of a tumor derived from, for example, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma

of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, multiple myeloma, Hodgkin's Disease, non-Hodgkin's lymphoma (NHL), primary mediastinal large B cell lymphoma (PMBC), diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL), transformed follicular lymphoma, splenic marginal zone lymphoma (SMZL), cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemia, acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia (ALL) (including non T cell ALL), chronic lymphocytic leukemia (CLL), solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T cell lymphoma, environmentally induced cancers including those induced by asbestos, other B cell malignancies, and combinations of said cancers. In some embodiments, the cancer is multiple myeloma. The particular cancer may be responsive to chemo- or radiation therapy or the cancer may be refractory. A refractor cancer refers to a cancer that is not amendable to surgical intervention and the cancer is either initially unresponsive to chemo- or radiation therapy or the cancer becomes unresponsive over time.

[0069] An “anti-tumor effect” as used herein, refers to a biological effect that may present as a decrease in tumor volume, a decrease in the number of tumor cells, a decrease in tumor cell proliferation, a decrease in the number of metastases, an increase in overall or progression-free survival, an increase in life expectancy, or amelioration of various physiological symptoms associated with the tumor. An anti-tumor effect may also refer to the prevention of the occurrence of a tumor, e.g., a vaccine.

[0070] A “cytokine,” as used herein, refers to a non-antibody protein that is released by one cell in response to contact with a specific antigen, wherein the cytokine interacts with a second cell to mediate a response in the second cell. “Cytokine” as used herein is meant to refer to proteins released by one cell population that act on another cell as intercellular mediators. A cytokine may be endogenously expressed by a cell or administered to a subject. Cytokines may be released by immune cells, including macrophages, B cells, T cells, and mast cells to propagate an immune response. Cytokines may induce various responses in the recipient cell. Cytokines may include homeostatic cytokines, chemokines, pro-inflammatory

cytokines, effectors, and acute-phase proteins. For example, homeostatic cytokines, including interleukin (IL) 7 and IL-15, promote immune cell survival and proliferation, and pro-inflammatory cytokines may promote an inflammatory response. Examples of homeostatic cytokines include, but are not limited to, IL-2, IL-4, IL-5, IL-7, IL-10, IL-12p40, IL-12p70, IL-15, and interferon (IFN) gamma. Examples of pro-inflammatory cytokines include, but are not limited to, IL-1a, IL-1b, IL-6, IL-13, IL-17a, tumor necrosis factor (TNF)-alpha, TNF-beta, fibroblast growth factor (FGF) 2, granulocyte macrophage colony-stimulating factor (GM-CSF), soluble intercellular adhesion molecule 1 (sICAM-1), soluble vascular adhesion molecule 1 (sVCAM-1), vascular endothelial growth factor (VEGF), VEGF-C, VEGF-D, and placental growth factor (PLGF). Examples of effectors include, but are not limited to, granzyme A, granzyme B, soluble Fas ligand (sFasL), and perforin. Examples of acute phase-proteins include, but are not limited to, C-reactive protein (CRP) and serum amyloid A (SAA).

[0071] "Chemokines" are a type of cytokine that mediates cell chemotaxis, or directional movement. Examples of chemokines include, but are not limited to, IL-8, IL-16, eotaxin, eotaxin-3, macrophage-derived chemokine (MDC or CCL22), monocyte chemotactic protein 1 (MCP-1 or CCL2), MCP-4, macrophage inflammatory protein 1α (MIP-1α, MIP-1a), MIP-1β (MIP-1b), gamma-induced protein 10 (IP-10), and thymus and activation regulated chemokine (TARC or CCL17).

[0072] A "therapeutically effective amount," "effective dose," "effective amount," or "therapeutically effective dosage" of a therapeutic agent, e.g., engineered CAR T cells, is any amount that, when used alone or in combination with another therapeutic agent, protects a subject against the onset of a disease or promotes disease regression evidenced by a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. The ability of a therapeutic agent to promote disease regression may be evaluated using a variety of methods known to the skilled practitioner, such as in human subjects during clinical trials, in animal model systems predictive of efficacy in humans, or by assaying the activity of the agent in in vitro assays.

[0073] The term "lymphocyte" as used herein includes natural killer (NK) cells, T cells, or B cells. NK cells are a type of cytotoxic (cell toxic) lymphocyte that represent a major component of the inherent immune system. NK cells reject tumors and cells infected by viruses. It works through the process of apoptosis or programmed cell death. They were

termed “natural killers” because they do not require activation in order to kill cells. T cells play a major role in cell-mediated-immunity (no antibody involvement). Its T cell receptors (TCR) differentiate themselves from other lymphocyte types. The thymus, a specialized organ of the immune system, is primarily responsible for the T cell’s maturation. There are six types of T cells, namely: Helper T cells (e.g., CD4+ cells), Cytotoxic T cells (also known as TC, cytotoxic T lymphocyte, CTL, T-killer cell, cytolytic T cell, CD8+ T cells or killer T cell), Memory T cells ((i) stem memory TSCM cells, like naive cells, are CD45RO−, CCR7+, CD45RA+, CD62L+ (L-selectin), CD27+, CD28+ and IL-7Rα+, but they also express large amounts of CD95, IL-2Rβ, CXCR3, and LFA-1, and show numerous functional attributes distinctive of memory cells); (ii) central memory TCM cells express L-selectin and the CCR7, they secrete IL-2, but not IFNγ or IL-4, and (iii) effector memory TEM cells, however, do not express L-selectin or CCR7 but produce effector cytokines like IFNγ and IL-4), Regulatory T cells (Tregs, suppressor T cells, or CD4+CD25+ regulatory T cells), Natural Killer T cells (NKT) and Gamma Delta T cells. B-cells, on the other hand, play a principal role in humoral immunity (with antibody involvement). It makes antibodies and antigens and performs the role of antigen-presenting cells (APCs) and turns into memory B-cells after activation by antigen interaction. In mammals, immature B-cells are formed in the bone marrow, where its name is derived from.

[0074] The term “genetically engineered” or “engineered” refers to a method of modifying the genome of a cell, including, but not limited to, deleting a coding or non-coding region or a portion thereof or inserting a coding region or a portion thereof. In some embodiments, the cell that is modified is a lymphocyte, e.g., a T cell, which may either be obtained from a patient or a donor. The cell may be modified to express an exogenous construct, such as, e.g., a chimeric antigen receptor (CAR) or a T cell receptor (TCR), which is incorporated into the cell's genome.

[0075] An “immune response” refers to the action of a cell of the immune system (for example, T lymphocytes, B lymphocytes, natural killer (NK) cells, macrophages, eosinophils, mast cells, dendritic cells and neutrophils) and soluble macromolecules produced by any of these cells or the liver (including Abs, cytokines, and complement) that results in selective targeting, binding to, damage to, destruction of, and/or elimination from a vertebrate's body of invading pathogens, cells or tissues infected with pathogens, cancerous or other abnormal cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

[0076] The term "immunotherapy" refers to the treatment of a subject afflicted with, or at risk of contracting or suffering a recurrence of, a disease by a method comprising inducing, enhancing, suppressing or otherwise modifying an immune response. Examples of immunotherapy include, but are not limited to, T cell therapies. T cell therapy may include adoptive T cell therapy, tumor-infiltrating lymphocyte (TIL) immunotherapy, autologous cell therapy, engineered autologous cell therapy (eACT™), and allogeneic T cell transplantation. However, one of skill in the art would recognize that the conditioning methods disclosed herein would enhance the effectiveness of any transplanted T cell therapy. Examples of T cell therapies are described in U.S. Patent Publication Nos. 2014/0154228 and 2002/0006409, U.S. Patent No. 7,741,465, U.S. Patent No. 6,319,494, U.S. Patent No. 5,728,388, and International Publication No. WO 2008/081035.

[0077] The T cells of the immunotherapy may come from any source known in the art. For example, T cells may be differentiated in vitro from a hematopoietic stem cell population, or T cells may be obtained from a subject. T cells may be obtained from, e.g., peripheral blood mononuclear cells (PBMCs), bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In addition, the T cells may be derived from one or more T cell lines available in the art. T cells may also be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as FICOLL™ separation and/or apheresis. Additional methods of isolating T cells for a T cell therapy are disclosed in U.S. Patent Publication No. 2013/0287748, which is herein incorporated by references in its entirety.

[0078] The term "engineered Autologous Cell Therapy," which may be abbreviated as "eACT™," also known as adoptive cell transfer, is a process by which a patient's own T cells are collected and subsequently genetically altered to recognize and target one or more antigens expressed on the cell surface of one or more specific tumor cells or malignancies. T cells may be engineered to express, for example, chimeric antigen receptors (CAR). CAR positive (+) T cells are engineered to express an extracellular single chain variable fragment (scFv) with specificity for a particular tumor antigen linked to an intracellular signaling part comprising at least one costimulatory domain and at least one activating domain. The CAR scFv may be designed to target, for example, CD19, which is a transmembrane protein expressed by cells in the B cell lineage, including all normal B cells and B cell malignances, including but not limited to diffuse large B-cell lymphoma (DLBCL) not otherwise specified,

primary mediastinal large B-cell lymphoma, high grade B-cell lymphoma, and DLBCL arising from follicular lymphoma, NHL, CLL, and non-T cell ALL. Example CAR T cell therapies and constructs are described in U.S. Patent Publication Nos. 2013/0287748, 2014/0227237, 2014/0099309, and 2014/0050708, and these references are incorporated by reference in their entirety.

[0079] A "patient" as used herein includes any human who is afflicted with a cancer (e.g., a lymphoma or a leukemia). The terms "subject" and "patient" are used interchangeably herein.

[0080] As used herein, the term "in vitro cell" refers to any cell which is cultured ex vivo. In particular, an in vitro cell may include a T cell.

