

CHIMERIC ANTIGEN RECEPTOR THERAPY T CELL EXPANSION KINETICS AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/713,994 filed August 2, 2018; and to U.S. Provisional Patent Application No. 62/756,391 filed November 6, 2018, each of which are incorporated by reference herein in their entirety.

SEQUENCE LISTING

[0002] This application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on July 22, 2019, is named K-1066_P2F_SL.txt and is 8 kilobytes in size.

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] Human T cell therapies rely on enriched or modified human T cells to target and kill cancer cells in a patient. To increase the ability of T cells to target and kill a particular cancer cell, methods have been developed to engineer T cells to express constructs which direct T cells to a particular target cancer cell. 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.

[0005] There is a need to understand how attributes of CAR-positive T cells (e.g., expansion kinetics) correlate with clinical outcomes.

SUMMARY

[0006] In one aspect, the disclosure provides a method of manufacturing an effective dose of engineered T cells comprising: (a) preparing a population of engineered T cells comprising a chimeric antigen receptor (CAR); (b) measuring the T cell expansion capability of the population; and (c) preparing an effective dose of engineered T cells for treating a malignancy in a patient in need thereof based on the T cell expansion capability of the population.

[0007] In some embodiments, the T cell expansion capability is measured during the manufacturing process.

[0008] In some embodiments, the T cell expansion capability is determined by measuring doubling time.

[0009] In some embodiments, the doubling time is between about 1 - 4.7 days, about 1.8 - 4.7 days, about 1 - 1.5 days, or less than about 1.5 days.

[0010] In some embodiments, the doubling time is about 1.3 days, about 1.5 days, or about 1.8 days.

[0011] In another aspect, the disclosure provides a method of manufacturing engineered T cells comprising: (a) expanding the engineered T cells in the presence of IL-2, wherein the engineered T cells comprise a chimeric antigen receptor (CAR); (b) measuring the doubling time of the population during the expansion process; (c) harvesting the engineered T cells after expansion; and (d) preparing an effective dose of engineered T cells based on the doubling time of the engineered T cells.

[0012] In some embodiments, the engineered T cells are expanded for about 2-7 days in the presence of IL-2.

[0013] In some embodiments, the doubling time is measured by determining the number of total viable cells at the start of expansion and at the time of harvesting the engineered T cells.

[0014] In another aspect, the disclosure provides a method of treating a malignancy in a patient comprising: (a) measuring levels of one or more attributes in a population of engineered T cells comprising a chimeric antigen receptor (CAR); (b) determining a patient's response to the treatment with the engineered T cells based on the measured levels of one or more attributes compared to a

reference level; and (c) administering a therapeutically effective dose of the engineered T cells to the patient.

[0015] In some embodiments, the one or more attributes is doubling time or T cell phenotype.

[0016] In some embodiments, the T cell phenotype is determined by percentage of CCR7 and CD45RA double positive cells.

[0017] In some embodiments, the doubling time is between about 1 - 4.7 days, about 1.8 - 4.7 days, about 1 - 1.5 days, or less than about 1.5 days.

[0018] In some embodiments, the chimeric antigen receptor targets a tumor antigen.

[0019] In some embodiments, the chimeric antigen receptor targets a tumor antigen selected from a tumor-associated surface antigen, such as 5T4, alphafetoprotein (AFP), B7-1 (CD80), B7-2 (CD86), BCMA, B-human chorionic gonadotropin, CA-125, carcinoembryonic antigen (CEA), carcinoembryonic antigen (CEA), CD123, CD133, CD138, CD19, CD20, CD22, CD23, CD24, CD25, CD30, CD33, CD34, CD4, CD40, CD44, CD56, CD8, CLL-1, c-Met, CMV-specific antigen, CS-1, CSPG4, CTLA-4, DLL3, disialoganglioside GD2, ductal-epithelial mucine, EBV-specific antigen, EGFR variant III (EGFRvIII), ELF2M, endoglin, ephrin B2, epidermal growth factor receptor (EGFR), epithelial cell adhesion molecule (EpCAM), epithelial tumor antigen, ErbB2 (HER2/neu), fibroblast associated protein (fap), FLT3, folate binding protein, GD2, GD3, glioma-associated antigen, glycosphingolipids, gp36, HBV- specific antigen, HCV-specific antigen, HER1-HER2, HER2-HER3 in combination, HERV-K, high molecular weight-melanoma associated antigen (HMW-MAA), HIV-1 envelope glycoprotein gp41, HPV-specific antigen, human telomerase reverse transcriptase, IGF1 receptor, IGF-II, IL-11Ralpha, IL-13R-a2, Influenza Virus-specific antigen; CD38, insulin growth factor (IGF1)-I, intestinal carboxyl esterase, kappa chain, LAGA-Ia, lambda chain, Lassa Virus-specific antigen, lectin-reactive AFP, lineage-specific or tissue specific antigen such as CD3, MAGE, MAGE-A1, major histocompatibility complex (MHC) molecule, major histocompatibility complex (MHC) molecule presenting a tumor-specific peptide epitope, M-CSF, melanoma-associated antigen, mesothelin, MN-CA IX, MUC-1, mut hsp70-2, mutated p53, mutated ras, neutrophil elastase, NKG2D, Nkp30, NY-ESO-1, p53, PAP, prostase, prostate specific antigen (PSA), prostate-carcinoma tumor antigen-1 (PCTA-1), prostate-specific antigen protein, STEAP1, STEAP2, PSMA, RAGE-1, ROR1, RU1, RU2 (AS), surface adhesion molecule, surviving and telomerase, TAG-72, the extra domain A (EDA) and extra domain B (EDB) of fibronectin and the Al

domain of tenascin-C (TnC A1), thyroglobulin, tumor stromal antigens, vascular endothelial growth factor receptor-2 (VEGFR2), virus-specific surface antigen such as an HIV-specific antigen (such as HIV gp120), as well as any derivate or variant of these surface antigens.

[0020] In some embodiments, the malignancy is a solid tumor, sarcoma, carcinoma, lymphoma, 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), chronic or acute leukemia, acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia (ALL) (including non T-cell ALL), chronic lymphocytic leukemia (CLL), T-cell lymphoma, one or more of B-cell acute lymphoid leukemia ("BALL"), T-cell acute lymphoid leukemia ("TALL"), acute lymphoid leukemia (ALL), chronic myelogenous leukemia (CML), B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma, follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, Marginal zone lymphoma, myelodysplasia and myelodysplastic syndrome, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, a plasma cell proliferative disorder (e.g., asymptomatic myeloma (smoldering multiple myeloma or indolent myeloma)), monoclonal gammopathy of undetermined significance (MGUS), plasmacytomas (e.g., plasma cell dyscrasia, solitary myeloma, solitary plasmacytoma, extramedullary plasmacytoma, and multiple plasmacytoma), systemic amyloid light chain amyloidosis, POEMS syndrome (also known as Crow-Fukase syndrome, Takatsuki disease, and PEP syndrome), or a combination thereof.

[0021] In some embodiments, the therapeutically effective dose is between $75\text{-}200 \times 10^6$ engineered T cells.

[0022] In some embodiments, the response is measured within about 1 month, about 3 months, about 6 months, about 9 months, or about 12 months after administration of the engineered T cells.

[0023] In some embodiments, the one or more attributes is measured prior to administration of the engineered T cells.

[0024] In some embodiments, the engineered T cells are autologous or allogeneic T cells.

[0025] In still another aspect, the disclosure provides a method of manufacturing or determining quality of a population of engineered T cells comprising: (a) preparing a population of

engineered T cells comprising a chimeric antigen receptor (CAR); (b) measuring the levels of one or more attributes of the population; and (c) determining whether the population is suitable for treating malignancy in a patient in need thereof based on the measured levels of one or more attributes compared to a reference level.

[0026] In another aspect, the disclosure provides a method of manufacturing an effective dose of engineered T cells comprising: (a) preparing a population of engineered T cells comprising a chimeric antigen receptor (CAR); (b) measuring the levels of one or more attributes of the population; and (c) preparing an effective dose of engineered T cells for treating malignancy in a patient in need thereof based on the measured levels of one or more attributes compared to a reference level.

