

iPSC-DERIVED EFFECTOR CELLS FOR AUTOIMMUNE DISEASE TREATMENT**RELATED APPLICATIONS**

[0001] The application claims priority to U.S. Provisional Application Serial No. 63/581,612, filed September 8, 2023, to U.S. Provisional Application Serial No. 63/557,427, filed February 23, 2024, and to U.S. Provisional Application Serial No. 63/644,427, filed May 8, 2024, the disclosures of which are hereby incorporated by reference in their entireties.

INCORPORATED BY REFERENCE OF SEQUENCE LISTING

[0002] The Sequence Listing titled 184143-654601_SL.xml, which was created on September 5, 2024 and is 20,563 bytes in size, is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0003] The present disclosure is broadly concerned with the field of off-the-shelf immunocellular products. More particularly, the present disclosure is concerned with strategies for developing multifunctional effector cells capable of delivering therapeutically relevant properties *in vivo*. The cell products developed under the present disclosure address critical limitations of patient-sourced cell therapies.

BACKGROUND OF THE INVENTION

[0004] The field of adoptive cell therapy is currently focused on using patient- and donor-sourced cells, which makes it particularly difficult to achieve consistent manufacturing of cancer immunotherapies and to deliver therapies to all patients who may benefit. There is also the need to improve the efficacy and persistence of adoptively transferred lymphocytes to promote favorable patient outcomes. Lymphocytes such as T cells and natural killer (NK) cells are potent anti-tumor effectors that play an important role in innate and adaptive immunity. However, the use of these immune cells for adoptive cell therapies remains challenging and has unmet needs for improvement. Therefore, significant opportunities remain to harness the full potential of T and NK cells, or other lymphocytes in adoptive immunotherapy.

SUMMARY OF THE INVENTION

[0005] There is a need for functionally improved effector cells that address issues ranging from response rate, cell exhaustion, loss of transfused cells (survival and/or persistence), off-target toxicity, off-target effect, to efficacy against various diseases.

[0006] It is an object of embodiments of the present invention to provide methods and compositions for adoptive cell therapy, wherein the adoptive cell therapy includes administering an adoptive cell therapy product generated from derivative non-pluripotent cells differentiated from a single cell derived iPSC (induced pluripotent stem cell) clonal line, which iPSC line comprises one or several genetic modifications in its genome. Said one or several genetic modifications include, in some embodiments, one or more of DNA insertion, deletion, and substitution, and which modifications are retained and remain functional in subsequently derived cells after differentiation, expansion, passaging and/or transplantation.

[0007] In one aspect, of the present invention provides a method of treating lupus or a symptom thereof in a subject. In some embodiments, the method comprises (a) administering to the subject one or more doses of chemotherapy; and (b) after step (a), administering to the subject one or more doses of an adoptive cell therapy product in a first effective amount; wherein the adoptive cell therapy product comprises an engineered T lineage effector cell comprising (i) expression of a CD19-CAR (chimeric antigen receptor) at a T-cell receptor α constant (TRAC) locus, and (ii) a T cell receptor (TCR) knockout.

[0008] In some embodiments, the method further comprises (c) after step (b), administering to the subject one or more doses of an additional chemotherapy, wherein the additional chemotherapy is the same or different as the chemotherapy of step (a); and (d) after step (c), administering to the subject one or more doses of an adoptive cell therapy product in a second effective amount that is the same or different as the first effective amount.

[0009] In some embodiments, the lupus or the symptom thereof comprises: (i) cutaneous lupus; (ii) lupus nephritis; (iii) neuropsychiatric lupus; or (iv) pericarditis, atherosclerosis, angina, Raynaud's syndrome, renal disease, metabolic syndrome, thyroid disorder, fibromyalgia, respiratory tract infection, skin infection, or urinary system infection.

[00010] In some embodiments, the chemotherapy comprises (a) one or more of cyclophosphamide (CY) or fludarabine (FLU); or (b) bendamustine. In some embodiments, the chemotherapy is administered one or more days prior to administering the adoptive cell therapy product; optionally wherein the chemotherapy is administered on one or more of (i) three days before the adoptive cell therapy product, (ii) four days before the adoptive cell therapy product, or (iii) five days before the adoptive cell therapy product. In some embodiments, the chemotherapy comprises cyclophosphamide administered at a daily dose of about 250 mg/m² to about 600 mg/m² and fludarabine administered at a daily dose of about 20 mg/m² to about 40 mg/m² for 3 consecutive days starting from about 4-6 days prior to day 1 of administering the adoptive cell therapy product. In some embodiments, the chemotherapy comprises bendamustine and is administered at a daily dose of about 30 mg/m² to about 150 mg/m² for 2

consecutive days starting from about 4-6 days prior to day 1 of administering the adoptive cell therapy product.

[00011] In some embodiments, the engineered T lineage effector cell is derived from an engineered induced pluripotent stem cell (iPSC) comprising a TCR knockout, and a polynucleotide encoding the CD19-CAR. In some embodiments, the first effective amount of the adoptive cell therapy product is about 1.8×10^8 cells to about 9×10^9 cells and is optionally escalated. In some embodiments, the first effective amount of the adoptive cell therapy product is about 3.6×10^8 cells, about 1×10^9 cells, about 3×10^9 cells, or about 9×10^9 . In some embodiments, the adoptive cell therapy product is cryopreserved, and then thawed prior to administering. In some embodiments, the adoptive cell therapy product is FT819.

[00012] In some embodiments, the subject has had a lupus diagnosis. In some embodiments, the subject is positive in at least one of (a) an anti-nuclear antibody; (b) an anti-dsDNA antibody; or (c) an anti-Smith antibody. In some embodiments, treating lupus comprises obtaining lupus remission or low activity in a subject. In some embodiments, remission or reduced disease activity is defined by DORIS (Definition of remission in systemic lupus erythematosus) complete remission, DORIS clinical remission on treatment, and/or LLDAS (lupus low disease activity state). In some embodiments, treating lupus comprises controlling lupus flare. In some embodiments, the treatment comprises longer disease-free intervals off immunosuppressive medications, decreases autoantibody production in the subject; and/or reconstitutes healthy B cell compartment in the subject as compared to B cells under active lupus. In some embodiments, the subject previously received one or more prior treatments comprising glucocorticoids, CY, mycophenolic acid or its derivatives, belimumab, methotrexate, azathioprine, anifrolumab, rituximab, obinutuzumab, cyclosporin, tacrolimus, or voclosporin. In some embodiments, the subject did not respond to the one or more prior treatments.

[00013] In one aspect, the present invention provides a method of treating lupus or a symptom thereof in a subject. In some embodiments, the method comprises (a) administering to the subject one or more doses of chemotherapy; and (b) after step (a), administering to the subject one or more doses of an adoptive cell therapy product in a first effective amount; wherein the adoptive cell therapy product comprises an engineered T lineage effector cell comprising (i) expression of a CD19-CAR (chimeric antigen receptor) at a T-cell receptor α constant (TRAC) locus, and (ii) a T cell receptor (TCR) knockout; and wherein the subject previously received and failed to respond to one or more prior treatments comprising glucocorticoids, CY, mycophenolic acid or its derivatives, belimumab, methotrexate, azathioprine, anifrolumab, rituximab, obinutuzumab, cyclosporin, tacrolimus, or voclosporin.

[00014] In another aspect, the present invention provides a method of treating lupus or a symptom thereof in a subject, the method comprising administering to the subject one or more doses of an adoptive cell therapy product in a first effective amount; wherein the adoptive cell therapy product comprises an engineered NK lineage effector cell comprising a CD38 knockout and expression of (i) a CD19-CAR (chimeric antigen receptor); (ii) an alloimmune defense receptor (ADR) targeting 4-1BB; (iii) a high-affinity, non-cleavable CD16 (hnCD16); and (iv) an interleukin-15 (IL15)/IL15 receptor fusion protein (IL15RF). In one embodiment, the method does not comprise administration of chemotherapy for lympho-conditioning to the patient.

BRIEF DESCRIPTION OF THE DRAWINGS

[00015] **FIG. 1A and FIG. 1B** show exemplary treatment schemas to evaluate FT819 as an allogeneic cell therapy in combination with a chemotherapy. Abbreviations are as follows: CY (cyclophosphamide), DLT (dose-limiting toxicity), FLU (fludarabine), LTFU (long-term follow-up), PTFU (post-treatment follow-up). According to the illustrated schema, subjects are followed in post-treatment follow-up for up to 2 years after the cycle of FT819 treatment, and followed in long-term follow-up for up to 15 years after the FT819 treatment.

[00016] **FIG. 2** shows an exemplary dose-escalation and dose expansion schema for the use of FT819 in lupus treatment. Illustrated Dose Level 1 (DL1) is set at 3.6×10^8 , and additional dose levels $> DL1$ ($\leq 3 \times$ the prior cleared dose level) may be explored. $DL0 = 1.8 \times 10^8$ cells/dose (to be assessed if DL1 exceeds MTD).

[00017] **FIG. 3** shows CD19+ B cell depletion observed in a 72-hour killing assay against PBMCS (E:T 1:1) derived from a blood sample of a patient with SLE.

[00018] **FIG. 4** shows that FT819 displays better targeting of CD19+ B cells from blood samples of SLE patients in comparison to autologous-manufactured CAR T cells. Curves from left to right in each graph correspond to the legend entries from top to bottom, respectively.

[00019] **FIG. 5** shows FT819 PK (mean $\pm$ SEM) at 90 and 360 million cells in a Phase 1 clinical trial.

[00020] **FIG. 6** shows B-cell depletion in peripheral blood with sustained suppression of B cells during FT819 treatment cycle in patients.

[00021] **FIG. 7** shows B-cell depletion observed following a single dose of FT819 to two patients who are resistant to Cy/Flu conditioning chemotherapy.

[00022] **FIG. 8** shows CD19+ B cell depletion following a single-dose FT819 with bendamustine as an alternative to Cy/Flu conditioning chemotherapy.

[00023] **FIG. 9** shows plasma cell depletion and B-cell reconstitution comprised of naïve B cells in 3 patients in 6-10 weeks following single-dose FT819.

[00024] **FIG. 10** shows that FT819 was detected and persisted in the bone marrow at Day 9, with complete elimination of CD19+ cells in the bone marrow and peripheral blood at the end of treatment cycle with standard conditioning chemotherapy followed by a single dose of FT819 at 90M cells.

[00025] **FIG. 11** shows CD19+ B cell depletion in the peripheral blood, >50% decrease in CD19+ tumor cells in secondary lymphoid tissue following a single dose of FT819, and complete metabolic response by FDG-PET with ~80% reduction of target lesion 1 after re-treatment with a single dose of FT819.

[00026] **FIG. 12** shows sustained depletion of CD19+ B cells in the peripheral blood and complete elimination of CD19+ masses in the liver following a single dose of FT819.

[00027] **FIG. 13** shows depletion of an SLE patient's CD19+ B cells in pre-treatment peripheral blood sample upon exposure to FT819 in a 24-hour co-culture assay for ex vivo translational assessment.