[0081] The terms "peptide," "polypeptide," and "protein" are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide contains at least two amino acids, and no limitation is placed on the maximum number of amino acids that may comprise a protein's or peptide's sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. "Polypeptides" include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. The polypeptides include natural peptides, recombinant peptides, synthetic peptides, or a combination thereof.

[0082] "Stimulation," as used herein, refers to a primary response induced by binding of a stimulatory molecule with its cognate ligand, wherein the binding mediates a signal transduction event. A "stimulatory molecule" is a molecule on a T cell, e.g., the T cell receptor (TCR)/CD3 complex, that specifically binds with a cognate stimulatory ligand present on an antigen present cell. A "stimulatory ligand" is a ligand that when present on an antigen presenting cell (e.g., an APC, a dendritic cell, a B-cell, and the like) may specifically bind with a stimulatory molecule on a T cell, thereby mediating a primary response by the T cell, including, but not limited to, activation, initiation of an immune response, proliferation, and the like. Stimulatory ligands include, but are not limited to, an anti-CD3 antibody, an

MHC Class I molecule loaded with a peptide, a superagonist anti-CD2 antibody, and a superagonist anti-CD28 antibody.

[0083] A "costimulatory signal," as used herein, refers to a signal, which in combination with a primary signal, such as TCR/CD3 ligation, leads to a T cell response, such as, but not limited to, proliferation and/or upregulation or down regulation of key molecules.

[0084] A "costimulatory ligand," as used herein, includes a molecule on an antigen presenting cell that specifically binds a cognate co-stimulatory molecule on a T cell. Binding of the costimulatory ligand provides a signal that mediates a T cell response, including, but not limited to, proliferation, activation, differentiation, and the like. A costimulatory ligand induces a signal that is in addition to the primary signal provided by a stimulatory molecule, for instance, by binding of a T cell receptor (TCR)/CD3 complex with a major histocompatibility complex (MHC) molecule loaded with peptide. A co-stimulatory ligand may include, but is not limited to, 3/TR6, 4-1BB ligand, agonist or antibody that binds Toll ligand receptor , B7-1 (CD80), B7-2 (CD86), CD30 ligand, CD40, CD7, CD70, CD83, herpes virus entry mediator (HVEM), human leukocyte antigen G (HLA-G), ILT4, immunoglobulin-like transcript (ILT) 3, inducible costimulatory ligand (ICOS-L), intercellular adhesion molecule (ICAM), ligand that specifically binds with B7-H3, lymphotoxin beta receptor, MHC class I chain-related protein A (MICA), MHC class I chain-related protein B (MICB), OX40 ligand, PD-L2, or programmed death (PD) L1. A co-stimulatory ligand includes, without limitation, an antibody that specifically binds with a co-stimulatory molecule present on a T cell, such as, but not limited to, 4-1BB, B7-H3, CD2, CD27, CD28, CD30, CD40, CD7, ICOS, ligand that specifically binds with CD83, lymphocyte function-associated antigen-1 (LFA-1), natural killer cell receptor C (NKG2C), OX40, PD-1, or tumor necrosis factor superfamily member 14 (TNFSF14 or LIGHT).

[0085] A "costimulatory molecule" is a cognate binding partner on a T cell that specifically binds with a costimulatory ligand, thereby mediating a costimulatory response by the T cell, such as, but not limited to, proliferation. Costimulatory molecules include, but are not limited to, A "costimulatory molecule" is a cognate binding partner on a T cell that specifically binds with a costimulatory ligand, thereby mediating a costimulatory response by the T cell, such as, but not limited to, proliferation. Costimulatory molecules include, but are not limited to, 4-1BB/CD137, B7-H3, BAFFR, BLAME (SLAMF8), BTLA, CD 33, CD 45, CD100 (SEMA4D), CD103, CD134, CD137, CD154, CD16, CD160 (BY55), CD18, CD19,

CD19a, CD2, CD22, CD247, CD27, CD276 (B7-H3), CD28, CD29, CD3 (alpha; beta; delta; epsilon; gamma; zeta), CD30, CD37, CD4, CD4, CD40, CD49a, CD49D, CD49f, CD5, CD64, CD69, CD7, CD80, CD83 ligand, CD84, CD86, CD8alpha, CD8beta, CD9, CD96 (Tactile), CDl-la, CDl-lb, CDl-lc, CDl-ld, CDS, CEACAM1, CRT AM, DAP-10, DNAM1 (CD226), Fc gamma receptor, GADS, GITR, HVEM (LIGHTR), IA4, ICAM-1, ICAM-1, ICOS, Ig alpha (CD79a), IL2R beta, IL2R gamma, IL7R alpha, integrin, ITGA4, ITGA4, ITGA6, ITGAD, ITGAE, ITGAL, ITGAM, ITGAX, ITGB2, ITGB7, ITGBl, KIRDS2, LAT, LFA-1, LFA-1, LIGHT, LIGHT (tumor necrosis factor superfamily member 14; TNFSF14), LTBR, Ly9 (CD229), lymphocyte function-associated antigen-1 (LFA-1 (CDl la/CD18), MHC class I molecule, NKG2C, NKG2D, NKp30, NKp44, NKp46, NKp80 (KLRF1), OX40, PAG/Cbp, PD-1, PSGL1, SELPLG (CD162), signaling lymphocytic activation molecule, SLAM (SLAMF1; CD150; IPO-3), SLAMF4 (CD244; 2B4), SLAMF6 (NTB-A; Lyl08), SLAMF7, SLP-76, TNF, TNFr, TNFR2, Toll ligand receptor, TRANCE/RANKL, VLA1, or VLA-6, or fragments, truncations, or combinations thereof.

[0086] The terms "reducing" and "decreasing" are used interchangeably herein and indicate any change that is less than the original. "Reducing" and "decreasing" are relative terms, requiring a comparison between pre- and post- measurements. "Reducing" and "decreasing" include complete depletions.

[0087] "Treatment" or "treating" of a subject refers to any type of intervention or process performed on, or the administration of an active agent to, the subject with the objective of reversing, alleviating, ameliorating, inhibiting, slowing down or preventing the onset, progression, development, severity or recurrence of a symptom, complication or condition, or biochemical indicia associated with a disease. In some embodiments, "treatment" or "treating" includes a partial remission. In another embodiment, "treatment" or "treating" includes a complete remission.

[0088] Various aspects of the disclosure are described in further detail in the following subsections.

Chimeric Antigen Receptors

[0089] Chimeric antigen receptors (CARs or CAR-Ts) are genetically engineered receptors. These engineered receptors may be readily inserted into and expressed by immune cells, including T cells in accordance with techniques known in the art. With a CAR, a single receptor may be programmed to both recognize a specific antigen and, when bound to that

antigen, activate the immune cell to attack and destroy the cell bearing that antigen. When these antigens exist on tumor cells, an immune cell that expresses the CAR may target and kill the tumor cell.

Engineered T cells and Use

[0090] A CD19-directed genetically modified autologous T cell immunotherapy indicated for the treatment of patients with relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy, including diffuse large B-cell lymphoma (DLBCL) not otherwise specified, primary mediastinal large B-cell lymphoma, high grade B-cell lymphoma, and DLBCL arising from follicular lymphoma. In some embodiments, the CD19-directed genetically modified autologous T cell immunotherapy is axicabtagene ciloleucel (Axi-cel™, YESCARTA®).

[0091] The cell of the present disclosure may be obtained through T cells obtained from a subject. T cells may be obtained from, e.g., peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In addition, the T cells may be derived from one or more T cell lines available in the art. T cells may also be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as FICOLL™ separation and/or apheresis. In some embodiments, the cells collected by apheresis are washed to remove the plasma fraction, and placed in an appropriate buffer or media for subsequent processing. In some embodiments, the cells are washed with PBS. As will be appreciated, a washing step may be used, such as by using a semiautomated flow through centrifuge, e.g., the CobeTM 2991 cell processor, the Baxter CytoMateTM, or the like. In some embodiments, the washed cells are resuspended in one or more biocompatible buffers, or other saline solution with or without buffer. In some embodiments, the undesired components of the apheresis sample are removed. Additional methods of isolating T cells for a T cell therapy are disclosed in U.S. Patent Pub. No. 2013/0287748, which is herein incorporated by references in its entirety.

[0092] In some embodiments, T cells are isolated from PBMCs by lysing the red blood cells and depleting the monocytes, e.g., by using centrifugation through a PERCOLL™ gradient. In some embodiments, a specific subpopulation of T cells, such as CD4+, CD8+, CD28+, CD45RA+, and CD45RO+ T cells is further isolated by positive or negative selection techniques known in the art. For example, enrichment of a T cell population by

negative selection may be accomplished with a combination of antibodies directed to surface markers unique to the negatively selected cells. In some embodiments, cell sorting and/or selection via negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells negatively selected may be used. For example, to enrich for CD4+ cells by negative selection, a monoclonal antibody cocktail typically includes antibodies to CD8, CD11b, CD14, CD16, CD20, and HLA-DR. In some embodiments, flow cytometry and cell sorting are used to isolate cell populations of interest for use in the present disclosure.

[0093] In some embodiments, PBMCs are used directly for genetic modification with the immune cells (such as CARs) using methods as described herein. In some embodiments, after isolating the PBMCs, T lymphocytes are further isolated, and both cytotoxic and helper T lymphocytes are sorted into naive, memory, and effector T cell subpopulations either before or after genetic modification and/or expansion.

[0094] In some embodiments, CD8+ cells are further sorted into naive, central memory, and effector cells by identifying cell surface antigens that are associated with each of these types of CD8+ cells. In some embodiments, the expression of phenotypic markers of central memory T cells includes CCR7, CD3, CD28, CD45RO, CD62L, and CD127 and are negative for granzyme B. In some embodiments, central memory T cells are CD8+, CD45RO+, and CD62L+ T cells. In some embodiments, effector T cells are negative for CCR7, CD28, CD62L, and CD127 and positive for granzyme B and perforin. In some embodiments, CD4+ T cells are further sorted into subpopulations. For example, CD4+ T helper cells may be sorted into naive, central memory, and effector cells by identifying cell populations that have cell surface antigens.