[0027] In yet another aspect, the disclosure provides a method of manufacturing an effective dose of engineered T cells comprising: (a) measuring the amount of one or more phenotype markers in a population of cells; and (b) preparing an effective dose of engineered T cells for treating a cancer in a patient in need thereof based on the measured amount of the one or more phenotype markers.

[0028] In some embodiments, one phenotype marker is CCR7 or CD45RA.

[0029] In some embodiments, the population of T cells is obtained from apheresis material.

[0030] In some embodiments, the method further comprises engineering the population of T cells to express a CAR.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] Figure 1A and 1B show that objective response rate (ORR) was associated with a shorter doubling time. Figure 1A shows a bar chart demonstrating ORR associated with shorter doubling time by quartile analysis. Figure 1B shows ORR associated with shorter doubling time using modelling by logistic regression.

[0032] Figures 2A and 2B show ongoing response (≥ 1 year) and doubling time (DT) in culture by quartile analysis (Fig. 2A) and modelling by logistic regression (Fig. 2B).

[0033] Figures 3A and 3B show grade ≥ 3 neurologic events and doubling time (DT) in culture by quartile analysis (Fig. 3A) and modelling by logistic regression (Fig. 3B).

[0034] Figures 4A and 4B show that increased CAR T cell engraftment *in vivo* may be associated with a shorter doubling time (DT) by quartile analysis (Fig. 4A) and simple linear regression (Fig. 4B). Figures 4C and 4D show that AUC₀₋₂₈ may be associated with doubling time by quartile analysis (Fig. 4C) and simple linear regression (Fig. 4D). AUC₀₋₂₈ was lower with longer doubling time (Spearman analysis, $r_s = -0.29$; SLR, $P = 0.06$)

[0035] Figures 5A-5D show that doubling time in culture measured pre-infusion may be associated with the percentage of CCR7⁺ CD45RA⁺ cells (Fig. 5A), CCR7⁺ cells (Fig. 5B), but not CCR7⁺ CD45RA⁻ T cells (Fig. 5C) or CD4:CD8 Ratio (Fig. 5D) in the product by simple linear regression.

DETAILED DESCRIPTION

[0036] The present disclosure is based in part on the surprising discovery that pre-infusion attributes (e.g., T cell fitness) of engineered CAR T cells may be associated with clinical efficacy and toxicity. In some embodiments, T cell fitness of engineered CAR T cells is measured by *in vivo* CAR T cell expansion rate. Additionally, the present disclosure provides pre-treatment characteristics of immune factors measured from the patient that may be associated with clinical efficacy and toxicity.

DEFINITIONS

[0037] In order for the present disclosure 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.

[0038] 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.

[0039] Unless specifically stated or obvious from context, as used herein, the term “or” is understood to be inclusive and covers both “or” and “and”.

[0040] 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).

[0041] 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.

[0042] 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.

[0043] 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.

[0044] 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.

[0045] 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.

[0046] 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., $\pm 10\%$). 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.

[0047] 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.

[0048] 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.

[0049] 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.

[0050] “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. Exemplary routes of administration for the compositions 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.

[0051] The term “antibody” (Ab) includes, without limitation, a glycoprotein immunoglobulin which binds specifically to an antigen. In general, an 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 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.

[0052] 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.

[0053] 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.

[0054] 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.

[0055] 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 blocks 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.

[0056] 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.

[0057] 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.

[0058] 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.

[0059] 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 refractory cancer refers to a cancer that is not amenable to surgical intervention and the cancer is either initially unresponsive to chemo- or radiation therapy or the cancer becomes unresponsive over time.

[0060] 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.

[0061] 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).

[0062] “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 1a (MIP-1 α , MIP-1a), MIP-1 β (MIP-1b), gamma-induced protein 10 (IP-10), and thymus and activation regulated chemokine (TARC or CCL17).

[0063] As used herein, “chimeric receptor” refers to an engineered surface expressed molecule capable of recognizing a particular molecule. Chimeric antigen receptors (CARs) and engineered T cell receptors (TCRs), 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.

[0064] 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. Such terms can be used interchangeably. 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.

[0065] 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-7R α ⁺, but they also express large amounts of CD95, IL-2R β , 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 IFN γ or IL-4, and (iii) effector memory TEM cells, however, do not express L-selectin or CCR7 but produce effector cytokines like IFN γ 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.

[0066] 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.

[0067] 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.

[0068] 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.

[0069] 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 reference in its entirety.

[0070] The term “engineered Autologous Cell Therapy,” or “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 malignancies, 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.

[0071] 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.

[0072] 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.

[0073] 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.

[0074] “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 presenting 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.

[0075] 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.

[0076] 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. In certain embodiments, 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).

[0077] 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, CD33, CD45, 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, CD40, CD40a, CD49a, CD49D, CD49f, CD5, CD64, CD69, CD7, CD80, CD83 ligand, CD84, CD86, CD8alpha, CD8beta, CD9, CD96 (Tactile), CD11a, CD11b, CD11c, CD11d, CDS, CEACAM1, CRT AM, DAP-10, DNAM1 (CD226), Fc gamma receptor, GADS, GITR, HVEM (LIGHTR), IA4, ICAM-1, ICOS, Ig alpha (CD79a), IL2R beta, IL2R gamma, IL7R alpha, integrin, ITGA4, ITGA6, ITGAD, ITGAE, ITGAL, ITGAM, ITGAX, ITGB2, ITGB7, ITGB1, KIRDS2, LAT, LFA-1, LIGHT (tumor necrosis factor superfamily member 14; TNFSF14), LTBR, Ly9 (CD229), lymphocyte function-associated antigen-1 (LFA-1 (CD11a/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.

[0078] 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.

[0079] “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.

[0080] As used herein, the term “polyfunctional T cells” refers to cells co-secreting at least two proteins from a pre-specified panel per cell coupled with the amount of each protein produced (i.e., combination of number of proteins secreted and at what intensity). In some embodiments, a single cell functional profile is determined for each evaluable population of engineered T cells. Profiles

may be categorized into effector (Granzyme B, IFN- γ , MIP-1 α , Perforin, TNF- α , TNF- β), stimulatory (GM-CSF, IL-2, IL-5, IL-7, IL-8, IL-9, IL-12, IL-15, IL-21), regulatory (IL-4, IL-10, IL-13, IL-22, TGF- β 1, sCD137, sCD40L), chemoattractive (CCL-11, IP-10, MIP-1 β , RANTES), and inflammatory (IL-1b, IL-6, IL-17A, IL-17F, MCP-1, MCP-4) groups. In some embodiments, the functional profile of each cell enables the calculation of other metrics, including a breakdown of each sample according to cell polyfunctionality (i.e., what percentage of cells are secreting multiple cytokines versus non-secreting or monofunctional cells), and a breakdown of the sample by functional groups (i.e., which mono- and polyfunctional groups are being secreted by cells in the sample, and their frequency).

[0081] Various aspects of the disclosure are described in further detail in the following subsections.

Pre-treatment Attributes

[0082] Pre-treatment attributes of the engineered cells (T cell attributes) and patient immune factors measured from a patient sample may be used to assess the probability of clinical outcomes including response and toxicity. Attributes associated with clinical outcomes are tumor related parameters (e.g., tumor burden, serum LDH as hypoxic / cell death marker, inflammatory markers associated with tumor burden and myeloid cell activity), T cell attributes (e.g., T cell fitness, functionality especially T1 related IFN γ production, and the total number of CD8 T cells infused) and CAR T cell engraftment measured by peak CAR T cell levels in blood at early time points.

[0083] Information extrapolated from T cell attributes and patient pre-treatment attributes may be used to determine, refine or prepare a therapeutically effective dose suitable for treating a malignancy (e.g., cancer). Furthermore, some T cell attributes and patient pre-treatment attributes may be used to determine whether a patient will develop adverse events after treatment with an engineered chimeric antigen receptor (CAR) immunotherapy (e.g., neurotoxicity (NT), cytokine release syndrome (CRS)). Accordingly, an effective adverse event management strategy may be determined (e.g., administration of tocilizumab, a corticosteroid therapy, or an anti-seizure medicine for toxicity prophylaxis based on the measured levels of the one or more attributes).