[00028] **FIG. 14** shows rapid, deep, and sustained CD19+ B cell depletion in SLE patient's peripheral blood throughout the treatment cycle with recovery of the T cell compartment post-treatment.

[00029] **FIG. 15** shows that FT522 demonstrates comparable-or-better CAR-mediated CD19+ B cell killing compared to TCR-less, allogeneic-like, primary CAR-T (pCAR-T) cells in a 24hr cytotoxicity assay with multiple E:T ratios and in two different SLE PBMC donors. The lower curve in each graph corresponds to results for FT522.

[00030] **FIG. 16A** illustrates an in vitro allogeneic reaction rechallenge assay in which SLE donor PBMCs are co-cultured with pCAR-T cells, FT596 (ADR-negative) or FT522 (ADR-armed) for 8 days, and redosed with effector cells and re-challenged with SLE donor PBMCs, and allowed to co-culture for a total of 14 days.

[00031] **FIG. 16B** shows T cell activation and allo-response to pCAR-T and FT596 cells, but not FT522 cells using flow cytometry of CD3+ SLE donor PBMC T cells on days 4 and 6 for CD25 and 4-1BB markers.

[00032] **FIG. 16C** shows the total SLE donor PBMC-derived T cells expand in response to an allogeneic challenge on day 6 and CD19+ cell counts prior to re-challenge. **FIG. 16D** shows that pCAR-T and FT596 cells are depleted upon completion of the allo-rechallenge assay, whereas FT522 cells continue to persist and CD19+ cell counts at the end of the assay. Cell counts were normalized to PBMC alone.

[00033] **FIG. 17A** shows that FT522 elicits a stronger anti-tumor effect compared to FT596 CAR iNK cells across tested E:T ratio in an in vitro killing assay. **FIG. 17B** shows that FT522 demonstrates higher PK at day 4 in comparison to FT596 at an equivalent dose and demonstrates higher overall AUC during treatment cycle in comparison to FT596 at single dose or three doses.

[00034] **FIG. 18 A** shows FT596 activity in eliminating peripheral blood B cells in a monotherapy to patients (n=9) in a BCL setting. **FIG. 18B** shows FT522 activity in eliminating peripheral blood B cells in combination regimen with rituximab.

DETAILED DESCRIPTION OF THE INVENTION

[00035] Genomic modification of iPSCs (induced pluripotent stem cells) can include one or more of polynucleotide insertion, deletion and substitution. Exogenous gene expression in genome-engineered iPSCs often encounters problems such as gene silencing or reduced gene expression after prolonged clonal expansion of the original genome-engineered iPSCs, after cell differentiation, and in dedifferentiated cell types from the cells derived from the genome-engineered iPSCs. On the other hand, direct engineering of primary immune cells such as T or NK cells can be challenging, and may present hurdles to the preparation and delivery of engineered immune cells for adoptive cell therapy. In various embodiments, the present invention provides an efficient, reliable, and targeted approach for stably integrating one or more exogenous genes, such as suicide genes or other functional modalities, which provide improved therapeutic properties relating to engraftment, trafficking, homing, migration, cytotoxicity, viability, maintenance, expansion, longevity, self-renewal, persistence, and/or survival, of iPSC derivative cells, including but not limited to HSCs (hematopoietic stem and progenitor cells), T cell progenitor cells, NK cell progenitor cells, T cells, NKT cells, NK cells.

[00036] Definitions

[00037] Unless otherwise defined herein, scientific and technical terms used in connection with the present application shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[00038] It should be understood that this invention is not limited to the particular methodology, protocols, and reagents, etc., described herein and as such may vary. The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is defined solely by the claims.

[00039] As used herein, the articles “a,” “an,” and “the” are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

[00040] The use of the alternative (e.g., “or”) should be understood to mean either one, both, or any combination thereof of the alternatives.

[00041] The term “and/or” should be understood to mean either one, or both of the alternatives.

[00042] As used herein, the term “about” or “approximately” refers to a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that varies by as much as 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% compared to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length. In one embodiment, the term “about” or “approximately” refers a range of quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length $\pm 15\%$, $\pm 10\%$, $\pm 9\%$, $\pm 8\%$, $\pm 7\%$, $\pm 6\%$, $\pm 5\%$, $\pm 4\%$, $\pm 3\%$, $\pm 2\%$, or $\pm 1\%$ of a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

[00043] As used herein, the term “substantially” or “essentially” refers to a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that is about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or higher compared to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length. In one embodiment, the terms “essentially the same” or “substantially the same” refer a range of quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that is about the same as a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

[00044] As used herein, the terms “substantially free of” and “essentially free of” are used interchangeably, and when used to describe a composition, such as a cell population or culture media, refer to a composition that is free of a specified substance or its source thereof, such as, 95% free, 96% free, 97% free, 98% free, 99% free of the specified substance or its source thereof, or is undetectable as measured by conventional means. The term “free of” or “essentially free of” a certain ingredient or substance in a composition also means that no such ingredient or substance is (1) included in the composition at any concentration, or (2) included in the composition at a functionally inert, low concentration. Similar meaning can be applied to the term “absence of,” where referring to the absence of a particular substance or its source thereof of a composition.

[00045] Throughout this specification, unless the context requires otherwise, the words “comprise,” “comprises” and “comprising” will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements. In particular embodiments, the terms “include,” “has,” “contains,” and “comprise” are used synonymously.

[00046] By “consisting of” is meant including, and limited to, whatever follows the phrase “consisting of.” Thus, the phrase “consisting of” indicates that the listed elements are required or mandatory, and that no other elements may be present.

[00047] By “consisting essentially of” is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase “consisting essentially of” indicates that the listed elements are required or mandatory, but that no other elements are optional and may or may not be present depending upon whether or not they affect the activity or action of the listed elements.

[00048] Reference throughout this specification to “one embodiment,” “an embodiment,” “a particular embodiment,” “a related embodiment,” “a certain embodiment,” “an additional embodiment,” or “a further embodiment” or combinations thereof means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, the appearances of the foregoing phrases in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more embodiments.

[00049] The term “*ex vivo*” refers generally to activities that take place outside an organism, such as experimentation or measurements done in or on living tissue in an artificial environment outside the organism, preferably with minimum alteration of the natural conditions. In particular embodiments, “*ex vivo*” procedures involve living cells or tissues taken from an organism and cultured in a laboratory apparatus, usually under sterile conditions, and typically for a few hours or up to about 24 hours, but including up to 48 or 72 hours or longer, depending on the circumstances. In certain embodiments, such tissues or cells can be collected and frozen, and later thawed for *ex vivo* treatment. Tissue culture experiments or procedures lasting longer than a few days using living cells or tissue are typically considered to be “*in vitro*,” though in certain embodiments, this term can be used interchangeably with *ex vivo*.

[00050] The term “*in vivo*” refers generally to activities that take place inside an organism.

[00051] As used herein, the terms “reprogramming” or “dedifferentiation” or “increasing cell potency” or “increasing developmental potency” refer to a method of increasing the potency of a cell or dedifferentiating the cell to a less differentiated state. For example, a cell that has an increased cell potency has more developmental plasticity (i.e., can differentiate into more cell types) compared to the same cell in the non-reprogrammed state. In other words, a reprogrammed cell is one that is in a less differentiated state than the same cell in a non-reprogrammed state.

[00052] As used herein, the term “differentiation” is the process by which an unspecialized (“uncommitted”) or less specialized cell acquires the features of a specialized cell such as, for example, a blood cell or a muscle cell. A differentiated or differentiation- induced cell is one that has taken on a more specialized (“committed”) position within the lineage of a cell. The term “committed”, when applied to the process of differentiation, refers to a cell that has proceeded in the differentiation pathway to a point where, under normal circumstances, it will continue to differentiate into a specific cell type or subset of cell types, and cannot, under normal circumstances, differentiate into a different cell type or revert to a less differentiated cell type. As used herein, the term “pluripotent” refers to the ability of a cell to form all lineages of the body or soma (i.e., the embryo proper). For example, embryonic stem cells are a type of pluripotent stem cells that are able to form cells from each of the three germ layers, the ectoderm, the mesoderm, and the endoderm. Pluripotency is a continuum of developmental potencies ranging from the incompletely or partially pluripotent cell (e.g., an epiblast stem cell or EpiSC), which is unable to give rise to a complete organism to the more primitive, more pluripotent cell, which is able to give rise to a complete organism (e.g., an embryonic stem cell).

[00053] As used herein, the term “induced pluripotent stem cells” or “iPSCs”, refers to stem cells that are produced *in vitro* from differentiated adult, neonatal or fetal cells that have been induced or changed, i.e., reprogrammed into cells capable of differentiating into tissues of all three germ or dermal layers: mesoderm, endoderm, and ectoderm. In some embodiments, the reprogramming process uses reprogramming factors and/or small molecule chemical driven methods. The iPSCs produced do not refer to cells as they are found in nature.

[00054] As used herein, the term “embryonic stem cell” refers to naturally occurring pluripotent stem cells of the inner cell mass of the embryonic blastocyst. Embryonic stem cells are pluripotent and give rise during development to all derivatives of the three primary germ layers: ectoderm, endoderm and mesoderm. They do not contribute to the extra-embryonic membranes or the placenta (i.e., are not totipotent).

[00055] As used herein, the term “multipotent stem cell” refers to a cell that has the developmental potential to differentiate into cells of one or more germ layers (i.e., ectoderm, mesoderm and endoderm), but not all three. Thus, a multipotent cell can also be termed a “partially differentiated cell.” Multipotent cells are known in the art, and examples of multipotent cells include adult stem cells, such as for example, hematopoietic stem cells and neural stem cells. “Multipotent” indicates that a cell may form many types of cells in a given lineage, but not cells of other lineages. For example, a multipotent hematopoietic cell can form the many different types of blood cells (red, white, platelets, etc.), but it cannot form neurons.

Accordingly, the term “multipotency” refers to the state of a cell with a degree of developmental potential that is less than totipotent and pluripotent.

[00056] Pluripotency can be determined, in part, by assessing pluripotency characteristics of the cells. Pluripotency characteristics include, but are not limited to: (i) pluripotent stem cell morphology; (ii) the potential for unlimited self-renewal; (iii) expression of pluripotent stem cell markers including, but not limited to SSEA1 (mouse only), SSEA3/4, SSEA5, TRA1-60/81, TRA1-85, TRA2-54, GCTM-2, TG343, TG30, CD9, CD29, CD133/prominin, CD140a, CD56, CD73, CD90, CD105, OCT4, NANOG, SOX2, CD30 and/or CD50; (iv) ability to differentiate to all three somatic lineages (ectoderm, mesoderm and endoderm); (v) teratoma formation consisting of the three somatic lineages; and (vi) formation of embryoid bodies consisting of cells from the three somatic lineages.