[0095] In some embodiments, the immune cells, e.g., T cells, are genetically modified following isolation using known methods, or the immune cells are activated and expanded (or differentiated in the case of progenitors) in vitro prior to being genetically modified. In another embodiment, the immune cells, e.g., T cells, are genetically modified with the chimeric antigen receptors described herein (e.g., transduced with a viral vector comprising one or more nucleotide sequences encoding a CAR) and then are activated and/or expanded in vitro. Methods for activating and expanding T cells are known in the art and are described, e.g., in U.S. Patent Nos. 6,905,874; 6,867,041; and 6,797,514; and PCT Publication No. WO 2012/079000, the contents of which are hereby incorporated by reference in their entirety. Generally, such methods include contacting PBMC or isolated T cells with a stimulatory

agent and costimulatory agent, such as anti-CD3 and anti-CD28 antibodies, generally attached to a bead or other surface, in a culture medium with appropriate cytokines, such as IL-2. Anti-CD3 and anti-CD28 antibodies attached to the same bead serve as a "surrogate" antigen presenting cell (APC). One example is The Dynabeads® system, a CD3/CD28 activator/stimulator system for physiological activation of human T cells. In other embodiments, the T cells are activated and stimulated to proliferate with feeder cells and appropriate antibodies and cytokines using methods such as those described in U.S. Patent Nos. 6,040,177 and 5,827,642 and PCT Publication No. WO 2012/129514, the contents of which are hereby incorporated by reference in their entirety.

[0096] In some embodiments, the T cells are obtained from a donor subject. In some embodiments, the donor subject is human patient afflicted with a cancer or a tumor. In some embodiments, the donor subject is a human patient not afflicted with a cancer or a tumor.

[0097] In some embodiments, the composition comprises a pharmaceutically acceptable carrier, diluent, solubilizer, emulsifier, preservative and/or adjuvant. In some embodiments, the composition comprises an excipient.

[0098] In some embodiments, the composition is selected for parenteral delivery, for inhalation, or for delivery through the digestive tract, such as orally. The preparation of such pharmaceutically acceptable compositions is within the ability of one skilled in the art. In some embodiments, buffers are used to maintain the composition at physiological pH or at a slightly lower pH, typically within a pH range of from about 5 to about 8. In some embodiments, when parenteral administration is contemplated, the composition is in the form of a pyrogen-free, parenterally acceptable aqueous solution comprising a composition described herein, with or without additional therapeutic agents, in a pharmaceutically acceptable vehicle. In some embodiments, the vehicle for parenteral injection is sterile distilled water in which composition described herein, with or without at least one additional therapeutic agent, is formulated as a sterile, isotonic solution, properly preserved. In some embodiments, the preparation involves the formulation of the desired molecule with polymeric compounds (such as polylactic acid or polyglycolic acid), beads or liposomes, that provide for the controlled or sustained release of the product, which are then be delivered via a depot injection. In some embodiments, implantable drug delivery devices are used to introduce the desired molecule.

[0099] In some embodiments, the methods of treating a cancer in a subject in need thereof comprise a T cell therapy. In some embodiments, the T cell therapy disclosed herein is engineered Autologous Cell Therapy (eACT™). According to this embodiment, the method may include collecting blood cells from the patient. The isolated blood cells (e.g., T cells) may then be engineered to express a CAR or a TCR disclosed herein. In a particular embodiment, the CAR T cells or the TCR T cells are administered to the patient. In some embodiments, the CAR T cells or the TCR T cells treat a tumor or a cancer in the patient. In some embodiments the CAR T cells or the TCR T cells reduce the size of a tumor or a cancer.

[0100] In some embodiments, the donor T cells for use in the T cell therapy are obtained from the patient (e.g., for an autologous T cell therapy). In other embodiments, the donor T cells for use in the T cell therapy are obtained from a subject that is not the patient. The T cells may be administered at a therapeutically effective amount. For example, a therapeutically effective amount of the T cells may be at least about 10^4 cells, at least about 10^5 cells, at least about 10^6 cells, at least about 10^7 cells, at least about 10^8 cells, at least about 10^9, or at least about 10^{10}. In another embodiment, the therapeutically effective amount of the T cells is about 10^4 cells, about 10^5 cells, about 10^6 cells, about 10^7 cells, or about 10^8 cells. In some embodiments, the therapeutically effective amount of the CAR T cells is about 2 X 10^6 cells/kg, about 3 X 10^6 cells/kg, about 4 X 10^6 cells/kg, about 5 X 10^6 cells/kg, about 6 X 10^6 cells/kg, about 7 X 10^6 cells/kg, about 8 X 10^6 cells/kg, about 9 X 10^6 cells/kg, about 1 X 10^7 cells/kg, about 2 X 10^7 cells/kg, about 3 X 10^7 cells/kg, about 4 X 10^7 cells/kg, about 5 X 10^7 cells/kg, about 6 X 10^7 cells/kg, about 7 X 10^7 cells/kg, about 8 X 10^7 cells/kg, or about 9 X 10^7 cells/kg. In some embodiments, the therapeutically effective amount of the CAR-positive viable T cells is between about 1 × 10^6 and about 2 × 10^6 CAR-positive viable T cells per kg body weight up to a maximum dose of about 1 x 10^8 CAR-positive viable T cells.

[0101] In some embodiments, the therapeutically effective amount of the CAR-positive viable T cells is between about 0.4 x 10^8 and about 2 x 10^8 CAR-positive viable T cells. In some embodiments, the therapeutically effective amount of the CAR-positive viable T cells is about 0.4 x 10^8, about 0.5 x 10^8, about 0.6 x 10^8, about 0.7 x 10^8, about 0.8 x 10^8, about 0.9 x 10^8, about 1.0 x 10^8, about 1.1 x 10^8, about 1.2 x 10^8, about 1.3 x 10^8, about 1.4 x 10^8, about 1.5 x 10^8, about 1.6 x 10^8, about 1.7 x 10^8, about 1.8 x 10^8, about 1.9 x 10^8, or about 2.0 x 10^8 CAR-positive viable T cells.

Methods of Treatment

[0102] The methods disclosed herein may be used to treat a cancer in a subject, reduce the size of a tumor, kill tumor cells, prevent tumor cell proliferation, prevent growth of a tumor, eliminate a tumor from a patient, prevent relapse of a tumor, prevent tumor metastasis, induce remission in a patient, or any combination thereof. In some embodiments, the methods induce a complete response. In other embodiments, the methods induce a partial response.

[0103] Cancers that may be treated include tumors that are not vascularized, not yet substantially vascularized, or vascularized. The cancer may also include solid or non-solid tumors. In some embodiments, the cancer is a hematologic cancer. In some embodiments, the cancer is of the white blood cells. In other embodiments, the cancer is of the plasma cells. In some embodiments, the cancer is leukemia, lymphoma, or myeloma. In some embodiments, the cancer is acute lymphoblastic leukemia (ALL) (including non T cell ALL), acute lymphoid leukemia (ALL), and hemophagocytic lymphohistocytosis (HLH)), B cell prolymphocytic leukemia, B-cell acute lymphoid leukemia ("BALL"), blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), chronic myeloid leukemia (CML), chronic or acute granulomatous disease, chronic or acute leukemia, diffuse large B cell lymphoma, diffuse large B cell lymphoma (DLBCL), follicular lymphoma, follicular lymphoma (FL), hairy cell leukemia, hemophagocytic syndrome (Macrophage Activating Syndrome (MAS), Hodgkin's Disease, large cell granuloma, leukocyte adhesion deficiency, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, Marginal zone lymphoma, monoclonal gammapathy of undetermined significance (MGUS), multiple myeloma, myelodysplasia and myelodysplastic syndrome (MDS), myeloid diseases including but not limited to acute myeloid leukemia (AML), non-Hodgkin's lymphoma (NHL), plasma cell proliferative disorders (e.g., asymptomatic myeloma (smoldering multiple myeloma or indolent myeloma), plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, plasmacytomas (e.g., plasma cell dyscrasia; solitary myeloma; solitary plasmacytoma; extramedullary plasmacytoma; and multiple plasmacytoma), POEMS syndrome (Crow-Fukase syndrome; Takatsuki disease; PEP syndrome), primary mediastinal large B cell lymphoma (PMBC), small cell- or a large cell-follicular lymphoma, splenic marginal zone lymphoma (SMZL), systemic amyloid light chain amyloidosis, T cell acute lymphoid leukemia ("TALL"), T cell lymphoma, transformed follicular lymphoma, Waldenstrom macroglobulinemia, or a combination thereof.

[0104] In some embodiments, the cancer is a myeloma. In some embodiments, the cancer is multiple myeloma. In some embodiments, the cancer is a leukemia. In some embodiments, the cancer is acute myeloid leukemia.

[0105] In some embodiments, the methods further comprise administering a chemotherapeutic. In some embodiments, the chemotherapeutic selected is a lymphodepleting (preconditioning) chemotherapeutic. Beneficial preconditioning treatment regimens, along with correlative beneficial biomarkers are described in U.S. Provisional Patent Applications 62/262,143 and 62/167,750 which are hereby incorporated by reference in their entirety herein. These describe, e.g., methods of conditioning a patient in need of a T cell therapy comprising administering to the patient specified beneficial doses of cyclophosphamide (between 200 mg/m^2/day and 2000 mg/m^2/day) and specified doses of fludarabine (between 20 mg/m^2/day and 900 mg/m^2/day). One such dose regimen involves treating a patient comprising administering daily to the patient about 500 mg/m^2/day of cyclophosphamide and about 60 mg/m^2/day of fludarabine for three days prior to administration of a therapeutically effective amount of engineered T cells to the patient.