[0084] In some embodiments, the pre-treatment attributes are attributes of the engineered T cells comprising one or more chimeric antigen receptors. In some embodiments, the pre-treatment attributes are T cell transduction rate, major T cell phenotype, numbers of CAR T cells and T cell subsets, fitness of CAR T cells, T cell functionality, T cell polyfunctionality, number of differentiated CAR+CD8+ T cells.

[0085] In some embodiments, the pre-treatment attributes are measured from a sample obtained from the patient (e.g., cerebrospinal fluid (CSF), blood, serum, or tissue biopsy). In some embodiments, the one or more pre-treatment attributes is tumor burden, levels of IL-6, or levels of LDH.

T cell Fitness

T cell fitness is the capability of cells to rapidly expand. In the context of engineered T cells, T cell fitness is a measurement of how fast the engineered T cell population expand pre-treatment. As described herein, T cell fitness is an attribute of engineered T cells that associates with clinical outcome. In some embodiments, T cell fitness is measured by doubling time or expansion rate. An exemplary derivation of T cell “fitness” measured as T cell population doubling time (DT) during the manufacturing process is shown below.

$$\text{Doubling Time} = \frac{\ln(2) \times \text{duration}}{\ln\left(\frac{\text{total viable cells at harvest}}{\text{total viable cells at Day 3}}\right)}$$

[0086] Recombinant IL-2 is used to drive polyclonal T cell expansion towards achieving the target dose. The shorter the DT, the higher engineered T cell fitness. Expansion rate may be calculated using the formula below.

$$\text{Expansion rate} = \ln(2) / \text{Doubling Time}$$

In the instances described above, the expansion rate is provided in units of “rate/day” or “/day.”

[0087] In one aspect, the present disclosure provides a method of treating a malignancy in a patient comprising measuring the doubling time (DT) in a population of engineered T cells comprising a chimeric antigen receptor (CAR). In some embodiments, the method further comprises determining whether the patient will respond to chimeric antigen receptor treatment based on the measured doubling time compared to a reference level. In some embodiments, the doubling time is measured during the manufacturing process. In some embodiments, the reference level of doubling time is 1.5 days. In some embodiments, the reference level of doubling time is 2 days. In some embodiments, the reference level of doubling time is 2.5 days. In some embodiments, the reference level of doubling time is about 1 day, about 1.1 days, about 1.2 days, about 1.3 days, about 1.4 days, about 1.5 days, about 1.6 days, about 1.7 days, about 1.8 days, about 1.9 days, about 2 days, about 2.1 days, about

2.2 days, about 2.3 days, about 2.4 days, about 2.5 days, about 2.6 days, about 2.7 days, about 2.8 days, about 2.9 days, about 3 days, about 3.1 days, about 3.2 days, about 3.3 days, about 3.4 days, about 3.5 days, about 3.6 days, about 3.7 days, about 3.8 days, about 3.9 days, about 4 days, about 4.1 days, about 4.2 days, about 4.3 days, about 4.4 days, about 4.5 days, about 4.6 days, about 4.7 days, about 4.8 days, about 4.9 days, about 5 days, about 6 days, or about 7 days.

[0088] In some embodiments, the reference level of doubling time is less than about 1 day, about 1.1 days, about 1.2 days, about 1.3 days, about 1.4 days, about 1.5 days, about 1.6 days, about 1.7 days, about 1.8 days, about 1.9 days, about 2 days, about 2.1 days, about 2.2 days, about 2.3 days, about 2.4 days, about 2.5 days, about 2.6 days, about 2.7 days, about 2.8 days, about 2.9 days, about 3 days, about 3.1 days, about 3.2 days, about 3.3 days, about 3.4 days, about 3.5 days, about 3.6 days, about 3.7 days, about 3.8 days, about 3.9 days, about 4 days, about 4.1 days, about 4.2 days, about 4.3 days, about 4.4 days, about 4.5 days, about 4.6 days, about 4.7 days, about 4.8 days, about 4.9 days, about 5 days, about 6 days, or about 7 days.

[0089] In some embodiments, the reference level of doubling time is greater than about 1 day, about 1.1 days, about 1.2 days, about 1.3 days, about 1.4 days, about 1.5 days, about 1.6 days, about 1.7 days, about 1.8 days, about 1.9 days, about 2 days, about 2.1 days, about 2.2 days, about 2.3 days, about 2.4 days, about 2.5 days, about 2.6 days, about 2.7 days, about 2.8 days, about 2.9 days, about 3 days, about 3.1 days, about 3.2 days, about 3.3 days, about 3.4 days, about 3.5 days, about 3.6 days, about 3.7 days, about 3.8 days, about 3.9 days, about 4 days, about 4.1 days, about 4.2 days, about 4.3 days, about 4.4 days, about 4.5 days, about 4.6 days, about 4.7 days, about 4.8 days, about 4.9 days, about 5 days, about 6 days, or about 7 days.

[0090] In some embodiments, the engineered T cells with a doubling time (DT) greater than about 1.5 days, about 1.6 days, about 1.7 days, about 1.8 days, about 1.9 days, or about 2 days, result in primary treatment failure. In some embodiments, engineered CAR T cells with a doubling time (DT) less than about 1.2 days, 1.3 days, 1.4 days, 1.5 days, about 1.6 days, about 1.7 days, about 1.8 days, about 1.9 days, or about 2 days, result in objective response in patients with high tumor burden.

[0091] In another aspect, the present disclosure provides a method of treating a malignancy in a patient comprising measuring the expansion rate of a population of engineered T cells comprising a chimeric antigen receptor (CAR). In some embodiments, the method further comprises determining whether the patient may respond to chimeric antigen receptor treatment based on the measured

expansion rate compared to a reference level. In some embodiments, the expansion rate is measured during the manufacturing process. In some embodiments, the reference level of expansion rate is 0.4/day, 0.45/day or 0.5/day. In some embodiments, the reference level of expansion rate is 0.3/day, 0.35/day or 0.4/day. In some embodiments, the reference level of expansion rate is 0.28/day. In some embodiments, the reference level of expansion rate is about 0.7/day, about 0.65/day, about 0.6/day, about 0.55/day, about 0.5/day, about 0.45/day, about 0.4/day, about 0.35/day, about 0.3/day, about 0.25/day, about 0.2/day, about 0.15/day, or about 0.1/day.

[0092] In some embodiments, the reference level of expansion rate is less than about 0.7/day, about 0.65/day, about 0.6/day, about 0.55/day, about 0.5/day, about 0.45/day, about 0.4/day, about 0.35/day, about 0.3/day, about 0.25/day, about 0.2/day, about 0.15/day, or about 0.1/day.

[0093] In some embodiments, the reference level of expansion rate is greater than about 0.7/day, about 0.65/day, about 0.6/day, about 0.55/day, about 0.5/day, about 0.45/day, about 0.4/day, about 0.35/day, about 0.3/day, about 0.25/day, about 0.2/day, about 0.15/day, or about 0.1/day.

[0094] In some embodiments, the engineered T cells with an expansion rate less than about 0.45/day, about 0.44/day, about 0.43/day, about 0.42/day, about 0.41/day, about 0.40/day, about 0.39/day, about 0.38/day, about 0.37/day, about 0.36/day, or about 0.35/day result in primary treatment failure. In some embodiments, engineered CAR T cells with an expansion rate greater than about 0.45/day, about 0.44/day, about 0.43/day, about 0.42/day, about 0.41/day, about 0.40/day, about 0.39/day, about 0.38/day, about 0.37/day, about 0.36/day, or about 0.35/day, result in objective response in patients with high tumor burden.

T cell phenotypes

[0095] In one aspect, the present disclosure provides a method of treating a malignancy in a patient comprising measuring the T cell phenotypes in a population of T cells obtained from a patient (e.g., apheresis material). In some embodiments, the method further comprises determining whether the patient will respond to chimeric antigen receptor treatment based on the measured percentage of specific T cell types. In some embodiments, the T cell phenotype is measured prior to engineering the cells to express a chimeric antigen receptor (CAR) (e.g., apheresis material). In some embodiments, the T cell phenotype is measured after engineering the cells to express a chimeric antigen receptor (CAR) (e.g., engineered T cells comprising a CAR).