[00057] Two types of pluripotency have previously been described: the “primed” or “metastable” state of pluripotency akin to the epiblast stem cells (EpiSC) of the late blastocyst, and the “naïve” or “ground” state of pluripotency akin to the inner cell mass of the early/preimplantation blastocyst. While both pluripotent states exhibit the characteristics as described above, the naïve or ground state further exhibits: (i) pre-inactivation or reactivation of the X-chromosome in female cells; (ii) improved clonality and survival during single-cell culturing; (iii) global reduction in DNA methylation; (iv) reduction of H3K27me3 repressive chromatin mark deposition on developmental regulatory gene promoters; and (v) reduced expression of differentiation markers relative to primed state pluripotent cells. Standard methodologies of cellular reprogramming in which exogenous pluripotency genes are introduced to a somatic cell, expressed, and then either silenced or removed from the resulting pluripotent cells are generally seen to have characteristics of the primed-state of pluripotency. Under standard pluripotent cell culture conditions such cells remain in the primed state unless the exogenous transgene expression is maintained, wherein characteristics of the ground-state are observed.

[00058] As used herein, the term “pluripotent stem cell morphology” refers to the classical morphological features of an embryonic stem cell. Normal embryonic stem cell morphology is characterized by being round and small in shape, with a high nucleus-to-cytoplasm ratio, the notable presence of nucleoli, and typical inter-cell spacing.

[00059] As used herein, the term “subject” refers to any animal, preferably a human patient, livestock, or other domesticated animal.

[00060] A “pluripotency factor,” or “reprogramming factor,” refers to an agent capable of increasing the developmental potency of a cell, either alone or in combination with other agents. Pluripotency factors include, without limitation, polynucleotides, polypeptides, and small

molecules capable of increasing the developmental potency of a cell. Exemplary pluripotency factors include, for example, transcription factors and small molecule reprogramming agents.

[00061] “Culture” or “cell culture” refers to the maintenance, growth and/or differentiation of cells in an *in vitro* environment. “Cell culture media,” “culture media” (singular “medium” in each case), “supplement” and “media supplement” refer to nutritive compositions that cultivate cell cultures.

[00062] “Cultivate” or “maintain” refers to the sustaining, propagating (growing) and/or differentiating of cells outside of tissue or the body, for example in a sterile plastic (or coated plastic) cell culture dish or flask. “Cultivation” or “maintaining” may utilize a culture medium as a source of nutrients, hormones and/or other factors helpful to propagate and/or sustain the cells.

[00063] As used herein, the term “mesoderm” refers to one of the three germinal layers that appears during early embryogenesis and which gives rise to various specialized cell types including blood cells of the circulatory system, muscles, the heart, the dermis, skeleton, and other supportive and connective tissues.

[00064] As used herein, the term “definitive hemogenic endothelium” (HE) or “pluripotent stem cell-derived definitive hemogenic endothelium” (iHE) refers to a subset of endothelial cells that give rise to hematopoietic stem and progenitor cells in a process called endothelial-to-hematopoietic transition. The development of hematopoietic cells in the embryo proceeds sequentially from lateral plate mesoderm through the hemangioblast to the definitive hemogenic endothelium and hematopoietic progenitors.

[00065] The terms “hematopoietic stem and progenitor cells,” “hematopoietic stem cells,” “hematopoietic progenitor cells,” or “hematopoietic precursor cells” refer to cells which are committed to a hematopoietic lineage but are capable of further hematopoietic differentiation and include, multipotent hematopoietic stem cells (hematoblasts), myeloid progenitors, megakaryocyte progenitors, erythrocyte progenitors, and lymphoid progenitors. Hematopoietic stem and progenitor cells (HSCs) are multipotent stem cells that give rise to all the blood cell types including myeloid (monocytes and macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, dendritic cells), and lymphoid lineages (T cells, B cells, NK cells). The term “definitive hematopoietic stem cell” as used herein, refers to CD34⁺ hematopoietic cells capable of giving rise to both mature myeloid and lymphoid cell types including T lineage cells, NK lineage cells and B lineage cells. Hematopoietic cells also include various subsets of primitive hematopoietic cells that give rise to primitive erythrocytes, megakarocytes and macrophages.

[00066] As used herein, the terms “T lymphocyte” and “T cell” are used interchangeably and refer to a principal type of white blood cell that completes maturation in the thymus and that

has various roles in the immune system, including the identification of specific foreign antigens in the body and the activation and deactivation of other immune cells. A T cell can be any T cell, such as a cultured T cell, e.g., a primary T cell, or a T cell from a cultured T cell line, e.g., Jurkat, SupT1, etc., or a T cell obtained from a mammal. The T cell can be a CD3⁺ cell. The T cell can be any type of T cell and can be of any developmental stage, including but not limited to, CD4⁺/CD8⁺ double positive T cells, CD4⁺ helper T cells (e.g., Th1 and Th2 cells), CD8⁺ T cells (e.g., cytotoxic T cells), peripheral blood mononuclear cells (PBMCs), peripheral blood leukocytes (PBLs), tumor infiltrating lymphocytes (TILs), memory T cells, naïve T cells, regulator T cells, gamma delta T cells ($\gamma\delta$ T cells), and the like. Additional types of helper T cells include cells such as Th3 (Treg), Th17, Th9, or Tfh cells. Additional types of memory T cells include cells such as central memory T cells (Tcm cells), effector memory T cells (Tem cells and TEMRA cells). The term “T cell” can also refer to a genetically engineered T cell, such as a T cell modified to express a T cell receptor (TCR) or a chimeric antigen receptor (CAR). A T cell or T cell-like effector cell can also be differentiated from a stem cell or progenitor cell (“a derived T cell” or “a derived T cell like effector cell”, or collectively, “a derivative T lineage cell”). A derived T cell like effector cell may have a T cell lineage in some respects, but at the same time has one or more functional features that are not present in a primary T cell. In this application, a T cell, a T cell like effector cell, a derived T cell, a derived T cell like effector cell, or a derivative T lineage cell, are collectively termed as “a T lineage cell”. In some embodiments, the derivative T lineage cell is an iPSC-derived T cell obtained by differentiating an iPSC, which cells are also referred to herein as “iT” cells.

[00067] “CD4⁺ T cells” refers to a subset of T cells that express CD4 on their surface and are associated with cell-mediated immune response. They are characterized by the secretion profiles following stimulation, which may include secretion of cytokines such as IFN-gamma, TNF-alpha, IL2, IL4 and IL10. “CD4” molecules are 55-kD glycoproteins originally defined as differentiation antigens on T-lymphocytes, but also found on other cells including monocytes/macrophages. CD4 antigens are members of the immunoglobulin supergene family and are implicated as associative recognition elements in MHC (major histocompatibility complex) class II-restricted immune responses. On T-lymphocytes they define the helper/inducer subset.

[00068] “CD8⁺ T cells” refers to a subset of T cells which express CD8 on their surface, are MHC class I-restricted, and function as cytotoxic T cells. “CD8” molecules are differentiation antigens found on thymocytes and on cytotoxic and suppressor T-lymphocytes. CD8 antigens are members of the immunoglobulin supergene family and are associative recognition elements in major histocompatibility complex class I-restricted interactions.

[00069] As used herein, the term “NK cell” or “Natural Killer cell” refers to a subset of peripheral blood lymphocytes defined by the expression of CD56 or CD16 and the absence of the T cell receptor (CD3). As used herein, the terms “adaptive NK cell” and “memory NK cell” are interchangeable and refer to a subset of NK cells that are phenotypically CD3⁻ and CD56⁺, expressing at least one of NKG2C and CD57, and optionally, CD16, but lack expression of one or more of the following: PLZF, SYK, FcεRγ, and EAT-2. In some embodiments, isolated subpopulations of CD56⁺ NK cells comprise expression of CD16, NKG2C, CD57, NKG2D, NCR ligands, NKp30, NKp40, NKp46, activating and inhibitory KIRs, NKG2A and/or DNAM-1. CD56⁺ can be dim or bright expression. An NK cell, or an NK cell-like effector cell may be differentiated from a stem cell or progenitor cell (“a derived NK cell” or “a derived NK cell like effector cell”, or collectively, “a derivative NK lineage cell”). A derivative NK cell like effector cell may have an NK cell lineage in some respects, but at the same time has one or more functional features that are not present in a primary NK cell. In this application, an NK cell, an NK cell like effector cell, a derived NK cell, a derived NK cell like effector cell, or a derivative NK lineage cell, are collectively termed as “an NK lineage cell”. In some embodiments, the derivative NK lineage cell is an iPSC-derived NK cell obtained by differentiating an iPSC, which cells are also referred to herein as “iNK” cells.

[00070] As used herein, the term “NKT cells” or “natural killer T cells” refers to CD1d-restricted T cells, which express a T cell receptor (TCR). Unlike conventional T cells that detect peptide antigens presented by conventional major histocompatibility (MHC) molecules, NKT cells recognize lipid antigens presented by CD1d, a non-classical MHC molecule. Two types of NKT cells are recognized. Invariant or type I NKT cells express a very limited TCR repertoire - a canonical α-chain (Vα24-Jα18 in humans) associated with a limited spectrum of β chains (Vβ11 in humans). The second population of NKT cells, called non-classical or non-invariant type II NKT cells, display a more heterogeneous TCR αβ usage. Type I NKT cells are considered suitable for immunotherapy. Adaptive or invariant (type I) NKT cells can be identified with the expression of at least one or more of the following markers, TCR Vα24-Jα18, Vβ11, CD1d, CD3, CD4, CD8, αGalCer, CD161 and CD56.

[00071] As used herein, the term “isolated” or the like refers to a cell, or a population of cells, which has been separated from its original environment, i.e., the environment of the isolated cells is substantially free of at least one component as found in the environment in which the “un-isolated” reference cells exist. The term includes a cell that is removed from some or all components as it is found in its natural environment, for example, isolated from a tissue or biopsy sample. The term also includes a cell that is removed from at least one, some or all components as the cell is found in non-naturally occurring environments, for example, isolated

form a cell culture or cell suspension. Therefore, an “isolated cell” is partly or completely separated from at least one component, including other substances, cells or cell populations, as it is found in nature or as it is grown, stored or subsisted in non-naturally occurring environments. Specific examples of isolated cells include partially pure cell compositions, substantially pure cell compositions and cells cultured in a medium that is non-naturally occurring. Isolated cells may be obtained by separating the desired cells, or populations thereof, from other substances or cells in the environment, or by removing one or more other cell populations or subpopulations from the environment.

[00072] As used herein, the term “purify” or the like refers to increasing purity. For example, the purity can be increased to at least 50%, 60%, 70%, 80%, 90%, 95%, 99%, or 100%.

[00073] As used herein, the term “encoding” refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or a mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (i.e., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as “encoding” the protein or other product of that gene or cDNA.