[0106] In some embodiments, the antigen binding molecule, transduced (or otherwise engineered) cells (such as CARs), and the chemotherapeutic agent are administered each in an amount effective to treat the disease or condition in the subject.

[0107] In some embodiments, compositions comprising CAR-expressing immune effector cells disclosed herein may be administered in conjunction with any number of chemotherapeutic agents. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide (CYTOXAN^{TM}); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaoramide and trimethylolomelamine resume; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins,

peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®; razoxane; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2, 2',2''-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g. paclitaxel (TAXOL™, Bristol-Myers Squibb) and doxetaxel (TAXOTERE®, Rhone-Poulenc Rorer); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS2000; difluoromethylomithine (DMFO); retinoic acid derivatives such as Targretin™ (bexarotene), Panretin™, (alitretinoin); ONTAK™ (denileukin diftitox); esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. In some embodiments, compositions comprising CAR- and/or TCR-expressing immune effector cells disclosed herein may be administered in conjunction with an anti-hormonal agent that acts to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Combinations of chemotherapeutic agents are also administered where appropriate, including, but not limited to CHOP, i.e., Cyclophosphamide (Cytoxan®), Doxorubicin (hydroxydoxorubicin), Vincristine (Oncovin®), and Prednisone.

[0108] In some embodiments, the chemotherapeutic agent is administered at the same time or within one week after the administration of the engineered cell or nucleic acid. In other embodiments, the chemotherapeutic agent is administered from 1 to 4 weeks or from 1 week to 1 month, 1 week to 2 months, 1 week to 3 months, 1 week to 6 months, 1 week to 9 months, or 1 week to 12 months after the administration of the engineered cell or nucleic acid. In some embodiments, the chemotherapeutic agent is administered at least 1 month before administering the cell or nucleic acid. In some embodiments, the methods further comprise administering two or more chemotherapeutic agents.

[0109] A variety of additional therapeutic agents may be used in conjunction with the compositions described herein. For example, potentially useful additional therapeutic agents include PD-1 inhibitors such as nivolumab (OPDIVO®), pembrolizumab (KEYTRUDA®), pembrolizumab, pidilizumab (CureTech), and atezolizumab (Roche).

[0110] Additional therapeutic agents suitable for use in combination with the compositions and methods disclosed herein include, but are not limited to, ibrutinib (IMBRUVICA®), ofatumumab (ARZERRA®), rituximab (RITUXAN®), bevacizumab (AVASTIN®), trastuzumab (HERCEPTIN®), trastuzumab emtansine (KADCYLA®), imatinib (GLEEVEC®), cetuximab (ERBITUX®), panitumumab (VECTIBIX®), catumaxomab, ibritumomab, ofatumumab, tositumomab, brentuximab, alemtuzumab, gemtuzumab, erlotinib, gefitinib, vandetanib, afatinib, lapatinib, neratinib, axitinib, masitinib, pazopanib, sunitinib, sorafenib, toceranib, lestaurtinib, axitinib, cediranib, lenvatinib, nintedanib, pazopanib, regorafenib, semaxanib, sorafenib, sunitinib, tivozanib, toceranib, vandetanib, entrectinib, cabozantinib, imatinib, dasatinib, nilotinib, ponatinib, radotinib, bosutinib, lestaurtinib, ruxolitinib, pacritinib, cobimetinib, selumetinib, trametinib, binimetinib, alectinib, ceritinib, crizotinib, aflibercept,adipotide, denileukin diftitox, mTOR inhibitors such as Everolimus and Temsirolimus, hedgehog inhibitors such as sonidegib and vismodegib, CDK inhibitors such as CDK inhibitor (palbociclib).

[0111] In some embodiments, the composition comprising CAR immune cells are administered with an anti-inflammatory agent. Anti-inflammatory agents or drugs may include, but are not limited to, steroids and glucocorticoids (including betamethasone, budesonide, dexamethasone, hydrocortisone acetate, hydrocortisone, hydrocortisone, methylprednisolone, prednisolone, prednisone, triamcinolone), nonsteroidal anti-inflammatory drugs (NSAIDS) including aspirin, ibuprofen, naproxen, methotrexate, sulfasalazine, leflunomide, anti-TNF medications, cyclophosphamide and mycophenolate.

Exemplary NSAIDs include ibuprofen, naproxen, naproxen sodium, Cox-2 inhibitors, and sialylates. Exemplary analgesics include acetaminophen, oxycodone, tramadol of proporxyphene hydrochloride. Exemplary glucocorticoids include cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, or prednisone. Exemplary biological response modifiers include molecules directed against cell surface markers (e.g., CD4, CD5, etc.), cytokine inhibitors, such as the TNF antagonists, (e.g., etanercept (ENBREL®), adalimumab (HUMIRA®) and infliximab (REMICADE®), chemokine inhibitors and adhesion molecule inhibitors. The biological response modifiers include monoclonal antibodies as well as recombinant forms of molecules. Exemplary DMARDs include azathioprine, cyclophosphamide, cyclosporine, methotrexate, penicillamine, leflunomide, sulfasalazine, hydroxychloroquine, Gold (oral (auranofin) and intramuscular), and minocycline.

[0112] In some embodiments, the compositions described herein are administered in conjunction with a cytokine. Examples of cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormones such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor (HGF); fibroblast growth factor (FGF); prolactin; placental lactogen; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors (NGFs) such as NGF-beta; platelet-growth factor; transforming growth factors (TGFs) such as TGF-alpha and TGF-beta; insulin-like growth factor-I and -II; erythropoietin (EPO, Epogen®, Procrit®); osteoinductive factors; interferons such as interferon-alpha, beta, and -gamma; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1alpha, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; IL-15, a tumor necrosis factor such as TNF-alpha or TNF-beta; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture, and biologically active equivalents of the native sequence cytokines.

Administration of CD19-directed genetically modified autologous T cell immunotherapy

Indications and Usage

[0113] In some embodiments, CD19-directed genetically modified autologous T cell immunotherapy indicated for the treatment of adult patients with relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy, including diffuse large B-cell lymphoma (DLBCL) not otherwise specified, primary mediastinal large B-cell lymphoma, high grade B-cell lymphoma, and DLBCL arising from follicular lymphoma. In some embodiments, CD19-directed genetically modified autologous T cell immunotherapy is not indicated for the treatment of patients with primary central nervous system lymphoma.

Dosage and Administration

[0114] In some embodiments, an infusion bag of CD19-directed genetically modified autologous T cell immunotherapy comprises a suspension of chimeric antigen receptor (CAR)-positive T cells in approximately 68 mL. The target dose may be between about 1×10^6 and about 2×10^6 CAR-positive viable T cells per kg body weight, with a maximum of 2×10^8 CAR-positive viable T cells. In some embodiments the CD19-directed genetically modified autologous T cell immunotherapy is Axi-cel™ (YESCARTA®, axicabtagene ciloleucel).

[0115] CD19-directed genetically modified autologous T cell immunotherapy is for autologous use. The patient's identity must match the patient identifiers on the CD19-directed genetically modified autologous T cell immunotherapy cassette and infusion bag. If the information on the patient-specific label does not match the intended patient, the CD19-directed genetically modified autologous T cell immunotherapy cannot be administered.

[0116] In some embodiments, the availability of CD19-directed genetically modified autologous T cell immunotherapy must be confirmed prior to starting the lymphodepleting regimen.

[0117] In some embodiments, the patient is pre-treated prior to CD19-directed genetically modified autologous T cell immunotherapy infusion with administration of lymphodepleting chemotherapy. In some embodiments, a lymphodepleting chemotherapy regimen of cyclophosphamide 500 mg/m^2 IV and fludarabine 30 mg/m^2 IV on the fifth, fourth, and third day before infusion of CD19-directed genetically modified autologous T cell immunotherapy is administered.

[0118] In some embodiments, the patient is premedicated prior to CD19-directed genetically modified autologous T cell immunotherapy infusion by oral administration of acetaminophen at a dose between about 500-1000 mg, about 600-1000 mg, about 700-1000 mg, about 800-1000 mg, about 900-1000 mg, about 500-900 mg, about 500-800 mg, about 500-700 mg, about 500-600 mg, about 600-900 mg, about 600-800 mg, about 600-700 mg, about 700-900 mg, about 700-800 mg, or about 800-900 mg. In some embodiments, the patient is premedicated prior to CD19-directed genetically modified autologous T cell immunotherapy infusion by oral administration of acetaminophen at a dose of about 500 mg, about 525 mg, about 550 mg, about 575 mg, about 600 mg, about 625 mg, about 650 mg, about 675 mg, about 700 mg, about 725 mg, about 750 mg, about 775 mg, about 800 mg, about 825 mg, about 850 mg, about 875 mg, about 900 mg, about 925 mg, about 950 mg, about 975 mg or about 1000 mg.

[0119] In some embodiments, the patient is premedicated prior to CD19-directed genetically modified autologous T cell immunotherapy infusion by administration of acetaminophen 650 mg by mouth and diphenhydramine 12.5 mg intravenously or by mouth approximately 1 hour before CD19-directed genetically modified autologous T cell immunotherapy infusion.

[0120] In some embodiments, the prophylactic use of systemic steroids is avoided as it may interfere with the activity of CD19-directed genetically modified autologous T cell immunotherapy.

Preparation of CD19-directed genetically modified autologous T cell immunotherapy for infusion

[0121] The timing of CD19-directed genetically modified autologous T cell immunotherapy thaw and infusion is coordinated. In some embodiments, the infusion time is confirmed in advance, and the start time of CD19-directed genetically modified autologous T cell immunotherapy thaw is adjusted such that it will be available for infusion when the patient is ready.