[0096] As described herein, the T cell phenotypes in manufacturing starting material (apheresis) may be associated with T cell fitness (DT). Total % of Tn-like and Tcm cells (CCR7+ cells) is inversely related to DT. The % of Tem (CCR7- CD45RA-) cells is directly associated with DT. Accordingly, in some embodiments, the pre-treatment attribute is the % of Tn-like and Tcm cells. In some embodiments, the % of Tn-like and Tcm cells is determined by the percentage of CCR7+ cells. In some embodiments, the percentage of CCR7+ cells is measured by flow cytometry.

[0097] In some embodiments, the pre-treatment attribute is the % of Tem (CCR7- CD45RA-) cells. In some embodiments, the % of Tem cells is determined by the percentage of CCR7- CD45RA- cells. In some embodiments, the percentage of CCR7- CD45RA- cells is measured by flow cytometry.

T1 Functionality

[0098] Engineered T cells may be characterized by their immune function characteristics. Methods of the present disclosure provide measuring levels of cytokine production *ex vivo*. In some embodiments, the cytokines are selected from the group consisting of IFN γ , TNF α , IL-12, MIP1 β , MIP1 α , IL-2, IL-4, IL-5, and IL-13. In some embodiments, the T cell functionality is measured by levels of Th1 cytokines.

[0099] In some embodiments, the Th1 cytokines are selected from the group consisting of IFN γ , TNF α , and IL-12. In some embodiments, T cell functionality is measured by levels of IFN γ production. In some embodiments, excess T cell IFN γ (pre-treatment attribute), and post-treatment T1 activity, are attributes that may be used to determine whether a patient will develop adverse events (e.g., neurotoxicity). In some embodiments, IFN γ levels produced by engineered CAR T cells are measured by co-culture prior to administration of engineered CAR T cells.

[0100] In some embodiments, engineered CAR T cells with lower co-culture IFN γ result in positive clinical efficacy outcome and reduced grade 3+ neurotoxicity. In one aspect, the present disclosure provides a method of treating a malignancy in a patient comprising measuring the levels of IFN γ produced by a population of engineered T cells comprising a chimeric antigen receptor (CAR). In some embodiments, the method further comprises determining whether the patient will respond to chimeric antigen receptor treatment based on the measured levels of IFN γ compared to a reference level. In some embodiments, the reference level is less than about 1 ng/ml, about 2 ng/ml, about 3 ng/ml, about 4 ng/ml, about 5 ng/ml, about 6 ng/ml, about 7 ng/ml, or about 8 ng/ml.

[0101] In some embodiments, engineered CAR T cells with excess IFN γ production show rapidly elevating rate of grade 3+ neurotoxicity and diminution of objective response rate. In one aspect, the present disclosure provides a method of treating a malignancy in a patient comprising measuring the levels of IFN γ produced by a population of engineered T cells comprising a chimeric antigen receptor (CAR). In some embodiments, the method further comprises determining whether the patient will develop an adverse event to chimeric antigen receptor treatment based on the measured levels of IFN γ compared to a reference level. In some embodiments, the reference level is greater than about 5 ng/ml, about 6 ng/ml, about 7 ng/ml, or about 8 ng/ml, about 9 ng/ml, about 10 ng/ml, or about 11 ng/ml.

[0102] As described herein, there is a direct association of early elevation of IFN γ in serum after CAR T cell infusion and rate of grade 3+ toxicities. In some embodiments, IFN γ elevation in serum post CAR T cell infusion (day 1/day 0 fold change) is measured. In some embodiments, day 1/day 0 serum IFN γ fold change greater than about 25 results in grade 3+ neurotoxicity. In some embodiments, day 1/day 0 serum IFN γ fold change greater than about 30, about 35, about 40, about 45, or about 50 results in grade 3+ neurotoxicity.

[0103] There is a direct association of early elevation of IFN γ related CXCL10 (IP-10) elevation in serum after CAR T cell infusion and rate of grade 3+ toxicities. In some embodiments, IFN γ related CXCL10 (IP-10) elevation in serum post CAR T cell infusion (day 1/day 0 fold change) is measured. In some embodiments, day 1/day 0 serum IFN γ related CXCL10 (IP-10) fold change greater than about 2.5 results in grade 3+ neurotoxicity. In some embodiments, day 1/day 0 serum IFN γ related CXCL10 (IP-10) fold change greater than about 3.0, about 3.5, about 4.0, about 4.5, or about 5.0 results in grade 3+ neurotoxicity.

Tumor Burden

[0104] Tumor related parameters (e.g., tumor burden, serum LDH as hypoxic / cell death marker, inflammatory markers associated with tumor burden and myeloid cell activity) may be associated with clinical outcomes. In one aspect, the present disclosure provides a method of treating a malignancy in a patient comprising measuring the tumor burden in a patient prior to administration of a chimeric antigen receptor treatment. In some embodiments, the method further comprises determining whether the patient will respond to chimeric antigen receptor treatment based on the levels

of tumor burden compared to a reference level. In some embodiments, the reference level is less than about 1,000 mm², about 2,000 mm², about 3,000 mm², about 4,000 mm².

[0105] As described herein, the higher the tumor burden, the higher the probability of relapse within 1 year post treatment in subjects who achieved an OR, and the higher the probability of grade 3+ neurotoxicity. In some embodiments, tumor burden may be used to assess the probability of relapse in patients who respond, if the pre-treatment tumor burden is greater than about 4,000 mm², about 5,000 mm², about 6,000 mm², about 7,000 mm², or about 8,000 mm².

Measuring Response

[0106] In some embodiments, methods described herein may provide a clinical benefit to a subject. In some embodiments, at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of patients achieve a clinical benefit. Clinical benefit may be objective response or durable clinical response defined as ongoing response at a median follow up time of 15.6 months. In some embodiments, response, levels of CAR T cells in blood, or immune related factors is determined by follow up at about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, or about 7 days after administration of engineered CAR T cells. In some embodiments, response, levels of CAR T cells in blood, or immune related factors is determined by follow up at about 1 week, about 2 weeks, about 3 weeks, or about 4 weeks after administration of engineered CAR T cells. In some embodiments, response, levels of CAR T cells in blood and/or immune related factors are determined by follow up at about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 13 months, about 14 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, or about 24 months after administration of a engineered CAR T cells. In some embodiments, response, levels of CAR T cells in blood and/or immune related factors are determined by follow up at about 1 year, about 1.5 years, about 2 years, about 2.5 years, about 3 years, about 4 years, or about 5 years after administration of engineered CAR T cells.

[0107] In some embodiments, objective response (OR) is determined per the revised IWG Response Criteria for Malignant Lymphoma (Cheson, 2007) and determined by IWG Response

Criteria for Malignant Lymphoma (Cheson et al. *Journal of Clinical Oncology* 32, no. 27 (September 2014) 3059-3067). Duration of Response is assessed. The Progression-Free Survival (PFS) by investigator assessment per Lugano Response Classification Criteria is evaluated.

Chimeric Antigen Receptors

[0108] Chimeric antigen receptors (CARs or CAR-Ts) are genetically engineered receptors. These engineered receptors may be 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. Chimeric antigen receptors may incorporate costimulatory (signaling) domains to increase their potency. See U.S. Patent Nos. 7,741,465, and 6,319,494, as well as Krause *et al.* and Finney *et al.* (supra), Song *et al.*, *Blood* 119:696-706 (2012); Kalos *et al.*, *Sci. Transl. Med.* 3:95 (2011); Porter *et al.*, *N. Engl. J. Med.* 365:725-33 (2011), and Gross *et al.*, *Annu. Rev. Pharmacol. Toxicol.* 56:59-83 (2016).

[0109] In some embodiments, a costimulatory domain which includes a truncated hinge domain ("THD") further comprises some or all of a member of the immunoglobulin family such as IgG1, IgG2, IgG3, IgG4, IgA, IgD, IgE, IgM, or fragment thereof.