[00074] A “construct” refers to a macromolecule or complex of molecules comprising a polynucleotide to be delivered to a host cell, either *in vitro* or *in vivo*. A “vector,” as used herein refers to any nucleic acid construct capable of directing the delivery or transfer of a foreign genetic material to target cells, where it can be replicated and/or expressed. Thus, the term “vector” comprises the construct to be delivered. A vector can be a linear or a circular molecule. A vector can be integrating or non-integrating. The major types of vectors include, but are not limited to, plasmids, episomal vectors, viral vectors, cosmids, and artificial chromosomes. Viral vectors include, but are not limited to, adenovirus vectors, adeno-associated virus vectors, retrovirus vectors, lentivirus vectors, Sendai virus vectors, and the like.

[00075] By “integration” it is meant that one or more nucleotides of a construct is stably inserted into the cellular genome, i.e., covalently linked to the nucleic acid sequence within the cell's chromosomal DNA. By “targeted integration” it is meant that the nucleotide(s) of a construct is inserted into the cell's chromosomal or mitochondrial DNA at a pre-selected site or “integration site”. The term “integration” as used herein further refers to a process involving

insertion of one or more exogenous sequences or nucleotides of the construct, with or without deletion of an endogenous sequence or nucleotide at the integration site. In the case, where there is a deletion at the insertion site, “integration” may further comprise replacement of the endogenous sequence or a nucleotide that is deleted with the one or more inserted nucleotides.

[00076] As used herein, the term “exogenous” is intended to mean that the referenced molecule or the referenced activity is introduced into, or is non-native to, the host cell. The molecule can be introduced, for example, by introduction of an encoding nucleic acid into the host genetic material such as by integration into a host chromosome or as non-chromosomal genetic material such as a plasmid. Therefore, the term as it is used in reference to expression of an encoding nucleic acid refers to introduction of the encoding nucleic acid in an expressible form into the cell. The term “endogenous” refers to a referenced molecule or activity that is present in the host cell. Similarly, the term when used in reference to expression of an encoding nucleic acid refers to expression of an encoding nucleic acid contained within the cell and not exogenously introduced.

[00077] As used herein, a “gene of interest” or “a polynucleotide sequence of interest” is a DNA sequence that is transcribed into RNA and in some instances translated into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences. A gene or polynucleotide of interest can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and synthetic DNA sequences. For example, a gene of interest may encode an miRNA, an shRNA, a native polypeptide (i.e., a polypeptide found in nature) or fragment thereof; a variant polypeptide (i.e., a mutant of the native polypeptide having less than 100% sequence identity with the native polypeptide) or fragment thereof; an engineered polypeptide or peptide fragment, a therapeutic peptide or polypeptide, an imaging marker, a selectable marker, and the like.

[00078] As used herein, the term “polynucleotide” refers to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides or analogs thereof. The sequence of a polynucleotide is composed of four nucleotide bases: adenine (A); cytosine (C); guanine (G); thymine (T); and uracil (U) for thymine when the polynucleotide is RNA. A polynucleotide can include a gene or gene fragment (for example, a probe, primer, EST or SAGE tag), exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes and primers. Polynucleotide also refers to both double- and single-stranded molecules.

[00079] As used herein, the terms “peptide,” “polypeptide,” and “protein” are used interchangeably and refer to a molecule having amino acid residues covalently linked by peptide

bonds. A polypeptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids of a polypeptide. As used herein, the terms refer to both short chains, which are also commonly 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 polypeptides or proteins. "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 polypeptides, recombinant polypeptides, synthetic polypeptides, or a combination thereof.

[00080] "Operably-linked" or "operatively linked," interchangeable with "operably connected" or "operatively connected," refers to the association of nucleic acid sequences on a single nucleic acid fragment (or amino acids in a polypeptide with multiple domains) so that the function of one is affected by the other. For example, a promoter is operably-linked with a coding sequence or functional RNA when it is capable of affecting the expression of that coding sequence or functional RNA (i.e., the coding sequence or functional RNA is under the transcriptional control of the promoter). Coding sequences can be operably-linked to regulatory sequences in sense or antisense orientation. As a further example, a receptor-binding domain can be operatively connected to an intracellular signaling domain, such that binding of the receptor to a ligand transduces a signal responsive to said binding.

[00081] "Fusion proteins" or "chimeric proteins", as used herein, are proteins created through genetic engineering to join two or more partial or whole polynucleotide coding sequences encoding separate proteins, and the expression of these joined polynucleotides results in a single peptide or multiple polypeptides with functional properties derived from each of the original proteins or fragments thereof. Between two neighboring polypeptides of different sources in the fusion protein, a linker (or spacer) peptide can be added.

[00082] As used herein, the term "genetic imprint" refers to genetic or epigenetic information that contributes to preferential therapeutic attributes in a source cell or an iPSC, and is retainable in the source cell derived iPSCs, and/or the iPSC-derived hematopoietic lineage cells. As used herein, "a source cell" is a non-pluripotent cell that may be used for generating iPSCs through reprogramming, and the source cell derived iPSCs may be further differentiated to specific cell types including any hematopoietic lineage cells. The source cell derived iPSCs, and differentiated cells therefrom are sometimes collectively called "derived" or "derivative" cells depending on the context. For example, derivative effector cells, or derivative NK lineage cells or derivative T lineage cells, as used throughout this application are cells differentiated from an iPSC, as compared to their primary counterpart obtained from natural/native sources

such as peripheral blood, umbilical cord blood, or other donor tissues. As used herein, the genetic imprint(s) conferring a preferential therapeutic attribute is incorporated into the iPSCs either through reprogramming a selected source cell that is donor-, disease-, or treatment response- specific, or through introducing genetically modified modalities to iPSCs using genomic editing. In the aspect of a source cell obtained from a specifically selected donor, disease or treatment context, the genetic imprint contributing to preferential therapeutic attributes may include any context-specific genetic or epigenetic modifications which manifest a retainable phenotype, i.e., a preferential therapeutic attribute, that is passed on to derivative cells of the selected source cell, irrespective of the underlying molecular events being identified or not. Donor-, disease-, or treatment response- specific source cells may comprise genetic imprints that are retainable in iPSCs and derived hematopoietic lineage cells, which genetic imprints include but are not limited to, prearranged monospecific TCR, for example, from a viral specific T cell or invariant natural killer T (iNKT) cell; trackable and desirable genetic polymorphisms, for example, homozygous for a point mutation that encodes for the high-affinity CD16 receptor in selected donors; and predetermined HLA requirements, i.e., selected HLA-matched donor cells exhibiting a haplotype with increased population. As used herein, preferential therapeutic attributes include improved engraftment, trafficking, homing, viability, self-renewal, persistence, immune response regulation and modulation, survival, and cytotoxicity of a derived cell. A preferential therapeutic attribute may also relate to antigen targeting receptor expression; HLA presentation or lack thereof; resistance to tumor microenvironment; induction of bystander immune cells and immune modulations; and/or resistance to treatment such as chemotherapy. When derivative cells having one or more therapeutic attributes are obtained from differentiating an iPSC that has genetic imprint(s) conferring a preferential therapeutic attribute incorporated thereto, such derivative cells are also called “synthetic cells”. For example, synthetic effector cells, or synthetic NK cells or synthetic T cells, as used throughout this application are cells differentiated from a genomically modified iPSC, as compared to their primary counterpart obtained from natural/native sources such as peripheral blood, umbilical cord blood, or other donor tissues. In some embodiments, a synthetic cell possesses one or more non-native cell functions when compared to its closest counterpart primary cell.

[00083] The term “enhanced therapeutic property” as used herein, refers to a therapeutic property of a cell that is enhanced as compared to a typical immune cell of the same general cell type. For example, an NK cell with an “enhanced therapeutic property” will possess an enhanced, improved, and/or augmented therapeutic property as compared to a typical, unmodified, and/or naturally occurring NK cell. Therapeutic properties of an immune cell may include, but are not limited to, cell engraftment, trafficking, homing, viability, self-renewal,

persistence, immune response regulation and modulation, survival, and cytotoxicity. Therapeutic properties of an immune cell are also manifested by antigen targeting receptor expression; HLA presentation or lack thereof; resistance to tumor microenvironment; induction of bystander immune cells and immune modulations; and/or resistance to treatment such as chemotherapy.

[00084] As used herein, the term “engager” refers to a molecule, e.g., a fusion polypeptide, which is capable of forming a link between an immune cell (e.g., a T cell, a NK cell, a NKT cell, a B cell, a macrophage, a neutrophil), and a target cell; and activating the immune cell.

Examples of engagers include, but are not limited to, bi-specific T cell engagers (BiTEs), bi-specific killer cell engagers (BiKEs), tri-specific killer cell engagers (TriKEs), or multi-specific killer cell engagers, or universal engagers compatible with multiple immune cell types.

[00085] As used herein, the term “surface triggering receptor” refers to a receptor capable of triggering or initiating an immune response, e.g., a cytotoxic response. Surface triggering receptors may be engineered, and may be expressed on effector cells, e.g., a T cell, an NK cell, an NKT cell, a B cell, a macrophage, or a neutrophil. In some embodiments, the surface triggering receptor facilitates bi- or multi- specific antibody engagement between the effector cells and a specific target cell (e.g., a B cell) independent of the effector cells’ natural receptors and cell types. Using this approach, one may generate iPSCs comprising a universal surface triggering receptor, and then differentiate such iPSCs into populations of various effector cell types that express the universal surface triggering receptor. By “universal”, it is meant that the surface triggering receptor can be expressed in, and activate, any effector cells irrespective of the cell type, and all effector cells expressing the universal receptor can be coupled or linked to the engagers recognizable by the surface triggering receptor, regardless of the engager’s target binding specificities. In some embodiments, engagers having the same targeting specificity are used to couple with the universal surface triggering receptor. In some embodiments, engagers having different targeting specificity are used to couple with the universal surface triggering receptor. As such, one or multiple effector cell types can be engaged to kill one specific type of cells in some cases, and to kill two or more types of target cells in some other cases. A surface triggering receptor generally comprises a co-stimulatory domain for effector cell activation and an anti-epitope that is specific to the epitope of an engager. A bi-specific engager is specific to the anti-epitope of a surface triggering receptor on one end, and is specific to a target cell antigen on the other end.

[00086] As used herein, the term “safety switch protein” refers to an engineered protein designed to prevent potential toxicity or otherwise adverse effects of a cell therapy. In some instances, the safety switch protein expression is conditionally controlled to address safety concerns for transplanted engineered cells that have permanently incorporated the gene encoding

the safety switch protein into its genome. This conditional regulation could be variable and might include control through a small molecule-mediated post-translational activation and tissue-specific and/or temporal transcriptional regulation. The safety switch protein could mediate induction of apoptosis, inhibition of protein synthesis, DNA replication, growth arrest, transcriptional and post-transcriptional genetic regulation and/or antibody-mediated depletion. In some instance, the safety switch protein is activated by an exogenous molecule, e.g., a prodrug, that when activated, triggers apoptosis and/or cell death of a therapeutic cell. Examples of safety switch proteins include, but are not limited to, suicide genes such as caspase 9 (or caspase 3 or 7), thymidine kinase, cytosine deaminase, B cell CD20, modified EGFR, and any combination thereof. In this strategy, a prodrug that is administered in the event of an adverse event is activated by the suicide-gene product and kills the transduced cell.