[0122] In some embodiments, the patient identity is confirmed prior to CD19-directed genetically modified autologous T cell immunotherapy thaw. Prior to CD19-directed genetically modified autologous T cell immunotherapy preparation, patient's identity is matched with the patient identifiers on the CD19-directed genetically modified autologous T cell immunotherapy cassette. In some embodiments, the CD19-directed genetically modified

autologous T cell immunotherapy product bag is not removed from the cassette if the information on the patient-specific label does not match the intended patient.

[0123] In some embodiments, once patient identification is confirmed, CD19-directed genetically modified autologous T cell immunotherapy product bag is removed from the cassette and the patient information on the cassette label is confirmed to match the bag label.

[0124] In some embodiments, the method comprises inspecting the product bag for any breaches of container integrity such as breaks or cracks before thawing. In some embodiments, the infusion bag is placed inside a second sterile bag per local guidelines.

[0125] In some embodiments, the method comprises thawing the CD19-directed genetically modified autologous T cell immunotherapy at approximately 37°C using either a water bath or dry thaw method until there is no visible ice in the infusion bag. In some embodiments, the method comprises mixing or agitating the contents of the bag to disperse clumps of cellular material. In some embodiments, the contents of the bag are gently mixed or agitated. In some embodiments, the method comprises inspecting the bag for the presence of visible cell clumps remaining and mixing or agitation is continued. Small clumps of cellular material should disperse with gentle manual mixing. In some embodiments, the method does not comprise a wash, spin down, and/or re-suspension of CD19-directed genetically modified autologous T cell immunotherapy in new media prior to infusion.

[0126] In some embodiments, once thawed, CD19-directed genetically modified autologous T cell immunotherapy may be stored at room temperature (20°C to 25°C) for up to 3 hours.

Administration

[0127] In some embodiments, the presently disclosed methods of administration of CD19-directed genetically modified autologous T cell immunotherapy comprise on or more of the following as steps or as considerations:

- Ensure that tocilizumab and emergency equipment are available prior to infusion and during the recovery period.
- Do NOT use a leukodepleting filter.
- Central venous access is recommended for the infusion of CD19-directed genetically modified autologous T cell immunotherapy.

- Confirm the patient's identity matches the patient identifiers on the CD19-directed genetically modified autologous T cell immunotherapy product bag.
- Prime the tubing with normal saline prior to infusion.
- Infuse the entire contents of the CD19-directed genetically modified autologous T cell immunotherapy bag within 30 minutes by either gravity or a peristaltic pump. CD19-directed genetically modified autologous T cell immunotherapy is stable at room temperature for up to 3 hours after thaw.
- Gently agitate the product bag during CD19-directed genetically modified autologous T cell immunotherapy infusion to prevent cell clumping.
- After the entire content of the product bag is infused, rinse the tubing with normal saline at the same infusion rate to ensure all product is delivered.
- CD19-directed genetically modified autologous T cell immunotherapy contains human blood cells that are genetically modified with replication incompetent retroviral vector. Follow universal precautions and local biosafety guidelines for handling and disposal to avoid potential transmission of infectious diseases.

Monitoring

[0128] In some embodiments, administration of CD19-directed genetically modified autologous T cell immunotherapy occurs at a certified healthcare facility.

[0129] In some embodiments, the methods disclosed herein comprise monitoring patients at least daily for 7 days at the certified healthcare facility following infusion for signs and symptoms of CRS and neurologic toxicities. In some embodiments, the methods disclosed herein comprise monitoring patients at least daily for 10 days at the certified healthcare facility following infusion for signs and symptoms of CRS and neurologic toxicities.

[0130] In some embodiments, patients are instructed to remain within proximity of the certified healthcare facility for at least 4 weeks following infusion.

Management of Severe Adverse Reactions

[0131] In some embodiments, the method comprises management of adverse reactions. In some embodiments, the adverse reaction is selected from the group consisting of

cytokine release syndrome (CRS), a neurologic toxicity, a hypersensitivity reaction, a serious infection, a cytopenia and hypogammaglobulinemia.

[0132] In some embodiments, the signs and symptoms of adverse reactions are selected from the group consisting of fever, hypotension, tachycardia, hypoxia, and chills, include cardiac arrhythmias (including atrial fibrillation and ventricular tachycardia), cardiac arrest, cardiac failure, renal insufficiency, capillary leak syndrome, hypotension, hypoxia, organ toxicity, hemophagocytic lymphohistiocytosis/macrophage activation syndrome (HLH/MAS), seizure, encephalopathy, headache, tremor, dizziness, aphasia, delirium, insomnia anxiety, anaphylaxis, febrile neutropenia, thrombocytopenia, neutropenia, and anemia.

Cytokine Release Syndrome

[0133] In some embodiments, the method comprises identifying CRS based on clinical presentation. In some embodiments, the method comprises evaluating for and treating other causes of fever, hypoxia, and hypotension. If CRS is observed or suspected, manage according to the recommendations in Table 1. Patients who experience ≥ Grade 2 CRS (e.g., hypotension, not responsive to fluids, or hypoxia requiring supplemental oxygenation) should be monitored with continuous cardiac telemetry and pulse oximetry. In some embodiments, for patients experiencing severe CRS, consider performing an echocardiogram to assess cardiac function. For severe or life-threatening CRS, intensive care supportive therapy may be considered. In some embodiments, a biosimilar or equivalent of tocilizumab may be used instead of tocilizumab in the methods disclosed herein.

Table 1. CRS Grading and Management Guidance

CRS Grade *(a)*	**Tocilizumab**	**Corticosteroids**
Grade 1 Symptoms require symptomatic treatment only (e.g., fever, nausea, fatigue, headache, myalgia, malaise).	N/A	N/A

Grade 2 Symptoms require and respond to moderate intervention. Oxygen requirement less than 40% FiO_2 or hypotension responsive to fluids or low-dose of one vasopressor or Grade 2 organ toxicity *(b)*.	Administer tocilizumab *(c)* 8 mg/kg IV over 1 hour (not to exceed 800 mg). Repeat tocilizumab every 8 hours as needed if not responsive to IV fluids or increasing supplemental oxygen. Limit to a maximum of 3 doses in a 24-hour period; maximum total of 4 doses if no clinical improvement in the signs and symptoms of CRS.	Manage per Grade 3 if no improvement within 24 hours after starting tocilizumab.
Grade 3 Symptoms require and respond to aggressive intervention. Oxygen requirement greater than or equal to 40% FiO_2 or hypotension requiring high-dose or multiple vasopressors or Grade 3 organ toxicity or Grade 4 transaminitis.	Per Grade 2	Administer methylprednisolone 1 mg/kg IV twice daily or equivalent dexamethasone (e.g., 10 mg IV every 6 hours). Continue corticosteroids use until the event is Grade 1 or less, then taper over 3 days. If not improving, manage as Grade 4.

Grade 4 Life-threatening symptoms. Requirements for ventilator support, continuous veno-venous hemodialysis (CVVHD) or Grade 4 organ toxicity (excluding transaminitis).	Per Grade 2	Administer methylprednisolone 1000 mg IV per day for 3 days; if improves, then manage as above. Consider alternate immunosuppressants if no improvement or if condition worsens.

(a) Lee DW et al., (2014). Current concepts in the diagnosis and management of cytokine release syndrome. Blood. *2014 Jul 10; 124(2): 188–195.*

(b) Refer to Table 2 for management of neurologic toxicity.

(c) Refer to ACEMTRA® (tocilizumab) Prescribing Information for details, https://www.gene.com/download/pdf/actemra_prescribing.pdf (last accessed Oct. 18, 2017). *Initial U.S. approval is indicated to be in 2010.*

Neurologic Toxicity

[0134] In some embodiments, the method comprises monitoring patients for signs and symptoms of neurologic toxicities (Table 2). In some embodiments, the method comprises ruling out other causes of neurologic symptoms. Patients who experience ≥ Grade 2 neurologic toxicities should be monitored with continuous cardiac telemetry and pulse oximetry. Provide intensive care supportive therapy for severe or life threatening neurologic toxicities. Consider non-sedating, anti-seizure medicines (e.g., levetiracetam) for seizure prophylaxis for any ≥ Grade 2 neurologic toxicities.

Table 2. Neurologic Toxicity Grading and Management Guidance

Grading Assessment	**Concurrent CRS**	**No concurrent CRS**
Grade 2	Administer tocilizumab per Table 1 for management of Grade 2 CRS. If no improvement within 24 hours after starting tocilizumab, administer	Administer dexamethasone 10 mg IV every 6 hours. Continue dexamethasone use until the event is Grade 1 or less, then taper over 3

Grading Assessment	Concurrent CRS	No concurrent CRS
	dexamethasone 10 mg IV every 6 hours if not already taking other steroids. Continue dexamethasone use until the event is Grade 1 or less, then taper over 3 days.	days.
	Consider non-sedating, anti-seizure medicines (e.g., levetiracetam) for seizure prophylaxis.	
Grade 3	Administer tocilizumab per Table 1 for management of Grade 2 CRS. In addition, administer dexamethasone 10 mg IV with the first dose of tocilizumab and repeat dose every 6 hours. Continue dexamethasone use until the event is Grade 1 or less, then taper over 3 days.	Administer dexamethasone 10 mg IV every 6 hours. Continue dexamethasone use until the event is Grade 1 or less, then taper over 3 days.
	Consider non-sedating, anti-seizure medicines (e.g., levetiracetam) for seizure prophylaxis.	
Grade 4	Administer tocilizumab per Table 1 for management of Grade 2 CRS. Administer methylprednisolone 1000 mg IV per day with first dose of tocilizumab and continue methylprednisolone 1000 mg IV per day for 2 more days; if improves, then manage as above.	Administer methylprednisolone 1000 mg IV per day for 3 days; if improves, then manage as above.
	Consider non-sedating, anti-seizure medicines (e.g., levetiracetam) for seizure prophylaxis.	