[0110] In some embodiments, the THD is derived from a human complete hinge domain ("CHD"). In other embodiments, the THD is derived from a rodent, murine, or primate (*e.g.*, non-human primate) CHD of a costimulatory protein. In some embodiments, the THD is derived from a chimeric CHD of a costimulatory protein.

[0111] The costimulatory domain for the CAR of the disclosure may further comprise a transmembrane domain and/or an intracellular signaling domain. The transmembrane domain may be fused to the extracellular domain of the CAR. The costimulatory domain may similarly be fused to the intracellular domain of the CAR. In some embodiments, the transmembrane domain that naturally is associated with one of the domains in a CAR is used. In some instances, the transmembrane domain is selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex. The transmembrane domain may be derived either from

a natural or from a synthetic source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein. Transmembrane regions of particular use in this disclosure may be derived from (i.e., comprise) 4-1BB/CD137, activating NK cell receptors, an Immunoglobulin protein, B7-H3, BAFFR, BLAME (SLAMF8), BTLA, CD100 (SEMA4D), CD103, CD160 (BY55), CD18, CD19, CD19a, CD2, CD247, CD27, CD276 (B7-H3), CD28, CD29, CD3 delta, CD3 epsilon, CD3 gamma, CD3 zeta, CD30, CD4, CD40, CD49a, CD49D, CD49f, CD69, CD7, CD84, CD8, CD8alpha, CD8beta, CD96 (Tactile), CD11a, CD11b, CD11c, CD11d, CDS, CEACAM1, CRT AM, cytokine receptor, DAP-10, DNAM1 (CD226), Fc gamma receptor, GADS, GITR, HVEM (LIGHTR), IA4, ICAM-1, Ig alpha (CD79a), IL-2R beta, IL-2R gamma, IL-7R alpha, inducible T cell costimulator (ICOS), integrins, ITGA4, ITGA6, ITGAD, ITGAE, ITGAL, ITGAM, ITGAX, ITGB2, ITGB7, ITGB1, KIRDS2, LAT, LFA-1, a ligand that specifically binds with CD83, LIGHT, LTBR, Ly9 (CD229), lymphocyte function-associated antigen-1 (LFA-1; CD11a/CD18), MHC class 1 molecule, NKG2C, NKG2D, NKp30, NKp44, NKp46, NKp80 (KLRF1), OX-40, PAG/Cbp, programmed death-1 (PD-1), PSGL1, SELPLG (CD162), Signaling Lymphocytic Activation Molecules (SLAM proteins), SLAM (SLAMF1; CD150; IPO-3), SLAMF4 (CD244; 2B4), SLAMF6 (NTB-A; Lyl08), SLAMF7, SLP-76, TNF receptor proteins, TNFR2, TNFSF14, a Toll ligand receptor, TRANCE/RANKL, VLA1, or VLA-6, or a fragment, truncation, or a combination thereof.

[0112] Optionally, short linkers may form linkages between any or some of the extracellular, transmembrane, and intracellular domains of the CAR. In some embodiments, the linker may be derived from repeats of glycine-glycine-glycine-glycine-serine (SEQ ID NO: 2) (G4S)_n or GSTSGSGKPGSGEGSTKG (SEQ ID NO: 1). In some embodiments, the linker comprises 3-20 amino acids and an amino acid sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to GSTSGSGKPGSGEGSTKG (SEQ ID NO: 1).

[0113] The linkers described herein, may also be used as a peptide tag. The linker peptide sequence may be of any appropriate length to connect one or more proteins of interest and is preferably designed to be sufficiently flexible so as to allow the proper folding and/or function and/or activity of one or both of the peptides it connects. Thus, the linker peptide may have a length of no more than 10, no more than 11, no more than 12, no more than 13, no more than 14, no more than 15, no more than 16, no more than 17, no more than 18, no more than 19, or no more than 20 amino acids. In some

embodiments, the linker peptide comprises a length of at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20 amino acids. In some embodiments, the linker comprises at least 7 and no more than 20 amino acids, at least 7 and no more than 19 amino acids, at least 7 and no more than 18 amino acids, at least 7 and no more than 17 amino acids, at least 7 and no more than 16 amino acids, at least 7 and no more than 15 amino acids, at least 7 and no more than 14 amino acids, at least 7 and no more than 13 amino acids, at least 7 and no more than 12 amino acids or at least 7 and no more than 11 amino acids. In certain embodiments, the linker comprises 15-17 amino acids, and in particular embodiments, comprises 16 amino acids. In some embodiments, the linker comprises 10-20 amino acids. In some embodiments, the linker comprises 14-19 amino acids. In some embodiments, the linker comprises 15-17 amino acids. In some embodiments, the linker comprises 15-16 amino acids. In some embodiments, the linker comprises 16 amino acids. In some embodiments, the linker comprises 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids.

[0114] In some embodiments, a spacer domain is used. In some embodiments, the spacer domain is derived from CD4, CD8a, CD8b, CD28, CD28T, 4-1BB, or other molecule described herein. In some embodiments, the spacer domains may include a chemically induced dimerizer to control expression upon addition of a small molecule. In some embodiments, a spacer is not used.

[0115] The intracellular (signaling) domain of the engineered T cells of the disclosure may provide signaling to an activating domain, which then activates at least one of the normal effector functions of the immune cell. Effector function of a T cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines.

[0116] In certain embodiments, suitable intracellular signaling domain include (*i.e.*, comprise), but are not limited to 4-1BB/CD137, activating NK cell receptors, an Immunoglobulin protein, B7-H3, BAFFR, BLAME (SLAMF8), BTLA, CD100 (SEMA4D), CD103, CD160 (BY55), CD18, CD19, CD19a, CD2, CD247, CD27, CD276 (B7-H3), CD28, CD29, CD3 delta, CD3 epsilon, CD3 gamma, CD30, CD4, CD40, CD49a, CD49D, CD49f, CD69, CD7, CD84, CD8, CD8alpha, CD8beta, CD96 (Tactile), CD11a, CD11b, CD11c, CD11d, CDS, CEACAM1, CRT AM, cytokine receptor, DAP-10, DNAM1 (CD226), Fc gamma receptor, GADS, GITR, HVEM (LIGHTR), IA4, ICAM-1, Ig alpha (CD79a), IL-2R beta, IL-2R gamma, IL-7R alpha, inducible T cell costimulator (ICOS), integrins, ITGA4, ITGA6, ITGAD, ITGAE, ITGAL, ITGAM, ITGAX, ITGB2, ITGB7, ITGB1, KIRDS2, LAT,

ligand that specifically binds with CD83, LIGHT, LTBR, Ly9 (CD229), Lyl08), lymphocyte function-associated antigen-1 (LFA-1; CD11a/CD18), MHC class 1 molecule, NKG2C, NKG2D, NKp30, NKp44, NKp46, NKp80 (KLRF1), OX-40, PAG/Cbp, programmed death-1 (PD-1), PSGL1, SELPLG (CD162), Signaling Lymphocytic Activation Molecules (SLAM proteins), SLAM (SLAMF1; CD150; IPO-3), SLAMF4 (CD244; 2B4), SLAMF6 (NTB-A, SLAMF7, SLP-76, TNF receptor proteins, TNFR2, TNFSF14, a Toll ligand receptor, TRANCE/RANKL, VLA1, or VLA-6, or a fragment, truncation, or a combination thereof.

Antigen Binding Molecules

[0117] Suitable CARs may bind to an antigen (such as a cell-surface antigen) by incorporating an antigen binding molecule that interacts with that targeted antigen. In some embodiments, the antigen binding molecule is an antibody fragment thereof, e.g., one or more single chain antibody fragment (“scFv”). A scFv is a single chain antibody fragment having the variable regions of the heavy and light chains of an antibody linked together. See U.S. Patent Nos. 7,741,465 and 6,319,494, as well as Eshhar *et al.*, *Cancer Immunol Immunotherapy* (1997) 45: 131-136. A scFv retains the parent antibody’s ability to interact specifically with target antigen. scFv’s are useful in chimeric antigen receptors because they may be engineered to be expressed as part of a single chain along with the other CAR components. *Id.* See also Krause *et al.*, *J. Exp. Med.*, Volume 188, No. 4, 1998 (619–626); Finney *et al.*, *Journal of Immunology*, 1998, 161: 2791–2797. It will be appreciated that the antigen binding molecule is typically contained within the extracellular portion of the CAR such that it is capable of recognizing and binding to the antigen of interest. Bispecific and multispecific CARs are contemplated within the scope of the disclosure, with specificity to more than one target of interest.