[00087] As used herein, the term “pharmaceutically active proteins or peptides” refers to proteins or peptides that are capable of achieving a biological and/or pharmaceutical effect on an organism. A pharmaceutically active protein has healing curative or palliative properties against a disease and may be administered to ameliorate relieve, alleviate, reverse or lessen the severity of a disease. A pharmaceutically active protein also has prophylactic properties and is used to prevent the onset of a disease or to lessen the severity of such disease or pathological condition when it does emerge. Pharmaceutically active proteins include an entire protein or peptide or pharmaceutically active fragments thereof. The term also includes pharmaceutically active analogs of the protein or peptide or analogs of fragments of the protein or peptide. The term pharmaceutically active protein also refers to a plurality of proteins or peptides that act cooperatively or synergistically to provide a therapeutic benefit. Examples of pharmaceutically active proteins or peptides include, but are not limited to, receptors, binding proteins, transcription and translation factors, antibodies or fragments thereof, growth factors, and/or cytokines.

[00088] As used herein, the term “signaling molecule” refers to any molecule that modulates, participates in, inhibits, activates, reduces, or increases, cellular signal transduction. “Signal transduction” refers to the transmission of a molecular signal in the form of chemical modification by recruitment of protein complexes along a pathway that ultimately triggers a biochemical event in the cell. Examples of signal transduction pathways are known in the art, and include, but are not limited to, G protein coupled receptor signaling, tyrosine kinase receptor signaling, integrin signaling, toll gate signaling, ligand-gated ion channel signaling, ERK/MAPK signaling pathway, Wnt signaling pathway, cAMP-dependent pathway, and IP3/DAG signaling pathway.

[00089] As used herein, the term “targeting modality” refers to a molecule, e.g., a polypeptide, that is genetically incorporated into a cell to promote antigen and/or epitope specificity that includes but is not limited to (i) antigen specificity as it relates to a unique chimeric antigen receptor (CAR) or T cell receptor (TCR), (ii) engager specificity as it relates to monoclonal antibodies or bispecific engagers, (iii) targeting of particular cells, and (iv) other targeting strategies in the absence of a specific antigen or surface molecule.

[00090] As used herein, the term “specific” or “specificity” can be used to refer to the ability of a molecule, e.g., a receptor or an engager, to selectively bind to a target molecule, in contrast to non-specific or non-selective binding.

[00091] The term “adoptive cell therapy” as used herein refers to a cell-based immunotherapy that relates to the transfusion of autologous or allogeneic lymphocytes, whether the immune cells are isolated from a human donor, or effector cells obtained from *in vitro* differentiation of a pluripotent cell; whether they are genetically modified or not; or whether they are primary donor cells or cells that have been passaged, expanded, or immortalized, *ex vivo*, after isolation from a donor.

[00092] As used herein, “lymphodepletion” and “lympho-conditioning” are used interchangeably to refer to the destruction of lymphocytes and T cells, typically prior to immunotherapy. The purpose of lympho-conditioning prior to the administration of an adoptive cell therapy is to promote homeostatic proliferation of effector cells as well as to eliminate regulatory immune cells and other competing elements of the immune system that compete for homeostatic cytokines. Thus, lympho-conditioning is typically accomplished by administering one or more chemotherapeutic agents to the subject prior to a first dose of the adoptive cell therapy. In various embodiments, lympho-conditioning precedes the first dose of the adoptive cell therapy by a few hours to a few days. Exemplary chemotherapeutic agents useful for lympho-conditioning include, but are not limited to, cyclophosphamide (CY), fludarabine (FLU), and those described below. However, a sufficient lymphodepletion through anti-CD38 mAb could provide an alternative conditioning process (e.g., for use in a T lineage cell therapy in accordance with various embodiments herein), without or with minimal need of a CY/FLU-based lympho-conditioning procedure, as further described herein.

[00093] As used herein, the term “outpatient” refers to a patient who is not hospitalized overnight, but who visits a hospital, clinic, or associated facility for diagnosis and/or treatment. Thus, an “outpatient setting,” as compared to an “inpatient setting” refers to an environment for providing ambulatory care or outpatient care to a patient where hospitalization for one or more days/nights is not required while receiving treatment and/or diagnosis, thereby reducing overall discomfort to the patient receiving treatment and/or diagnosis, while reducing overall cost for

such treatment and/or diagnosis with relative ease in management and coordination. Additionally, an outpatient setting is more readily accessible to a larger population of patients and increases patient availability and patient compliance with a treatment protocol during a trial or course of treatment.

[00094] As used herein, “induction therapy,” also called “first-line therapy,” “primary therapy,” or “primary treatment,” refers to a first treatment given to a patient for a particular disease. It is often part of a standard set of treatments, such as surgery followed by chemotherapy and radiation. Thus, an “induction attempt” or “attempt of induction therapy” refers to an initial attempt at treating a particular disease using known and/or conventional therapeutic approaches for the particular disease.

[00095] A “therapeutically sufficient amount,” as used herein, includes within its meaning a non-toxic but sufficient and/or effective amount of a particular therapeutic agent and/or pharmaceutical composition to which it is referring to provide a desired therapeutic effect. The exact amount required will vary from subject to subject, depending on factors such as the patient's general health, the patient's age and the stage and severity of the condition being treated. In particular embodiments, a therapeutically sufficient amount is sufficient and/or effective to ameliorate, reduce, and/or improve at least one symptom associated with a disease or condition of the subject being treated. A therapeutically sufficient amount may be an amount delivered in a single dose, or refer to a cumulative amount administered over a plurality of doses that may be separated in time according to a dosing schedule.

[00096] Differentiation of pluripotent stem cells requires a change in the culture system, such as changing the stimuli agents in the culture medium or the physical state of the cells. The most conventional strategy utilizes the formation of embryoid bodies (EBs) as a common and critical intermediate to initiate lineage-specific differentiation. “Embryoid bodies” are three-dimensional clusters that have been shown to mimic embryo development as they give rise to numerous lineages within their three-dimensional area. Through the differentiation process, typically a few hours to days, simple EBs (for example, aggregated pluripotent stem cells elicited to differentiate) continue maturation and develop into a cystic EB at which time, typically days to a few weeks, they are further processed to continue differentiation. EB formation is initiated by bringing pluripotent stem cells into close proximity with one another in three-dimensional multilayered clusters of cells. Typically, this is achieved by one of several methods including allowing pluripotent cells to sediment in liquid droplets, sedimenting cells into “U” bottomed well-plates or by mechanical agitation. To promote EB development, the pluripotent stem cell aggregates require further differentiation cues, as aggregates maintained in pluripotent culture maintenance medium do not form proper EBs. As such, the pluripotent stem

cell aggregates need to be transferred to differentiation medium that provides eliciting cues towards the lineage of choice. EB-based culture of pluripotent stem cells typically results in generation of differentiated cell populations (i.e., ectoderm, mesoderm and endoderm germ layers) with modest proliferation within the EB cell cluster. Although proven to facilitate cell differentiation, EBs, however, give rise to heterogeneous cells in variable differentiation states because of the inconsistent exposure of the cells in the three-dimensional structure to the differentiation cues within the environment. In addition, EBs are laborious to create and maintain. Moreover, cell differentiation through EB formation is accompanied with modest cell expansion, which also contributes to low differentiation efficiency.

[00097] In comparison, “aggregate formation,” as distinct from “EB formation,” can be used to expand the populations of pluripotent stem cell derived cells. For example, during aggregate-based pluripotent stem cell expansion, culture media are selected to maintain proliferation and pluripotency. Cell proliferation generally increases the size of the aggregates, forming larger aggregates, which can be mechanically or enzymatically dissociated into smaller aggregates to maintain cell proliferation within the culture and increase numbers of cells. As distinct from EB culture, cells cultured within aggregates in maintenance culture media maintain markers of pluripotency. The pluripotent stem cell aggregates require further differentiation cues to induce differentiation.

[00098] As used herein, “monolayer differentiation” is a term referring to a differentiation method distinct from differentiation through three-dimensional multilayered clusters of cells, i.e., “EB formation.” Monolayer differentiation, among other advantages disclosed herein, avoids the need for EB formation to initiate differentiation. Because monolayer culturing does not mimic embryo development such as is the case with EB formation, differentiation towards specific lineages is deemed to be minimal as compared to all three germ layer differentiation in EB formation.

[00099] As used herein, a “dissociated cell” or “single dissociated cell” refers to a cell that has been substantially separated or purified away from other cells or from a surface (e.g., a culture plate surface). For example, cells can be dissociated from an animal or tissue by mechanical or enzymatic methods. Alternatively, cells that aggregate *in vitro* can be enzymatically or mechanically dissociated from each other, such as by dissociation into a suspension of clusters, single cells or a mixture of single cells and clusters. In yet another alternative embodiment, adherent cells can be dissociated from a culture plate or other surface. Dissociation thus can involve breaking cell interactions with extracellular matrix (ECM) and substrates (e.g., culture surfaces), or breaking the ECM between cells.

[000100] As used herein, a “master cell bank” or “MCB” refers to a clonal master engineered iPSC line, which is a clonal population of iPSCs that have been engineered to comprise one or more therapeutic attributes, have been characterized, tested, qualified, and expanded, and have been shown to reliably serve as the starting cellular material for the production of cell-based therapeutics through directed differentiation in manufacturing settings. In various embodiments, an MCB is maintained, stored, and/or cryopreserved in multiple vessels to prevent genetic variation and/or potential contamination by reducing and/or eliminating the total number of times the iPS cell line is passaged, thawed or handled during the manufacturing processes.

[000101] “Functional” as used in the context of genomic editing or modification of iPSC, and derived non-pluripotent cells differentiated therefrom, or genomic editing or modification of non-pluripotent cells and derived iPSCs reprogrammed therefrom, refers to (1) at the gene level—successful knocked-in, knocked-out, knocked-down gene expression, transgenic or controlled gene expression such as inducible or temporal expression at a desired cell development stage, which is achieved through direct genomic editing or modification, or through “passing-on” via differentiation from or reprogramming of a starting cell that is initially genomically engineered; or (2) at the cell level—successful removal, addition, or alteration of a cell function/characteristic via (i) gene expression modification obtained in said cell through direct genomic editing, (ii) gene expression modification maintained in said cell through “passing-on” via differentiation from or reprogramming of a starting cell that is initially genomically engineered; (iii) down-stream gene regulation in said cell as a result of gene expression modification that only appears in an earlier development stage of said cell, or only appears in the starting cell that gives rise to said cell via differentiation or reprogramming; or (iv) enhanced or newly attained cellular function or attribute displayed within the mature cellular product, initially derived from the genomic editing or modification conducted at the iPSC, progenitor or dedifferentiated cellular origin.