Dosage Forms And Strengths

[0135] In some embodiments, CD19-directed genetically modified autologous T cell immunotherapy is available as a cell suspension for infusion.

[0136] In some embodiments, a single dose of CD19-directed genetically modified autologous T cell immunotherapy comprises a target dose between about 1×10^6 and about 2×10^6 CAR-positive viable T cells per kg of body weight (or maximum of 2×10^8 CAR-positive viable T cells for patients 100 kg and above) in approximately 68 mL suspension in an infusion bag. In some embodiments, the CD19-directed genetically modified autologous T cell immunotherapy is axicabtagene ciloleucel (YESCARTA®).

[0137] In some embodiments, a single dose of CD19-directed genetically modified autologous T cell immunotherapy is present in a container. Such container may be sterile. In some embodiments, the container is an infusion bag. In some embodiments, the infusion bag volume is about 100 mL, 150 mL, 200 mL, 250 mL, 300 mL, 500 mL, 750 mL, 1,000 mL, 1,500 mL, 2,000 mL or 3,000 mL.

Risk Evaluation and Mitigation Strategy (REMS)

[0138] Because of the risk of CRS and neurologic toxicities, in some embodiments, CD19-directed genetically modified autologous T cell immunotherapy is available through a restricted program under a Risk Evaluation and Mitigation Strategy (REMS). Typical components of the REMS are:

- Healthcare facilities that dispense and administer CD19-directed genetically modified autologous T cell immunotherapy must be enrolled and comply with the REMS requirements.
- Certified healthcare facilities must have on-site, immediate access to tocilizumab, and ensure that a minimum of two doses of tocilizumab are available for each patient for infusion within 2 hours after CD19-directed genetically modified autologous T cell immunotherapy infusion, if needed for treatment of CRS.
- Certified healthcare facilities must ensure that healthcare providers who prescribe, dispense or administer CD19-directed genetically modified autologous T cell immunotherapy are trained about the management of CRS and neurologic toxicities.

Cytokine Release Syndrome (CRS)

[0139] In some embodiments, the health care facility ensures that two doses of tocilizumab are available prior to infusion of CD19-directed genetically modified autologous T cell immunotherapy. In some embodiments, the health care facility ensures that four doses of tocilizumab are available prior to infusion of CD19-directed genetically modified autologous T cell immunotherapy. In some embodiments, the method comprises monitoring patients at least daily for 7 days at the certified healthcare facility following infusion for signs and symptoms of CRS. In some embodiments, the method comprises monitoring patients at least daily for 7-10 days at the certified healthcare facility following infusion for signs and symptoms of CRS. In some embodiments, the method comprises monitoring patients at least daily for 8 days at the certified healthcare facility following infusion for signs and symptoms of CRS. In some embodiments, the method comprises monitoring patients at least daily for 9 days at the certified healthcare facility following infusion for signs and symptoms of CRS. In some embodiments, the method comprises monitoring patients at least daily for 10 days at the certified healthcare facility following infusion for signs and symptoms of CRS. In some embodiments, the method comprises monitoring patients for signs or symptoms of CRS for 4 weeks after infusion. In some embodiments, the method comprises counseling patients to seek immediate medical attention should signs or symptoms of CRS occur at any time. In some embodiments, the method comprises instituting treatment with supportive care, tocilizumab or tocilizumab and corticosteroids as indicated at the first sign of CRS.

Neurologic Toxicities

[0140] In some embodiments, the method comprises monitoring patients at least daily for 7 days at the certified healthcare facility following infusion for signs and symptoms of neurologic toxicities. In some embodiments, the method comprises monitoring patients at least daily for 7-10 days at the certified healthcare facility following infusion for signs and symptoms of CRS. In some embodiments, the method comprises monitoring patients at least daily for 10 days at the certified healthcare facility following infusion for signs and symptoms of CRS. In some embodiments, the method comprises monitoring patients for signs or symptoms of neurologic toxicities for 4 weeks after infusion and treat promptly.

Hypersensitivity Reactions

[0141] Allergic reactions may occur with the infusion of CD19-directed genetically modified autologous T cell immunotherapy. In some embodiments, serious hypersensitivity

reactions including anaphylaxis, may be due to dimethyl sulfoxide (DMSO) or residual gentamicin in CD19-directed genetically modified autologous T cell immunotherapy.

Viral Reactivation

[0142] In some embodiments, Hepatitis B virus (HBV) reactivation, in some cases resulting in fulminant hepatitis, hepatic failure and death, may occur in patients treated with drugs directed against B cells. In some embodiments, the method comprises performing screening for HBV, HCV, and HIV in accordance with clinical guidelines before collection of cells for manufacturing.

Prolonged Cytopenias

[0143] In some embodiments, patients may exhibit cytopenias for several weeks following lymphodepleting chemotherapy and CD19-directed genetically modified autologous T cell immunotherapy infusion. In some embodiments, the method comprises monitoring blood counts after CD19-directed genetically modified autologous T cell immunotherapy infusion.

Hypogammaglobulinemia

[0144] In some embodiments, B-cell aplasia and hypogammaglobulinemia may occur in patients receiving treatment with CD19-directed genetically modified autologous T cell immunotherapy. In some embodiments, the method comprises monitoring immunoglobulin levels after treatment with CD19-directed genetically modified autologous T cell immunotherapy and managing using infection precautions, antibiotic prophylaxis and immunoglobulin replacement.

[0145] In some embodiments, vaccination with live virus vaccines is not recommended for at least 6 weeks prior to the start of lymphodepleting chemotherapy, during CD19-directed genetically modified autologous T cell immunotherapy treatment, and until immune recovery following treatment with CD19-directed genetically modified autologous T cell immunotherapy.

Secondary Malignancies

[0146] In some embodiments, patients treated with CD19-directed genetically modified autologous T cell immunotherapy may develop secondary malignancies. In some embodiments, the method comprises monitoring life-long for secondary malignancies.

Tumour lysis syndrome (TLS)

[0147] Patients treated with CD19-directed genetically modified autologous T cell immunotherapy may develop TLS, which may be severe. To minimise risk of TLS, in some embodiments, the method comprises evaluating patients for elevated uric acid or high tumour burden and administering allopurinol, or an alternative prophylaxis, prior to axicabtagene ciloleucel infusion. Signs and symptoms of TLS should be monitored and events managed according to standard guidelines.

Effects on Ability to Drive and Use Machines

[0148] Due to the potential for neurologic events, including altered mental status or seizures, patients receiving CD19-directed genetically modified autologous T cell immunotherapy are at risk for altered or decreased consciousness or coordination in the 8 weeks following CD19-directed genetically modified autologous T cell immunotherapy infusion. In some embodiments, the method comprises advising patients to refrain from driving and engaging in hazardous occupations or activities, such as operating heavy or potentially dangerous machinery, during this initial period.

Storage and Handling

[0149] In some embodiments, CD19-directed genetically modified autologous T cell immunotherapy is supplied in an infusion bag containing approximately 68 mL of frozen suspension of genetically modified autologous T cells in 5% DMSO and 2.5% albumin (human). In some embodiments, CD19-directed genetically modified autologous T cell immunotherapy is supplied in an infusion bag containing approximately 68 mL of frozen suspension of genetically modified autologous T cells in 5% DMSO and 2.5% albumin (human) (NDC 71287-119-01). In some embodiments, CD19-directed genetically modified autologous T cell immunotherapy comprises Cryostor CS10. In some embodiments, CD19-directed genetically modified autologous T cell immunotherapy comprises 300 mg sodium per infusion. In some embodiments, CD19-directed genetically modified autologous T cell immunotherapy is supplied in an infusion bag containing approximately 50-100 mL, 50-90 mL, 50-80 mL, 50-70 mL, 60-70 mL, 60-75 mL, or 65-75 mL, of suspension of genetically modified autologous T cells in 5% DMSO and 2.5% albumin (human). In some embodiments, CD19-directed genetically modified autologous T cell immunotherapy is supplied in an infusion bag containing less than 100 mL, less than 90 mL, less than 80 mL, less than 70 mL, less than 70 mL, less than 72 mL, or less than 75 mL, of suspension of

genetically modified autologous T cells in 5% DMSO and 2.5% albumin (human). In some embodiments, CD19-directed genetically modified autologous T cell immunotherapy is supplied in an infusion bag containing greater than 50 mL, greater than 60 mL, greater than 65 mL, greater than 66 mL, greater than 67 mL, or greater than 68 mL, of suspension of genetically modified autologous T cells in 5% DMSO and 2.5% albumin (human). In some embodiments, the suspension is frozen.

[0150] In some embodiments, the CD19-directed genetically modified autologous T cell immunotherapy infusion bag is supplied in ethylene-vinyl acetate cryostorage bag with sealed addition tube and two available spike ports, containing approximately 68 mL of cell dispersion.

[0151] In some embodiments, the CD19-directed genetically modified autologous T cell immunotherapy infusion bag is individually packed in a metal cassette. In some embodiments, the CD19-directed genetically modified autologous T cell immunotherapy infusion bag is individually packed in a metal cassette (NDC 71287-119-02). In some embodiments, the CD19-directed genetically modified autologous T cell immunotherapy infusion bag is stored in the vapor phase of liquid nitrogen. In some embodiments, the CD19-directed genetically modified autologous T cell immunotherapy infusion bag is supplied in a liquid nitrogen dry shipper.

[0152] In some embodiments, the method comprises matching the identity of the patient with the patient identifiers on the cassette and infusion bag upon receipt. In some embodiments, CD19-directed genetically modified autologous T cell immunotherapy is stored frozen in the vapor phase of liquid nitrogen (less than or equal to minus 150°C). In some embodiments, the CD19-directed genetically modified autologous T cell immunotherapy is thaw before using.