[0118] In some embodiments, the polynucleotide encodes a CAR comprising a THD of the present disclosure and an antigen binding molecule that specifically binds to a target antigen. In some embodiments, the target antigen is a tumor antigen. In some embodiments, the antigen is selected from a tumor-associated surface antigen, such as 5T4, alphafetoprotein (AFP), B7-1 (CD80), B7-2 (CD86), BCMA, B-human chorionic gonadotropin, CA-125, carcinoembryonic antigen (CEA), CD123, CD133, CD138, CD19, CD20, CD22, CD23, CD24, CD25, CD30, CD33, CD34, CD4, CD40, CD44, CD56, CD8, CLL-1, c-Met, CMV-specific antigen, CS-1, CSPG4, CTLA-4, DLL3, disialoganglioside GD2, ductal-epithelial mucine, EBV-specific antigen, EGFR variant III (EGFRvIII), ELF2M,

endoglin, ephrin B2, epidermal growth factor receptor (EGFR), epithelial cell adhesion molecule (EpCAM), epithelial tumor antigen, ErbB2 (HER2/neu), fibroblast associated protein (fap), FLT3, folate binding protein, GD2, GD3, glioma-associated antigen, glycosphingolipids, gp36, HBV-specific antigen, HCV-specific antigen, HER1-HER2, HER2-HER3 in combination, HERV-K, high molecular weight-melanoma associated antigen (HMW-MAA), HIV-1 envelope glycoprotein gp41, HPV-specific antigen, human telomerase reverse transcriptase, IGF1 receptor, IGF-II, IL-11Ralpha, IL-13R-a2, Influenza Virus-specific antigen; CD38, insulin growth factor (IGF1)-I, intestinal carboxyl esterase, kappa chain, LAGA-Ia, lambda chain, Lassa Virus-specific antigen, lectin-reactive AFP, lineage-specific or tissue specific antigen such as CD3, MAGE, MAGE-A1, major histocompatibility complex (MHC) molecule, major histocompatibility complex (MHC) molecule presenting a tumor-specific peptide epitope, M-CSF, melanoma-associated antigen, mesothelin, MN-CA IX, MUC-1, mut hsp70-2, mutated p53, mutated ras, neutrophil elastase, NKG2D, Nkp30, NY-ESO-1, p53, PAP, prostase, prostate specific antigen (PSA), prostate-carcinoma tumor antigen-1 (PCTA-1), prostate-specific antigen protein, STEAP1, STEAP2, PSMA, RAGE-1, ROR1, RU1, RU2 (AS), surface adhesion molecule, surviving and telomerase, TAG-72, the extra domain A (EDA) and extra domain B (EDB) of fibronectin and the A1 domain of tenascin-C (TnC A1), thyroglobulin, tumor stromal antigens, vascular endothelial growth factor receptor-2 (VEGFR2), virus-specific surface antigen such as an HIV-specific antigen (such as HIV gpl20), as well as any derivate or variant of these surface antigens.

Engineered T cells and Uses

[0119] 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 semi-automated flow through centrifuge, e.g., the Cobe™ 2991 cell processor, the Baxter CytoMate™, 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.

[0120] 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.

[0121] 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.

[0122] 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 expression of CCR7, CD3, CD28, CD45RO, CD62L, and CD127 and 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.

[0123] 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.

[0124] 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.

[0125] In some embodiments, a composition comprising engineered T cells comprises a pharmaceutically acceptable carrier, diluent, solubilizer, emulsifier, preservative and/or adjuvant. In some embodiments, the composition comprises an excipient.

[0126] 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.

[0127] 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 disclosed herein. In a particular embodiment, the CAR T cells are administered to the patient. In some embodiments, the CAR T cells treat a tumor or a cancer in the patient. In some embodiments the CAR T cells reduce the size of a tumor or a cancer.

[0128] 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.

[0129] In some embodiments, the engineered T cells are administered at a therapeutically effective amount. For example, a therapeutically effective amount of the engineered 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 T cells is about 2×10^6 cells/kg, about 3×10^6 cells/kg, about 4×10^6 cells/kg, about 5×10^6 cells/kg, about 6×10^6 cells/kg, about 7×10^6 cells/kg, about 8×10^6 cells/kg, about 9×10^6 cells/kg, about 1×10^7 cells/kg, about 2×10^7 cells/kg, about 3×10^7 cells/kg, about 4×10^7 cells/kg, about 5×10^7 cells/kg, about 6×10^7 cells/kg, about 7×10^7 cells/kg, about 8×10^7 cells/kg, or about 9×10^7 cells/kg.

[0130] In some embodiments, the therapeutically effective amount of the engineered viable T cells is between about 1×10^6 and about 2×10^6 engineered viable T cells per kg body weight up to a maximum dose of about 1×10^8 engineered viable T cells.

Methods of Treatment

[0131] 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.

[0132] 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 gammopathy 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.

[0133] In some embodiments, the cancer is a myeloma. In some embodiments, the cancer is multiple myeloma. In some embodiments, the cancer is leukemia. In some embodiments, the cancer is acute myeloid leukemia.

[0134] 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/m²/day and 2000 mg/m²/day) and specified doses of fludarabine (between 20 mg/m²/day and 900 mg/m²/day). One such dose regimen involves treating a patient comprising administering daily to the patient about 500 mg/m²/day of cyclophosphamide and about 60 mg/m²/day of fludarabine for three days prior to administration of a therapeutically effective amount of engineered T cells to the patient.

[0135] 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.

[0136] 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 (CYTOXANTM); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphoramide and trimethylol melamine; 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, carabycin, 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, epitio stanol, mepitio stanol, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestabucil; 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; Polysaccharide K (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 (TAXOLTM, 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 TargretinTM (bexarotene), PanretinTM, (alitretinoin); ONTAKTM (denileukin diftitox); esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. In some embodiments, compositions comprising CAR-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.

[0137] 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.

[0138] 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®), pidilizumab (CureTech), and atezolizumab (Roche).

[0139] 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, and CDK inhibitors such as CDK inhibitor (palbociclib).

[0140] In some embodiments, a composition comprising engineered CAR T cells are administered with an anti-inflammatory agent. Anti-inflammatory agents or drugs 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 or propoxyphene 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.

[0141] 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.

Monitoring

[0142] In some embodiments, administration of chimeric receptor T cell immunotherapy occurs at a certified healthcare facility.

[0143] 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.

[0144] In some embodiments, patients are instructed to remain within proximity of the certified healthcare facility for at least 4 weeks following infusion.

Prevention or Management of Severe Adverse Reactions

[0145] In some embodiments, the present disclosure provides methods of preventing the development or reducing the severity of adverse reactions based on the levels of one or more attributes. In this respect, the disclosed method may comprise administering a “prophylactically effective amount” of tocilizumab, a corticosteroid therapy, or an anti-seizure medicine for toxicity prophylaxis. The pharmacologic and/or physiologic effect may be prophylactic, i.e., the effect completely or partially prevents a disease or symptom thereof. A “prophylactically effective amount” may refer to an amount effective, at dosages and for periods of time necessary, to achieve a desired prophylactic result (e.g., prevention of onset of adverse reactions).

[0146] 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.

[0147] 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 (CRS)

[0148] In some embodiments, the method comprises preventing or reducing the severity of CRS in a chimeric receptor treatment. In some embodiments, the engineered CAR T cells are deactivated after administration to the patient.

[0149] 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. Patients who experience $\geq$ 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.

[0150] 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 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 Toxicity (NT)

[0151] In some embodiments, the method comprises monitoring patients for signs and symptoms of neurologic toxicities. In some embodiments, the method comprises ruling out other causes of neurologic symptoms. Patients who experience $\geq$ 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.