[000102] The term “ligand” refers to a substance that forms a complex with a target molecule to produce a signal by binding to a site on the target. The ligand may be a natural or artificial substance capable of specific binding to the target. The ligand may be in the form of a protein, a peptide, an antibody, an antibody complex, a conjugate, a nucleic acid, a lipid, a polysaccharide, a monosaccharide, a small molecule, a nanoparticle, an ion, a neurotransmitter, or any other molecular entity capable of specific binding to a target. The target to which the ligand binds, may be a protein, a nucleic acid, an antigen, a receptor, a protein complex, or a cell. A ligand that binds to and alters the function of the target and triggers a signaling response

is called “agonistic” or “an agonist”. A ligand that binds to a target and blocks or reduces a signaling response is “antagonistic” or “an antagonist.”

[000103] The term “antibody” encompasses antibodies and antibody fragments that contain at least one binding site that specifically binds to a particular target of interest, wherein the target may be an antigen, or a receptor that is capable of interacting with certain antibodies. The term “antibody” includes, but is not limited to, an immunoglobulin molecule or an antigen-binding or receptor-binding portion thereof. For example, an NK cell can be activated by the binding of an antibody or the Fc region of an antibody to its Fc-gamma receptors (FcγR), thereby triggering the ADCC (antibody-dependent cellular cytotoxicity) mediated effector cell activation. A specific piece or portion of an antigen or receptor, or a target in general, to which an antibody binds is known as an epitope or an antigenic determinant. The term antibody also includes, but is not limited to, native antibodies and variants thereof, fragments of native antibodies and variants thereof, peptibodies and variants thereof, and antibody mimetics that mimic the structure and/or function of an antibody or a specified fragment or portion thereof, including single chain antibodies and fragments thereof. An antibody may be a murine antibody, a human antibody, a humanized antibody, a camel IgG, single variable new antigen receptor (VNAR), shark heavy-chain antibody (Ig-NAR), a chimeric antibody, a recombinant antibody, a single-domain antibody (dAb), an anti-idiotypic antibody, a bi-specific-, multi-specific- or multimeric-antibody, or antibody fragment thereof. Anti-idiotypic antibodies are specific for binding to an idiotope of another antibody, wherein the idiotope is an antigenic determinant of an antibody. A bi-specific antibody may be a BiTE (bi-specific T cell engager) or a BiKE (bi-specific killer cell engager), and a multi-specific antibody may be a TriKE (tri-specific Killer cell engager). Non-limiting examples of antibody fragments include Fab, Fab', F(ab')₂, F(ab')₃, Fv, Fabc, pFc, Fd, single chain fragment variable (scFv), tandem scFv (scFv)₂, single chain Fab (scFab), disulfide stabilized Fv (dsFv), minibody, diabody, triabody, tetrabody, single-domain antigen binding fragments (sdAb), camelid heavy-chain IgG and Nanobody® fragments, recombinant heavy-chain-only antibody (VHH), and other antibody fragments that maintain the binding specificity of the antibody.

[000104] “Fc receptors,” abbreviated “FcR”, are classified based on the type of antibody that they recognize. For example, those that bind the most common class of antibody, IgG, are called Fc-gamma receptors (FcγR), those that bind IgA are called Fc-alpha receptors (FcαR) and those that bind IgE are called Fc-epsilon receptors (FcεR). The classes of FcRs are also distinguished by the cells that express them (macrophages, granulocytes, natural killer cells, T and B cells) and the signaling properties of each receptor. Fc-gamma receptors (FcγR) includes several members,

FcγRI (CD64), FcγRIIA (CD32), FcγRIIB (CD32), FcγRIIIA (CD16a), FcγRIIIB (CD16b), which differ in their antibody affinities due to their different molecular structures.

[000105] CD16, a FcγR receptor, has been identified to have two isoforms, Fc receptors FcγRIIIa (CD16a) and FcγRIIIB (CD16b). CD16a is a transmembrane protein expressed by NK cells, which binds monomeric IgG attached to target cells to activate NK cells and facilitate antibody-dependent cell-mediated cytotoxicity (ADCC). “High affinity CD16,” “non-cleavable CD16,” or “high affinity non-cleavable CD16” (abbreviated as hnCD16), as used herein, refers to a natural or non-natural variant of CD16. The wildtype CD16 has low affinity and is subject to ectodomain shedding, a proteolytic cleavage process that regulates the cells surface density of various cell surface molecules on leukocytes upon NK cell activation. F176V and F158V are exemplary CD16 polymorphic variants having high affinity. A CD16 variant having the cleavage site (position 195-198) in the membrane-proximal region (position 189-212) altered or eliminated is not subject to shedding. The cleavage site and the membrane-proximal region are described in detail in WO2015/148926 and US Pat. No. 10,464,989, the complete disclosures of which are incorporated herein by reference. The CD16 S197P variant is an engineered non-cleavable version of CD16. A CD16 variant comprising both F158V and S197P has high affinity and is non-cleavable. Another exemplary high affinity and non-cleavable CD16 (hnCD16) variant is an engineered CD16 comprising an ectodomain originated from one or more of the 3 exons of the CD64 ectodomain.

[000106] As used herein, “FT819” refers to an engineered T cell therapy generated from a clonal master engineered iPSC line, and is engineered with multiple modalities for enhanced innate immunity: (1) anti-CD19-CAR at TRAC locus; and (2) T cell receptor (TCR) knockout.

I. Cells and Compositions Useful for Adoptive Cell Therapies with Enhanced Properties

[000107] Provided herein is a strategy to systematically engineer the regulatory circuitry of a clonal iPSC without impacting the differentiation potency and cell development biology of the iPSC and its derivative cells, while enhancing the therapeutic properties of the derivative cells differentiated from the iPSC. The iPSC-derived cells are functionally improved and suitable for adoptive cell therapies following a combination of selective modalities being introduced to the cells at the level of iPSC through genomic engineering. It was previously unclear whether altered iPSCs comprising one or more provided genetic edits still have the capacity to enter cell development, and/or to mature and generate functional differentiated cells while retaining modified activities and/or properties. Unanticipated failures during directed cell differentiation from iPSCs have been attributed to aspects including, but not limited to, development stage

specific gene expression or lack thereof, requirements for HLA complex presentation, protein shedding of introduced surface expressing modalities, and the need for reconfiguration of differentiation protocols enabling phenotypic and/or functional change in the cell. The present application has shown that the one or more selected genomic modifications as provided herein does not negatively impact iPSC differentiation potency, and the functional effector cells derived from the engineered iPSC have enhanced and/or acquired therapeutic properties attributable to the individual or combined genomic modifications retained in the effector cells following the iPSC differentiation. Further, all genomic modifications and combinations thereof as may be described in the context of iPSC and iPSC-derived effector cells are applicable to primary sourced cells, including primary immune cells such as T, NK, or immunoregulatory cells, whether cultured or expanded, the modification of which results in engineered immune cells useful for adoptive cell therapy.

[000108] Further, while CAR-T cells have been shown to be effective and potent in treating several hematologic malignancies, engineered autologous T cell therapies have had many limitations. In addition to antigen accessibility, antigen uniformity and antigen specificity, and antigen heterogeneity are significant barriers to the successful development of CAR-T cell treatments. In addition, inherent genetic engineering variability seen with patient- and donor-derived immune cells limits the wide application of CAR-T cell therapy. The present application provides an off-the-shelf adoptive cell therapy setting using effector cells derived from engineered iPSCs to treat autoimmune diseases, including systemic lupus erythematosus (SLE), lupus nephritis, rheumatoid arthritis (RA), and scleroderma.

1. TCR Knockout

[000109] In some embodiments, a polynucleotide encoding one or more modalities described herein (e.g., one or more of a CAR, CXCR2, IL7RF, or TGF β -SRR) inserted in a TCR constant region (e.g., TRAC or TRBC), leading to TCR knockout, and optionally placing expression of the one or more modalities under the control of the endogenous TCR promoter. The disruption of the constant region of TCR α or TCR β (TRAC or TRBC) produces a TCR^{neg} cell. TCR^{neg} cells do not require HLA matching, have reduced alloreactivity, and are able to prevent GvHD (Graft versus Host Disease) when used in allogeneic adoptive cell therapies. In some embodiments, polynucleotides encoding different modalities described herein are inserted at different sites. Additional insertion sites include, but are not limited to, AAVS1, CCR5, ROSA26, collagen, HTRP, H11, PH12, GAPDH, RUNX1, B2M, TAP1, TAP2, tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, NKG2A, NKG2D, CD25, CD38, CD44, CD58, CD54, CD56, CD69, CD71, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, and TIGIT.

[000110] In one embodiment as provided herein, the TCR knockout in an iPSC line is a bi-allelic knockout. When two or more transgenes are to be inserted at a selected location in a TCR locus, a linker sequence, for example, a 2A linker or IRES, is placed between any two transgenes. The 2A linker encodes a self-cleaving peptide derived from FMDV, ERAV, PTV-I, and TaV (referred to as “F2A”, “E2A”, “P2A”, and “T2A”, respectively), allowing for separate proteins to be produced from a single translation. In some embodiments, insulators may be included in the construct to reduce the risk of transgene and/or exogenous promoter silencing.

2. *Chimeric Antigen Receptor (CAR) expression*

[000111] Applicable to the genetically engineered immune cells, iPSCs and derivative effector cells thereof may be any CAR design known in the art. CAR is a fusion protein generally including an ectodomain that comprises a target binding region (for example, an antigen recognition domain), a transmembrane domain, and an endodomain. In some embodiments, the ectodomain can further include a signal peptide or leader sequence and/or a spacer. In some embodiments, the endodomain can further comprise a signaling peptide that activates the effector cell expressing the CAR. In some embodiments, the signaling peptide of the endodomain (or intracellular domain) comprises a full length or at least a portion of a polypeptide of 2B4, CD2, CD3 ζ , CD3 ζ 1XX, CD8, CD28, CD28H, CD137 (4-1BB), CS1, DAP10, DAP12, DNAM1, FcERI γ , IL2R γ , IL7R, IL21R, IL2R β (IL15R β), IL7, IL12, IL15, IL21, KIR2DS2, Nkp30, Nkp44, Nkp46, NKG2C, or NKG2D. In one embodiment, the signaling peptide of a CAR comprises an amino acid sequence that has at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to at least one ITAM (immunoreceptor tyrosine-based activation motif) of CD3 ζ . Exemplary N-terminal signal peptides include MALPVTALLLPLALLLHA (SEQ ID NO: 7; CD8asp) or MDFQVQIFSFLISASVIMSR (SEQ ID NO: 8; IgKsp), or any signal peptide sequence or functional variants thereof known in the art.

[000112] In some embodiments, the antigen recognition domain can specifically bind an antigen. In some embodiments, the CAR is suitable to activate T, NK or NKT cells expressing said CAR. In some embodiments, the CAR is T cell specific for comprising T-specific signaling components. In certain embodiments, said T cells are derived from CAR-encoding iPSCs comprising one or more additional modifications as described herein, and the derivative T cells may comprise T helper cells, cytotoxic T cells, memory T cells, regulatory T cells, natural killer T cells, $\alpha\beta$ T cells, $\gamma\delta$ T cells, or a combination thereof.