EXAMPLES

Example 1: Clinical Studies of Relapsed or Refractory Large B-Cell Lymphoma

[0153] A single-arm, open-label, multicenter trial evaluated the efficacy of a single infusion of Axi-cel™ (YESCARTA®) in adult patients with relapsed or refractory aggressive B-cell non-Hodgkin lymphoma. Eligible patients had refractory disease to the most recent therapy or relapse within 1 year after autologous hematopoietic stem cell transplantation (HSCT). The study excluded patients with prior allogeneic HSCT, any history of central

nervous system lymphoma, ECOG performance status of 2 or greater, absolute lymphocyte count less than 100/μL, creatinine clearance less than 60 mL/min, hepatic transaminases more than 2.5 times the upper limit of normal, cardiac ejection fraction less than 50%, or active serious infection.

[0154] Following lymphodepleting chemotherapy, Axi-cel™ was administered as a single IV infusion at a target dose of 2×10^6 CAR-positive viable T cells/kg (maximum permitted dose: 2×10^8 cells). The lymphodepleting regimen consisted of cyclophosphamide 500 mg/m^2 IV and fludarabine 30 mg/m^2 IV, both given on the fifth, fourth, and third day before Axi-cel™. Bridging chemotherapy between leukapheresis and lymphodepleting chemotherapy was not permitted. All patients were hospitalized for Axi-cel™ infusion and for a minimum of 7 days afterward.

[0155] Of 111 patients who underwent leukapheresis, 101 received Axi-cel™. Of the patients treated, the median age was 58 years (range: 23 to 76), 67% were male, and 89% were white. Most (76%) had DLBCL, 16% had transformed follicular lymphoma, and 8% had primary mediastinal large B-cell lymphoma. The median number of prior therapies was 3 (range: 1 to 10), 77% of the patients had refractory disease to a second or greater line of therapy, and 21% had relapsed within 1 year of autologous HSCT.

[0156] One out of 111 patients did not receive the product due to manufacturing failure. Nine other patients were not treated, primarily due to progressive disease or serious adverse reactions following leukapheresis. The median time from leukapheresis to product delivery was 17 days (range: 14 to 51 days), and the median time from leukapheresis to infusion was 24 days (range: 16 to 73 days). The median dose was 2.0×10^6 CAR-positive viable T cells/kg (range: 1.1 to 2.2×10^6 cells/kg).

[0157] Efficacy was established on the basis of complete remission (CR) rate and duration of response (DOR), as determined by an independent review committee (Table 3 and Table 4). The median time to response was 0.9 months (range: 0.8 to 6.2 months). Response durations were longer in patients who achieved CR, as compared to patients with a best response of partial remission (PR) (Table 4). Of the 52 patients who achieved CR, 14 initially had stable disease (7 patients) or PR (7 patients), with a median time to improvement of 2.1 months (range: 1.6 to 5.3 months).

Table 3. Response Rate

	Recipients of Axi-cel™ (N = 101)
Objective Response Rate[a] (95% CI)	73 (72%) (62, 81)
Complete Remission Rate (95% CI)	52 (51%) (41, 62)
Partial Remission Rate (95% CI)	21 (21%) (13, 30)

CI, confidence interval.

[a]*Per 2007 revised International Working Group criteria, as assessed by the independent review committee.*

Table 4. Duration of Response

	From N of 101
Number of Responders	73
DOR (Months)[a] Median[b] (95% CI) Range[c]	9.2 (5.4, NE) 0.03+, 14.4+
DOR if Best Response is CR (Months) Median[b] (95% CI) Range	NE (8.1, NE) 0.4, 14.4+
DOR if Best Response is PR (Months) Median[b] (95% CI) Range	2.1 (1.3, 5.3) 0.03+, 8.4+
Median Follow-up for DOR (Months)[a, b]	7.9

CR, complete remission; DOR, duration of response; NE, not estimable; PR, partial remission.

[a]*Among all responders. DOR is measured from the date of first objective response to the date of progression or death from relapse or toxicity.*

[b]*Kaplan-Meier estimate.*

[c]*A + sign indicates a censored value.*

Example 2: Pharmacodynamics and Pharmacokinetics After Axi-cel™ infusion

[0158] After Axi-cel™ infusion, pharmacodynamic responses were evaluated over a 4-week interval by measuring transient elevation of cytokines, chemokines and other molecules in blood. Levels of cytokines and chemokines such as IL-6, IL-8, IL-10, IL-15, TNF-α, IFN-γ, and sIL2Rα were analyzed. Peak elevation was observed within the first 14 days after infusion, and levels generally returned to baseline within 28 days. Due to the on-target effect of Axi-cel™, a period of B-cell aplasia is expected.

[0159] Following infusion of Axi-cel™, anti-CD19 CAR T cells exhibited an initial rapid expansion followed by a decline to near baseline levels by 3 months. Peak levels of anti-CD19 CAR T cells occurred within the first 7-14 days after Axi-cel™ infusion. Age (range: 23 – 76 years) and gender had no significant impact on $AUC_{(0\text{-}28d)}$ and Cmax of Axi-cel™.

[0160] The number of anti-CD19 CAR T cells in blood was positively associated with objective response (complete remission (CR) or partial remission (PR)). The median anti-CD19 CAR T-cell Cmax levels in responders (n=73) were 205% higher compared to the corresponding level in nonresponders (n=23) (43.6 cells/μL vs 21.2 cells/μL). Median AUC Day 0-28 in responding patients (n=73) was 251% of the corresponding level in nonresponders (n=23) (557.1 days*cells/μL vs. 222.0 days*cells/μL).

[0161] Some patients required tocilizumab and corticosteroids for management of CRS and neurologic toxicities. Patients treated with tocilizumab (n=44) had 262% and 232% higher anti-CD19 CAR T cells as measured by $AUC_{(0\text{-}28d)}$ and Cmax respectively, as compared to patients who did not receive tocilizumab (n=57). Similarly, patients that received corticosteroids (n=26) had 217% and 155% higher $AUC_{(0\text{-}28d)}$ and Cmax compared to patients who did not receive corticosteroids (n=75).

Example 3: Management of adverse reactions after CD19-directed genetically modified autologous T cell immunotherapy

[0162] Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

[0163] The safety data described in this section reflect exposure to Axi-cel™ in the clinical trial (Study 1) in which 108 patients with relapsed/refractory B-cell NHL received CAR-positive T cells based on a recommended dose which was weight-based. Patients with a history of CNS disorders (such as seizures or cerebrovascular ischemia) or autoimmune disease requiring systemic immunosuppression were ineligible. The median duration of follow up was 8.7 months. The median age of the study population was 58 years (range: 23 to 76 years); 68% were men. The baseline ECOG performance status was 43% with ECOG 0, and 57% with ECOG 1.

[0164] The most common adverse reactions (incidence ≥ 20%) include CRS, fever, hypotension, encephalopathy, tachycardia, fatigue, headache, decreased appetite, chills, diarrhea, febrile neutropenia, infections-pathogen unspecified, nausea, hypoxia, tremor, cough, vomiting, dizziness, constipation, and cardiac arrhythmias. Serious adverse reactions occurred in 52% of patients. The most common serious adverse reactions (> 2%) include encephalopathy, fever, lung infection, febrile neutropenia, cardiac arrhythmia, cardiac failure, urinary tract infection, renal insufficiency, aphasia, cardiac arrest, Clostridium difficile infection, delirium, hypotension, and hypoxia.

[0165] The most common (≥ 10%) Grade 3 or higher reactions include febrile neutropenia, fever, CRS, encephalopathy, infections-pathogen unspecified, hypotension, hypoxia and lung infections.

[0166] Forty-five percent (49/108) of patients received tocilizumab after infusion of Axi-cel™.

[0167] Table 5 summarizes the adverse reactions that occurred in at least 10% of patients treated with Axi-cel™ and Table 6 describes the laboratory abnormalities of Grade 3 or 4 that occurred in at least 10% of patients.

Table 5. Summary of Adverse Reactions Observed in at Least 10% of the Patients Treated with Axi-cel™ in Study 1

Adverse Reaction	Any Grade (%)	Grade 3 or Higher (%)
Cardiac Disorders		
Tachycardia[a]	57	2
Arrhythmia[b]	23	7
Gastrointestinal Disorders		
Diarrhea	38	4
Nausea	34	0
Vomiting	26	1
Constipation	23	0
Abdominal pain[c]	14	1
Dry mouth	11	0
General Disorders And Administration Site Conditions		
Fever	86	16
Fatigue[d]	46	3
Chills	40	0
Edema[e]	19	1
Immune System Disorders		
Cytokine release syndrome	94	13
Hypogammaglobulinemia[f]	15	0
Infections And Infestations		
Infections-pathogen unspecified	26	16
Viral infections	16	4
Bacterial Infections	13	9
Investigations		
Decreased appetite	44	2
Weight decreased	16	0
Dehydration	11	3
Musculoskelatal And Connective Tissue Disorders		
Motor dysfunction[g]	19	1
Pain in extremity[h]	17	2
Back pain	15	1
Muscle pain	14	1
Arthralgia	10	0
Nervous System Disorders		
Encephalopathy[i]	57	29
Headache[j]	45	1
Tremor	31	2
Dizziness[k]	21	1

Adverse Reaction	Any Grade (%)	Grade 3 or Higher (%)
Aphasia[l]	18	6
Psychiatric Disorders		
Delirium[m]	17	6
Respiratory, Thoracic And Mediastinal Disorders		
Hypoxia[n]	32	11
Cough[o]	30	0
Dyspnea[p]	19	3
Pleural effusion	13	2
Renal and Urinary Disorders		
Renal insufficiency	12	5
Vascular Disorders		
Hypotension[q]	57	15
Hypertension	15	6
Thrombosis[r]	10	1

The following events were also counted in the incidence of CRS: tachycardia, arrhythmia, fever, chills, hypoxia, renal insufficiency, and hypotension.