[0152] 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 for signs or symptoms of neurologic toxicities for 4 weeks after infusion.

Secondary Malignancies

[0153] In some embodiments, patients treated with CD19-directed genetically modified autologous T cell immunotherapy may develop secondary malignancies. In certain 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.

[0154] All publications, patents, and patent applications 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 disclosure. 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.

[0155] The present disclosure is further illustrated by the following examples, which should not be construed as further limiting. The contents of all references cited throughout this application are expressly incorporated herein by reference.

EXAMPLES

EXAMPLE 1: Pre-infusion T Cell Expansion Kinetics May Be Associated With CAR T Cell Expansion and Clinical Outcomes

[0156] In this study, the objective response rate (ORR) and the CAR T cell levels (peak and area under the curve from days 0–28 [AUC0-28]) were examined for product cell population doubling time (DT), a measure of product T cell expansion kinetics. DT, measured between day 3 and final day of manufacturing, depended on the rates of cell proliferation and death during incubation in recombinant interleukin (IL)-2-supplemented medium. The T cell phenotypes were evaluated by flow cytometry. Associations were evaluated using logistic regression (P values) and pairwise Spearman analysis (r_s values) and visualized using quartile analysis bar charts and logistic regression predicted probability curves.

[0157] Patients treated with axicabtagene ciloleucel, an autologous anti-CD19 chimeric antigen receptor (CAR) T cell therapy exhibited shorter product cell population DT and had higher

ORR ($P = 0.025$). Patients with the lowest product DT quartile ($DT \leq 1.33$ days) had 100% ORR. Patients with the highest product DT quartile ($DT \geq 1.79$ days) had 73% ORR. DT was also correlated or associated with greater CAR T cell expansion post-infusion (peak CAR T cell levels, $r_s = -0.27$; AUC_{0-28d} , $r_s = -0.29$; quartile analysis). Of the seventeen non-responders, twelve exhibited $DT > 1.5$ days. A specific CD4/CD8 T cell ratio of 1:1 was not required for achieving lowest DT and maximal CAR T cell expansion or ORR.

[0158] Pre-infusion product T cell expansion kinetics, as measured by DT during manufacturing in the presence of IL-2-supplemented medium, may be correlated or associated with ORR and *in vivo* CAR T cell expansion in the treated patients. Reduced product DT may limit *in vivo* CAR T cell expansion. Indices related to product DT, a component of T cell fitness, may be suitable to predict clinical performance and the optimization of CAR T cell therapy through optimizing manufacturing and/or utilizing combination approaches.

EXAMPLE 2: Manufacturing of chimeric antigen receptor (CAR) T cell therapy

[0159] Apheresis material was enriched for T cells at the start of the manufacturing process. T cells were activated by stimulation with anti-CD3 monoclonal antibody (OKT3) in the presence of IL-2 for 2 days. Activated T cells were transduced to introduce the CAR gene by retroviral transduction. To achieve the desired dose of CAR-positive cells, the transduced T cells were expanded in the presence of interleukin 2 (IL-2) for 4-6 days. T cell doubling time was measured from day 3 through the end of the manufacturing process, when transduced T cells were grown with medium containing recombinant IL-2. Pre-treatment expansion kinetics of CAR-positive T cells were characterized by doubling time as follows:

$$\text{Doubling Time} = \frac{\ln(2) \times \text{duration}}{\ln\left(\frac{\text{total viable cells at harvest}}{\text{total viable cells at Day 3}}\right)}$$

[0160] Major T cell phenotypes were evaluated by flow cytometry. Tumor immune microenvironment was evaluated pre-treatment, utilizing nanostring and a pre-specified Immunosign21 index. Objective response rate (ORR) (CR + PR) was evaluated using International Working Group Response Criteria for Malignant Lymphoma (Cheson BD, et al. *J Clin Oncol.* 2007;25:579-586. Neelapu SS, Locke FL, et al. *N Engl J Med.* 2017;377:2531-2544). Blood CAR T cell levels (peak and area under the curve from days 0–28 [AUC_{0-28}]) were measured in blood using

polymerase chain reaction as described (Neelapu SS, Locke FL, et al. *N Engl J Med*. 2017;377:2531-2544. Neelapu SS, Locke FL, et al. ASH 2017. Abstract #578).

[0161] Associations were evaluated using pairwise Spearman analysis (r_s values) and logistic regression with nominal P values < 0.05 (not adjusted for multiplicity), which were considered statistically significant. Associations were visualized using quartile analysis bar charts and logistic regression predicted probability curves. Doubling times of CAR-positive T cells measured pre-treatment with axicabtagene ciloleucel from 91 patients who were treated with axicabtagene ciloleucel were included in the analysis shown in Table 1.

Table 1: Doubling times of CAR-positive T cell products measured pre-treatment and outcome

Variable	Total Evaluable Patients (N = 91)
Median doubling time, days (range)	1.52 (1.04 – 4.67)
Q1, Q3	1.33 – 1.75
Objective Response Rate (ORR), n (%)	76 (83.5)
Complete response (CR)	52 (57.1)
Partial response (PR)	24 (26.4)
Stable disease (SD)	9 (9.9)
Progressive disease (PD)	4 (4.4)
Not Evaluable	2 (2.2)

[0162] Patients treated with CAR-positive T cell products having reduced doubling time had increased ORR ($P = 0.025$; Figures 1A and Figure 1B). Patients in the doubling time ≤ 1.33 days (Q1) had 100% ORR, and patients in the doubling time ≥ 1.79 days (Q3) had $< 75\%$ ORR (Figure 1A). 71% (12/17) of patients who did not respond to the treatment had a product doubling time more than 1.5 days. Figures 2A and Figure 2B show ongoing response (≥ 1 year) and doubling time in culture during the manufacturing process by quartile analysis (Figure 2A) and modeling by logistic regression (Figure 2B). Figures 3A and 3B show Grade ≥ 3 Neurologic Events, % and doubling time in culture by quartile analysis (Figure 3A) and modelling by logistic regression (Figure 3B).

[0163] Statistical comparison between clinical outcome groups based on doubling time (DT) in culture measured pre-treatment is shown in Table 2.

Table 2: Statistical comparison between clinical outcome groups based on doubling time (DT)

Outcome	Comparison	Odds Ratio (95% CI)	P Value
Neurologic events	Grade ≥ 3 vs grade ≤ 2	0.845 (0.405, 1.761)	0.653
	Grade ≥ 2 vs grade ≤ 1	0.762 (0.390, 1.490)	0.427
CRS	Grade ≥ 3 vs grade ≤ 2	1.426 (0.667, 3.050)	0.360
	Grade ≥ 2 vs grade ≤ 1	0.880 (0.476, 1.630)	0.685
Best response	Responder vs nonresponder	0.448 (0.222, 0.903)	0.025
Ongoing response	Relapsed vs ongoing response	1.447 (0.504, 4.152)	0.492
	Relapsed/nonresponder vs ongoing response	3.729 (1.266, 10.988)	0.017

[0164] Results suggested that increased CAR T cell engraftment *in vivo* may be associated with reduced doubling time by quartile analysis (Figure 4A) and simple linear regression (Figure 4B). Figures 4C and 4D show AUC₀₋₂₈ associated with doubling time by quartile analysis (Figure 4C) and simple linear regression (4D). AUC₀₋₂₈ was reduced with increased doubling time (Spearman analysis, $r_s = -0.29$; SLR, $P = 0.06$). Results suggested that doubling time in culture measured pre-infusion may be associated with the percentage of CCR7⁺ CD45RA⁺ and CCR7⁺, as shown in Figures 5A and 5B, respectively. Figures 5C and 5D show analysis of doubling time and percentage of CCR7⁺ CD45RA⁺ T cells (Figure 5C) or CD4:CD8 Ratio (Figure 5D) in CAR-positive T cell product by simple linear regression.