[000113] In various embodiments, the antigen recognition region comprises a murine antibody, a human antibody, a humanized antibody, a camel Ig, a single variable new antigen

receptor (VNAR), a shark heavy-chain antibody (Ig-NAR), a chimeric antibody, a recombinant antibody, a single-domain antibody (dAb), an anti-idiotypic antibody, a bi-specific-, multi-specific- or multimeric- antibody, or antibody fragment thereof. Anti-idiotypic antibodies are specific for binding to an idiotope of another antibody, wherein the idiotope is an antigenic determinant of an antibody. A bi-specific antibody may be a BiTE (bi-specific T cell engager) or a BiKE (bi-specific killer cell engager), and a multi-specific antibody may be a TriKE (tri-specific Killer cell engager). Non-limiting examples of antibody fragments include Fab, Fab', F(ab')₂, F(ab')₃, Fv, Fabc, pFc, Fd, single chain fragment variable (scFv), tandem scFv (scFv)₂, single chain Fab (scFab), disulfide stabilized Fv (dsFv), minibody, diabody, triabody, tetrabody, single-domain antigen binding fragments (sdAb), camelid heavy-chain IgG and Nanobody® fragments, recombinant heavy-chain-only antibody (VHH), and other antibody fragments that maintain the binding specificity of the antibody. In some embodiments an antigen binding domain of a CAR comprises CDR1, CDR2, and CDR3 of a heavy chain (H-CDRs) of an antibody or fragments thereof. In some embodiments, the antigen binding domain of a CAR comprising the H-CDRs of an antibody further comprises the CDRs of a light chain (L-CDRs) of the antibody.

[000114] In some embodiments, the scFV is an scFV selected based on having specific binding affinity for a target (e.g., CD19). In some embodiments, the scFV comprises one or more sequences of an antigen-binding fragment of an antibody selected based on having specific binding affinity for the target (e.g., CD19). In some embodiments the antigen binding domain of the CAR comprises a single chain variable fragment (scFV) comprising a heavy chain and a light chain having a sequence identity of at least 85%, 90%, 95%, 99%, 100%, or any percentage in-between, when compared to the exemplary sequences represented by SEQ ID NO: 1 and SEQ ID NO: 2, respectively. In some embodiments, the scFV comprises an amino acid sequence of at least 90% identity to SEQ ID NO: 1 or 2. In some embodiments, the scFV comprises an amino acid sequence of at least 95% identity to SEQ ID NO: 1 or 2. In some embodiments, the scFV comprises the amino acid sequence of SEQ ID NO: 1. In some embodiments, the scFV comprises the amino acid sequence of SEQ ID NO: 2. In some embodiments, the scFV comprises the amino acid sequences of both SEQ ID NO: 1 and 2.

SEQ ID NO: 1

AELVRPGSSVKISCKASGYAFSSYWMNWVKQRPGQGLEWIGQIYPGDGDTNYNGKFKGQATLT
ADKSSSTAYMQLSGLTSEDSAVYFCARKTISSVDFYF

SEQ ID NO: 2

KFMSTSVGDRVSVTCKASQNVGTNVAWYQQKPGQSPKPLIYSATYRNSGVPDRFTGSGSGTDF
TLTITNVQSKDLADYFCQYNNRYPYTS

[000115] In some embodiments, the CAR further comprises a transmembrane domain, such as a transmembrane domain of CD28. In some embodiments, the CAR further comprises a costimulatory domain, such as a co-stimulatory domain of CD28. In some embodiments, the CAR further comprises an activation domain, such as an activation domain of CD3ζ1XX. In one embodiment, the CAR provided herein comprises an amino acid sequence of at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to SEQ ID NO: 3, wherein the linker in the ectodomain and the spacer between the ectodomain and transmembrane domain may vary in length and sequence. In some embodiments, the CAR comprises an amino acid sequence of at least about 90% identity to SEQ ID NO: 3, wherein the linker in the ectodomain and the spacer between the ectodomain and transmembrane domain may vary in length and sequence. In some embodiments, the CAR comprises an amino acid sequence of at least about 95% identity to SEQ ID NO: 3, wherein the linker in the ectodomain and the spacer between the ectodomain and transmembrane domain may vary in length and sequence. In some embodiments, the CAR comprises the amino acid sequence of SEQ ID NO: 3. In some embodiments, the CAR provided herein recognizes a CD19 antigen of B cells. In some embodiments, the CAR provided herein recognizes a CD19 antigen of autoreactive B cells.

SEQ ID NO: 3

ESKYGPPCPPCPGGGSSGGSGGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNG
OPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHREALHNHYTQKSLSLGLKMFHWL
VVVGGVLACYSLLVTVAFIIFWVRSKRSLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS
RVKFSRSADAPAYQOGONOLYNELNLGRREEYDVLDRRGRDPEMGKPRRKNPQEGLFNELQDKD
MAEAFSEIGMKGERRRGKGHDGLFQGLSTATKDTFDALHMQALPPR

(*spacer*- CD28 TM- CD28 Costim-CD3ζ1XX *activation*)

[000116] In one embodiment, the CAR provided herein comprises an amino acid sequence of at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to SEQ ID NO: 6, wherein the linker in the ectodomain and the spacer between the ectodomain and transmembrane domain may vary in length and sequence. In some embodiments, the CAR comprises an amino acid sequence of at least about 90% identity to SEQ ID NO: 6, wherein the linker in the ectodomain and the spacer between the ectodomain and transmembrane domain may vary in length and sequence. In some embodiments, the CAR comprises an amino acid sequence of at least about 95% identity to SEQ ID NO: 6, wherein the linker in the ectodomain and the spacer between the ectodomain and transmembrane domain

may vary in length and sequence. In some embodiments, the CAR comprises the amino acid sequence of SEQ ID NO: 6. In some embodiments, the CAR provided herein recognizes a CD19 antigen.

SEQ ID NO: 6

MALPVTALLPLALLLHAEVKLQQSGAELVRPGSSVKISCKASGYAFSSYWMNWVKQRPGQGL
EWIGQIYPGDGDTNYNGKFKGQATLTADKSSSTAYMQLSGLTSEDSAVYFCARKTISSVDFYF
DYWGQGTITVTVSSGGGGSGGGSGGGGSDIELTQSPKFMSTSVGDRVSVTCKASQNVGTNVA
WYQQKPGQSPKPLIYSATYRNSGVPDRFTGSGSGTDFTLTITNVQSKDLADYFCQQYNRYPYTS
GGGTTKLEIKRAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKPFVVLVVVGGVLAC
YSLVTVAFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSAD
APAYQQGQNQLYNELNLGRREEYDVLDRRGRDPENGGKPRRKNPQEGFLNELQDKMAEAF
SEIGMKGERRRGKGGHDLFQGLSTATKDTFDALHMQALPPR

3. *CD16 knock-in*

[000117] CD16 has been identified as two isoforms, Fc receptors FcγRIIIa (CD16a; NM_000569.6) and FcγRIIIb (CD16b; NM_000570.4). CD16a is a transmembrane protein expressed by NK cells, which binds monomeric IgG attached to target cells to activate NK cells and facilitate antibody-dependent cell-mediated cytotoxicity (ADCC). CD16b is exclusively expressed by human neutrophils. “High affinity CD16,” “non-cleavable CD16,” “high affinity non-cleavable CD16,” or “hnCD16,” as used herein, refers to various CD16 variants. The wildtype CD16 has low affinity and is subject to ectodomain shedding, a proteolytic cleavage process that regulates the cells surface density of various cell surface molecules on leukocytes upon NK cell activation. F176V (also called F158V in some publications) is an exemplary CD16 polymorphic variant having high affinity; whereas an S197P variant is an example of a genetically engineered non-cleavable version of CD16. An engineered CD16 variant comprising both F176V and S197P has high affinity and is non-cleavable, which was described in greater detail in International Pub. No. WO2015/148926 and US Pat. No. 10,464,989, the complete disclosures of which are incorporated herein by reference. In some embodiments, the hnCD16 comprises an amino acid sequence of at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to SEQ ID NO: 4. In some embodiments, the hnCD16 comprises an amino acid sequence of at least about 90% identity to SEQ ID NO: 4. In some embodiments, the hnCD16 comprises an amino acid sequence of at least about 95% identity to SEQ ID NO: 4. In some embodiments, the hnCD16 comprises the amino acid sequence of SEQ ID NO: 4.

SEQ ID NO: 4

MWQLLLPTALLLLVSAGMRTE₁DLPKAVVFLEPQWYRVLEKDSVTLKCQGAYSPEDNSTQWFH
 NESLISSQASSYFIDAATVDDSGEYRCQTNLSTLSDPVQLEVHIGWLLLQAPRWVFKEEDPIHLRC
 HSWKNTALHKV₂TYLQNGKGRKYFHHNSDFYIPKATLKDSGSYFCRGLVGSKNV₃SSETVNITITQ
 GLAVPTISSFFPPGYQVSFCLVMVLLFAVDTGLYFSVKTNIRSSTRDWKDHKFKWRKDPQDK

[000118] In some embodiments, the primary-sourced or derived effector cells comprising the exogenous CD16 or variant thereof are T lineage cells. In some embodiments, the exogenous CD16 or functional variants thereof comprised in iPSC or effector cells has high affinity in binding to a ligand that triggers downstream signaling upon such binding. Non-limiting examples of ligands binding to the exogenous CD16 or functional variants thereof include not only ADCC antibodies or fragments thereof, but also to bi-, tri-, or multi- specific engagers or binders that recognize the CD16 extracellular binding domains of the exogenous CD16. Examples of bi-, tri-, or multi- specific engagers or binders are further described below in this application. As such, at least one of the aspects of the present application provides a derivative effector cell or a cell population thereof, preloaded with one or more pre-selected ADCC antibodies through an exogenous CD16 expressed on the derivative effector cell, in an amount sufficient for therapeutic use in a treatment of a condition, a disease, or an infection as further detailed this application, wherein the exogenous CD16 comprises an extracellular binding domain of a CD16 having F176V and S197P.

[000119] ADCC is a mechanism of NK cell mediated lysis through the binding of CD16 to antibody-coated target cells. However, the endogenous CD16 expressed by primary NK cells gets cleaved from the cellular surface following NK cell activation. The various non-cleavable versions of CD16 avoid CD16 shedding and maintain constant expression. Non-cleavable CD16 provides antibody-dependent cell-mediated cytotoxicity (ADCC), and the engagement of bi-, tri-, or multi- specific engagers to a T cell. In derivative T cells, non-cleavable CD16 increases expression of TNF α and CD107a, indicative of improved cell functionality. The additional high affinity characteristics of the introduced hnCD16 in derived T cells also enables *in vitro* loading of an ADCC antibody to the T cell through hnCD16 before administering the cell to a subject in need of a cell therapy.