[a]*Tachycardia includes tachycardia, sinus tachycardia.*

[b]*Arrhythmia includes arrhythmia, atrial fibrillation, atrial flutter, atrioventricular block, bundle branch block right, electrocardiogram QT prolonged, extra-systoles, heart rate irregular, supraventricular extra systoles, supraventricular tachycardia, ventricular arrhythmia, ventricular tachycardia.*

[c]*Abdominal pain includes abdominal pain, abdominal pain lower, abdominal pain upper.*

[d]*Fatigue includes fatigue, malaise.*

[e]*Edema includes face edema, generalized edema, local swelling, localized edema, edema, edema genital, edema peripheral, periorbital edema, peripheral swelling, scrotal edema.*

[f]*Hypogammaglobulinemia includes hypogammaglobulinemia, blood immunoglobulin D decreased, blood immunoglobulin G decreased.*

[g]*Motor dysfunction includes muscle spasms, muscular weakness.*

[h]*Pain in extremity includes pain not otherwise specified, pain in extremity.*

[i]*Encephalopathy includes cognitive disorder, confusional state, depressed level of consciousness, disturbance in attention, encephalopathy, hypersomnia, leukoencephalopathy, memory impairment, mental status changes, paranoia, somnolence, stupor.*

[j]*Headache includes headache, head discomfort, sinus headache, procedural headache.*

[k]*Dizziness includes dizziness, presyncope, syncope.*

[l]*Aphasia includes aphasia, dysphasia.*

[m]*Delirium includes agitation, delirium, delusion, disorientation, hallucination, hyperactivity, irritability, restlessness.*

[n]*Hypoxia includes hypoxia, oxygen saturation decreased.*

[o]*Cough includes cough, productive cough, upper-airway cough syndrome.*

[p]*Dyspnea includes acute respiratory failure, dyspnea, orthopnea, respiratory distress.*

[q]*Hypotension includes diastolic hypotension, hypotension, orthostatic hypotension.*

[r]*Thrombosis includes deep vein thrombosis, embolism, embolism venous, pulmonary embolism, splenic infarction, splenic vein thrombosis, subclavian vein thrombosis, thrombosis, thrombosis in device.*

[0168] Other clinically important adverse reactions that occurred in less than 10% of patients treated with Axi-cel™ include the following:

- *Blood and lymphatic system disorders:* Coagulopathy (2%)
- *Cardiac disorders:* Cardiac failure (6%) and cardiac arrest (4%)
- *Immune system disorders*: Hemophagocytic lymphohistiocytosis/macrophage activation syndrome (HLH/MAS) (1%), hypersensitivity (1%)
- *Infections and infestations disorders:* Fungal infections (5%)
- *Nervous system disorders:* Ataxia (6%), seizure (4%), dyscalculia (2%), and myoclonus (2%)
- *Respiratory, thoracic and mediastinal disorders:* Pulmonary edema (9%)
- *Skin and subcutaneous tissue disorders:* Rash (9%)
- *Vascular disorders:* Capillary leak syndrome (3%)

[0169] Laboratory Abnormalities:

Table 6. Grade 3 or 4 Laboratory Abnormalities Occurring in ≥ 10% of Patients in Study 1 Following Treatment with Axi-cel based on CTCAE (N=108)

	Grades 3 or 4 (%)
Lymphopenia	100
Leukopenia	96
Neutropenia	93
Anemia	66
Thrombocytopenia	58
Hypophosphatemia	50
Hyponatremia	19
Uric acid increased	13
Direct Bilirubin increased	13
Hypokalemia	10
Alanine Aminotransferase increased	10

Cytokine Release Syndrome

[0170] CRS, including fatal or life-threatening reactions, occurred following treatment with Axi-cel™. In Study 1, CRS occurred in 94% (101/108) of patients receiving Axi-cel™, including ≥ Grade 3 (Lee grading system[1]) CRS in 13% (14/108) of patients. Among patients who died after receiving Axi-cel™, four had ongoing CRS events at the time of death. The median time to onset was 2 days (range: 1 to 12 days) and the median duration of CRS was 7 days (range: 2 to 58 days). Key manifestations of CRS include fever (78%), hypotension (41%), tachycardia (28%), hypoxia (22%), and chills (20%). Serious events that may be associated with CRS include cardiac arrhythmias (including atrial fibrillation and ventricular tachycardia), cardiac arrest, cardiac failure, renal insufficiency, capillary leak syndrome, hypotension, hypoxia, and hemophagocytic lymphohistiocytosis/macrophage activation syndrome (HLH/MAS).

Neurologic Toxicities

[0171] Neurologic toxicities, that were fatal or life-threatening, occurred following treatment with Axi-cel™. Neurologic toxicities occurred in 87% of patients. Ninety-eight percent of all neurologic toxicities occurred within the first 8 weeks of Axi-cel™ infusion, with a median time to onset of 4 days (range: 1 to 43 days). The median duration of neurologic toxicities was 17 days. Grade 3 or higher neurologic toxicities occurred in 31% of patients.

[0172] The most common neurologic toxicities included encephalopathy (57%), headache (44%), tremor (31%), dizziness (21%), aphasia (18%), delirium (17%), insomnia (9%) and anxiety (9%). Prolonged encephalopathy lasting up to 173 days was noted. Serious events including leukoencephalopathy and seizures occurred with Axi-cel™. Fatal and serious cases of cerebral edema have occurred in patients treated with Axi-cel™.

Serious Infections

[0173] Severe or life-threatening infections occurred in patients after Axi-cel™ infusion. In Study 1, infections (all grades) occurred in 38% of patients. Grade 3 or higher infections occurred in 23% of patients. Grade 3 or higher infections with an unspecified pathogen occurred in 16% of patients, bacterial infections in 9%, and viral infections in 4%. Axi-cel™ should not be administered to patients with clinically significant active systemic infections. Monitor patients for signs and symptoms of infection before and after Axi-cel™

infusion and treat appropriately. Administer prophylactic anti-microbials according to local guidelines.

[0174] Febrile neutropenia was observed in 36% of patients after Axi-cel™ infusion and may be concurrent with CRS. In the event of febrile neutropenia, evaluate for infection and manage with broad spectrum antibiotics, fluids and other supportive care as medically indicated.

Immunogenicity

[0175] Axi-cel™ has the potential to induce anti-product antibodies. The immunogenicity of Axi-cel™ has been evaluated using an enzyme-linked immunosorbent assay (ELISA) for the detection of binding antibodies against FMC63, the originating antibody of the anti-CD19 CAR. Three patients tested positive for pre-dose anti-FMC63 antibodies at baseline and months 1, 3, or 6 in Study 1. There is no evidence that the kinetics of initial expansion and persistence of Axi-cel™, or the safety or effectiveness of Axi-cel™, was altered in these patients.

[0176] In Study 1, Grade 3 or higher cytopenias not resolved by Day 30 following Axi-cel™ infusion occurred in (28%) of patients and included thrombocytopenia (18%), neutropenia (15%), and anemia (3%). Monitor blood counts after Axi-cel™ infusion.

[0177] In Study 1, hypogammaglobulinemia occurred in 15% of patients.

[0178] All publications, patents, patent applications, and references, including prescribing information, that are mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. However, the citation of a reference herein should not be construed as an acknowledgement that such reference is prior art to the present invention. To the extent that any of the definitions or terms provided in the references incorporated by reference differ from the terms and discussion provided herein, the present terms and definitions control.

CLAIMS

1. Use of an axicabtagene ciloleucel suspension in the manufacture of a medicament for treating relapsed or refractory diffuse large B-cell lymphoma (DLBCL) not otherwise specified, primary mediastinal large B-cell lymphoma, high grade B-cell lymphoma, or DLBCL arising from follicular lymphoma after two or more lines of systemic therapy in a patient;

wherein the medicament is to be administered by intravenous infusion at a dose between about 1×10^6 and about 2×10^6 CAR-positive viable T cells per kg body weight up to a maximum dose of about 1×10^8 CAR-positive viable T cells; and

the patient is to be monitored for signs and symptoms of cytokine release syndrome (CRS) at least daily, for a period of only 7-10 days following the administration of the medicament, if CRS Grade 2 is observed, the patient is to be administered tocilizumab 8 mg/kg IV over 1 hour.

2. The use of claim 1, wherein following administering the medicament, if CRS Grade 3 is observed, tocilizumab is to be administered at a dose of 8 mg/kg IV over 1 hour, tocilizumab every 8 hours as needed, and methylprednisolone 1 mg/kg IV is to be administered at least two times or equivalent dexamethasone (10 mg IV every 6 hour) is to be administered.

3. The use of claim 1, wherein following administering the medicament, if CRS Grade 4 is observed, tocilizumab is to be administered at a dose of about 8 mg/kg IV over 1hour, repeating tocilizumab every 8 hours as needed; and about 1,000 mg IV methylprednisolone is to be administered per day for 3 days.

4. The use of any one of claims 1-3, wherein the intravenous infusion time for the axicabtagene ciloleucel suspension infusion is between 15 and 120 minutes.

5. The use of any one of claims 1-4, wherein the axicabtagene ciloleucel suspension infusion volume is between 50 and 100 mL.

6. The use of any one of claims 1-5, wherein the suspension further comprises albumin.

7. The use of any one of claims 1-6, wherein the suspension further comprises DMSO.

8. The use of any one of claims 1-7, further comprising measuring cytokine and chemokine levels.

9. The use of any one of claims 1-8, wherein monitoring for signs and symptoms of CRS comprises or consists of using a CRS grading system and observing CRS Grades.

10. The use of any one of claims 1-9, wherein the CRS is monitored for only 7 days following administration.

11. The use of any one of claims 1-9, wherein the CRS is monitored for a maximum of 10 days following administration.