[0165] Taken together, the study showed that intrinsic T cell fitness and measured pre-treatment may be associated with *in vivo* expansion of CAR T cell products and clinical outcome. The rate of product T cell expansion, quantified as cell population doubling time under polyclonal stimulation during the manufacturing process, may be associated with CAR T cell expansion measured post-treatment. The study also showed that product T cell doubling time (DT) and measured pre-treatment may be associated with clinical objective response. Treatment failures may be associated with products with a doubling time of > 1.5 days measured pre-treatment. Product T cell expansion rate, measured pre-treatment, may be associated with percent of CCR7 CD45RA double-positive cells in product cells. Increased levels of CCR7⁺ T cells and CCR7⁺ CD45RA⁺ naïve T cells in product

may be associated with increased expansion rate and reduced doubling time in culture. Indices related to product T cell fitness, comprising the kinetics of T cell expansion, may be useful in characterizing CAR T cell products and for guiding the optimization of the treatment modality.

CLAIMS

What is claimed:

1. A method of manufacturing an effective dose of engineered T cells comprising:
 - (a) preparing a population of engineered T cells comprising a chimeric antigen receptor (CAR);
 - (b) measuring the T cell expansion capability of the population; and
 - (c) preparing an effective dose of engineered T cells for treating a malignancy in a patient in need thereof based on the T cell expansion capability of the population.
2. The method of claim 1, wherein the T cell expansion capability is measured during the manufacturing process.
3. The method of claim 1 or 2, wherein the T cell expansion capability is determined by measuring doubling time.
4. The method of claim 3, wherein the doubling time is between about 1 - 4.7 days, about 1.8 - 4.7 days, about 1 - 1.5 days, less than about 1.3 days, or less than about 1.5 days.
5. A method of manufacturing engineered T cells comprising
 - (a) expanding the engineered T cells in the presence of IL-2, wherein the engineered T cells comprise a chimeric antigen receptor (CAR);
 - (b) measuring the doubling time of the population during the expansion process;
 - (c) harvesting the engineered T cells after expansion; and
 - (d) preparing an effective dose of engineered T cells based on the doubling time of the engineered T cells.
6. The method of claim 5, wherein the engineered T cells are expanded for about 2-7 days in the presence of IL-2.
7. The method of claim 6, wherein the doubling time is measured by determining the number of total viable cells at the start of expansion and at the time of harvesting the engineered T cells.
8. A method of treating a malignancy in a patient comprising:
 - (a) measuring levels of one or more attributes in a population of engineered T cells comprising a chimeric antigen receptor (CAR);

- (b) determining a patient's response to treatment with the engineered T cells based on the measured levels of one or more attributes compared to a reference level; and
 - (c) administering a therapeutically effective dose of the engineered T cells to the patient.
9. The method of claim 8, wherein the one or more attributes is doubling time or T cell phenotype.
 10. The method of claim 9, wherein the T cell phenotype is determined by percentage of CCR7 and CD45RA double positive cells.
 11. The method of claim 10, wherein the doubling time is between about 1 - 4.7 days, about 1.8 - 4.7 days, about 1 - 1.5 days, or less than about 1.5 days.
 12. The method of any one of the preceding claims, wherein the chimeric antigen receptor targets a tumor antigen.
 13. The method of any one of the preceding claims, wherein the chimeric antigen receptor targets a tumor antigen selected from a tumor-associated surface antigen, such as 5T4, alphafetoprotein (AFP), B7-1 (CD80), B7-2 (CD86), BCMA, B-human chorionic gonadotropin, CA-125, carcinoembryonic antigen (CEA), CD123, CD133, CD138, CD19, CD20, CD22, CD23, CD24, CD25, CD30, CD33, CD34, CD4, CD40, CD44, CD56, CD8, CLL-1, c-Met, CMV-specific antigen, CS-1, CSPG4, CTLA-4, DLL3, disialoganglioside GD2, ductal-epithelial mucine, EBV-specific antigen, EGFR variant III (EGFRvIII), ELF2M, endoglin, ephrin B2, epidermal growth factor receptor (EGFR), epithelial cell adhesion molecule (EpCAM), epithelial tumor antigen, ErbB2 (HER2/neu), fibroblast associated protein (fap), FLT3, folate binding protein, GD2, GD3, glioma-associated antigen, glycosphingolipids, gp36, HBV-specific antigen, HCV-specific antigen, HER1-HER2, HER2-HER3 in combination, HERV-K, high molecular weight-melanoma associated antigen (HMW-MAA), HIV-1 envelope glycoprotein gp41, HPV-specific antigen, human telomerase reverse transcriptase, IGF1 receptor, IGF-II, IL-11Ralpha, IL-13R-a2, Influenza Virus-specific antigen; CD38, insulin growth factor (IGF1)-I, intestinal carboxyl esterase, kappa chain, LAGA-Ia, lambda chain, Lassa Virus-specific antigen, lectin-reactive AFP, lineage-specific or tissue specific antigen such as CD3, MAGE, MAGE-A1, major histocompatibility complex (MHC) molecule, major histocompatibility complex (MHC) molecule presenting a tumor-specific peptide epitope, M-CSF, melanoma-associated antigen, mesothelin, MN-CA IX, MUC-1, mut hsp70-2, mutated p53, mutated ras, neutrophil elastase, NKG2D, Nkp30, NY-ESO-1, p53, PAP, prostase, prostate specific antigen (PSA), prostate-carcinoma tumor antigen-1 (PCTA-1), prostate-specific antigen protein, STEAP1, STEAP2, PSMA, RAGE-1, ROR1, RU1, RU2 (AS), surface

adhesion molecule, survivin and telomerase, TAG-72, the extra domain A (EDA) and extra domain B (EDB) of fibronectin and the A1 domain of tenascin-C (TnC A1), thyroglobulin, tumor stromal antigens, vascular endothelial growth factor receptor-2 (VEGFR2), virus-specific surface antigen such as an HIV-specific antigen (such as HIV gp120), as well as any derivate or variant of these surface antigens.

14. The method of any one of the preceding claims, wherein the malignancy is a solid tumor, sarcoma, carcinoma, lymphoma, 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), chronic or acute leukemia, acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia (ALL) (including non T cell ALL), chronic lymphocytic leukemia (CLL), T-cell lymphoma, one or more of B-cell acute lymphoid leukemia ("BALL"), T-cell acute lymphoid leukemia ("TALL"), acute lymphoid leukemia (ALL), chronic myelogenous leukemia (CML), B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma, follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, Marginal zone lymphoma, myelodysplasia and myelodysplastic syndrome, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, a plasma cell proliferative disorder (e.g., asymptomatic myeloma (smoldering multiple myeloma or indolent myeloma), monoclonal gammopathy of undetermined significance (MGUS), plasmacytomas (e.g., plasma cell dyscrasia, solitary myeloma, solitary plasmacytoma, extramedullary plasmacytoma, and multiple plasmacytoma), systemic amyloid light chain amyloidosis, POEMS syndrome (also known as Crow-Fukase syndrome, Takatsuki disease, and PEP syndrome), or a combination thereof.
15. The method of any one of the preceding claims wherein the therapeutically effective dose is between $75\text{-}200 \times 10^6$ engineered T cells.
16. A method of manufacturing or determining quality of a population of engineered T cells comprising:
 - (a) preparing a population of engineered T cells comprising a chimeric antigen receptor (CAR);
 - (b) measuring the levels of one or more attributes of the population; and

(c) determining whether the population is suitable for treating malignancy in a patient in need thereof based on the measured levels of one or more attributes compared to a reference level.

17. A method of manufacturing an effective dose of engineered T cells comprising:

- (a) preparing a population of engineered T cells comprising a chimeric antigen receptor (CAR);
- (b) measuring the levels of one or more attributes of the population; and
- (c) preparing an effective dose of engineered T cells for treating malignancy in a patient in need thereof based on the measured levels of one or more attributes compared to a reference level.

18. A method of manufacturing an effective dose of engineered T cells comprising:

- (a) measuring the amount of one or more phenotype markers in a population of cells; and
- (b) preparing an effective dose of engineered T cells for treating a cancer in a patient in need thereof based on the measured amount of the one or more phenotype markers.

19. The method of claim 18, wherein the one or more phenotype markers is CCR7 or CD45RA.

20. The method of any of the preceding claims, further comprising engineering the population of T cells to express a CAR.