4. Exogenously introduced cytokine signaling complex

[000120] By avoiding systemic high-dose administration of clinically relevant cytokines, the risk of dose-limiting toxicities due to such a practice is reduced while cytokine mediated cell autonomy being established. To achieve lymphocyte autonomy without the need to additionally administer soluble cytokines, a cytokine signaling complex comprising a partial or full peptide

of one or more of IL2, IL4, IL6, IL7, IL9, IL10, IL11, IL12, IL15, IL18, IL21, and/or their respective receptors may be introduced to the cell to enable cytokine signaling with or without the expression of the cytokine itself, thereby maintaining or improving cell growth, proliferation, expansion, and/or effector function with reduced risk of cytokine toxicities. In some embodiments, the introduced cytokine and/or its respective native or modified receptor for cytokine signaling (signaling complex) are expressed on the cell surface. In some embodiments, the cytokine signaling is constitutively activated. In some embodiments, the activation of the cytokine signaling is inducible. In some embodiments, the activation of the cytokine signaling is transient and/or temporal. In some embodiments, the transient/temporal expression of a cell surface cytokine/cytokine receptor is through an expression construct carried by a retrovirus, Sendai virus, an adenovirus, an episome, mini-circle, or RNAs, including mRNA.

[000121] In other various embodiments, the cytokine signaling complex comprises an IL7 receptor fusion (IL7RF) comprising a full or partial length of IL7 and a full or partial length of IL7 receptor. The transmembrane (TM) domain can be native to the IL7 receptor or may be modified or replaced with a transmembrane domain of any other membrane bound proteins. In one embodiment, a native (or wildtype) or modified IL7R may be fused to IL7 at the C-terminus through a linker, enabling constitutive signaling and maintaining membrane-bound IL7. In some embodiments, such a construct comprises an amino acid sequence of at least 75%, 80%, 85%, 90%, 95% or 99% identity to SEQ ID NO: 5, with transmembrane domain, signal peptide and linker being flexible and varying in length and/or sequences. In some embodiments, the IL7 construct comprises an amino acid sequence of at least 85% identity to SEQ ID NO: 5, with transmembrane domain, signal peptide and linker being flexible and varying in length and/or sequences. In some embodiments, the IL7 construct comprises an amino acid sequence of at least 90% identity to SEQ ID NO: 5, with transmembrane domain, signal peptide and linker being flexible and varying in length and/or sequences. In some embodiments, the IL7 construct comprises an amino acid sequence of at least 95% identity to SEQ ID NO: 5, with transmembrane domain, signal peptide and linker being flexible and varying in length and/or sequences. In some embodiments, the IL7 construct comprises the amino acid sequence of SEQ ID NO: 5. In some embodiments, such a construct comprises an amino acid sequence of at least 85%, 90%, 95% or 99% identity to SEQ ID NO: 5, excluding the amino acids of the exemplary transmembrane domain, signal peptide and linker indicated in SEQ ID NO: 5.

SEQ ID NO: 5

**MDWTWILFLVAAATRVHSDCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRH
ICDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALG**

EAQPTKSLEENKSLKEQKKLNDLCFLKRLLEIKTCWNKILMGTKEHSGGGSGGGSGGG
 GSGGGSGGGSLQESGYAQNGLDEDAELDDYSFSCYSQLEVNGSQHSLTCAFEDPDVNITNLEF
 EICGALVEVKCLNFRKLQEIYFIETKKFLLIGKSNICVKVGEKSLTCKKIDLTIVKPEAPFDLSVV
 YREGANDFVVTFTNTSHLQKKYVKVLMHDVAYRQEKDENKWTHTVNLSTKLTLQRKLQPA
 MYEIKVRSIPDHYFKGFWEWSPSYFRTPEINNSSGEMDPILLTISILSFFSVALLVILACVLWKK
 RIKPIVWPSLPDHKKTLHLCKKPRKNLNVSFNPESFLDCQIHRVDDIQARDEVEGFLODTFPQQ
 LEESEKQRLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNG
 PHVYQDLLLSLGTNTLPPFSLQSGILTLNPVAQGGPILTSLSNQEAYVTMSSFYQNNQ

(*Signal peptide-IL7-linker-IL7R*; transmembrane domain (TM), signal peptide and linker can vary in length and sequences)

[000122] In another embodiment, a native or modified common receptor γ C is fused to IL7 at the C-terminus through a linker for constitutive and membrane-bound cytokine signaling complex. In addition, engineered IL7R that forms a homodimer in the absence of IL7 is useful for producing constitutive signaling of the cytokine as well.

[000123] One having ordinary skill in the art would appreciate that the signal peptide and the linker sequences above are illustrative and in no way limit their variations suitable for use as a signal peptide or linker. There are many suitable signal peptide or linker sequences known and available to those in the art. The ordinary skilled in the art understands that the signal peptide and/or linker sequences may be substituted for another sequence without altering the activity of the functional peptide led by the signal peptide or linked by the linker.

[000124] In some embodiments, the cytokine signaling complex comprises an IL15 receptor fusion (IL15RF) comprising a full or partial length of IL15 and a full or partial length of IL15 receptor. The transmembrane (TM) domain can be native to the IL15 receptor or may be modified or replaced with a transmembrane domain of any other membrane bound proteins. In some embodiments, IL15 and IL15R α are co-expressed by using a self-cleaving peptide, mimicking trans-presentation of IL15, without eliminating cis-presentation of IL15. In other embodiments, IL15R α is fused to IL15 at the C-terminus through a linker, mimicking trans-presentation without eliminating cis-presentation of IL15 as well as ensuring that IL15 is membrane-bound. In other embodiments, IL15R α with truncated intracellular domain is fused to IL15 at the C-terminus through a linker, mimicking trans-presentation of IL15, maintaining IL15 membrane-bound, and additionally eliminating cis-presentation and/or any other potential signal transduction pathways mediated by a normal IL15R through its intracellular domain. In other embodiments, IL15R α is fused to IL15 without an intracellular domain (IL15 Δ), as described in International Pub. Nos. WO 2019/191495 and WO 2019/126748, the entire disclosure of each of which is incorporated herein by reference.

[000125] In various embodiments, such a truncated construct comprises an amino acid sequence of at least 75%, 80%, 85%, 90%, 95% or 99% identity to SEQ ID NO: 9. In one embodiment of the truncated IL15/IL15R α , the construct does not comprise the last 4 amino acid residues (KSRQ) of SEQ ID NO: 9, and comprises an amino acid sequence of at least 75%, 80%, 85%, 90%, 95% or 99% identity to SEQ ID NO: 10.

SEQ ID NO: 9

MDWTWILFLVAAATRVHSGIHVFILGCF SAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVHP
 SCKVTAMKCFLLLELQVISLES GDASIHDTVENLIILANNSSLSSNGNVTESGCKECELEEKNIKEFLQSF
 VHIVQMFINTSSGGGSGGGGSGGGGSGGGGSGGGSLQITCPPPMSVEHADIWVKSYSLYSRERYICNSGF
 KRKAGTSSLTECVLNKATNVAHWTTPSLKCIRD PALVHQRPAAPPSTVTTAGVTPQPESLSPSGKEPAASS
 PSSNNTAATTAAIVPGSQLMP SKSPSTGTTEISSHESHGTPSQTTAKNWELTASASHQPPGVYPQGHSD
 TTVAI STSTVLLCGLSAV SLLACYLKSRQ

(379 a.a.; signal and linker peptides are underlined)

SEQ ID NO: 10

MDWTWILFLVAAATRVHSGIHVFILGCF SAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVHP
 SCKVTAMKCFLLLELQVISLES GDASIHDTVENLIILANNSSLSSNGNVTESGCKECELEEKNIKEFLQSF
 VHIVQMFINTSSGGGSGGGGSGGGGSGGGGSGGGSLQITCPPPMSVEHADIWVKSYSLYSRERYICNSGF
 KRKAGTSSLTECVLNKATNVAHWTTPSLKCIRD PALVHQRPAAPPSTVTTAGVTPQPESLSPSGKEPAASS
 PSSNNTAATTAAIVPGSQLMP SKSPSTGTTEISSHESHGTPSQTTAKNWELTASASHQPPGVYPQGHSD
 TTVAI STSTVLLCGLSAV SLLACYL

(375 a.a.; signal and linker peptides are underlined)

[000126] One having ordinary skill in the art would appreciate that the signal peptide and the linker sequences above are illustrative and in no way limit their variations suitable for use as a signal peptide or linker. There are many suitable signal peptide or linker sequences known and available to those in the art. The ordinary skilled in the art understands that the signal peptide and/or linker sequences may be substituted for another sequence without altering the activity of the functional peptide led by the signal peptide or linked by the linker.

[000127] In iPSCs and derivative cells therefrom comprising both CAR and exogenous cytokine and/or cytokine receptor signaling (signaling complex or “IL”), the CAR and IL may be expressed in separate constructs. In some embodiments, IL may be co-expressed in a bi-cistronic construct comprising both CAR and IL, or both hnCD16 and IL. In some further embodiments, the signaling complex can be linked to either the 5' or the 3' end of a CAR or hnCD16 expression construct through a self-cleaving 2A coding sequence. As such, an IL signaling complex (e.g., IL15 or IL7 signaling complex) and CAR may be in a single open reading frame (ORF). In some embodiments, a bi-cistronic design allows for coordinated IL signaling complex expression with CAR or hnCD16, both in timing and quantity, and under the

same control mechanism that may be chosen to incorporate, for example, an inducible promoter or promoter with temporal or spatial specificity for the expression of the single ORF. In one embodiment, the signaling complex is comprised in a CAR-2A-IL or an IL-2A-CAR construct. In one embodiment, the signaling complex is comprised in a hnCD16-2A-IL or an IL-2A-hnCD16 construct. When CAR-2A-IL or IL-2A-CAR, or hnCD16-2A-IL or IL-2A-hnCD16, is expressed, the self-cleaving 2A peptide allows the expressed CAR and IL or hnCD16 and IL, to dissociate, and the dissociated IL can then be presented at the cell surface, with the transmembrane domain anchored in the cell membrane. Self-cleaving peptides are found in members of the Picornaviridae virus family, including aphthoviruses such as foot-and-mouth disease virus (FMDV), equine rhinitis A virus (ERAV), *Thosea asigna* virus (TaV) and porcine tescho virus- 1 (PTV-I) (Donnelly, et al, *J. Gen. Virol*, 82, 1027-101 (2001); Ryan, et al., *J. Gen. Virol.*, 72, 2727-2732 (2001)), and cardioviruses such as Theilovirus (e.g., Theiler's murine encephalomyelitis) and encephalomyocarditis viruses. The 2A peptides derived from FMDV, ERAV, PTV-I, and TaV are sometimes also referred to as "F2A", "E2A", "P2A", and "T2A", respectively.

[000128] In light of the above, the present application provides an iPSC, an iPS cell line cell, or a population thereof, or a derivative functional cell obtained from differentiating the iPSC, wherein each cell comprises a polynucleotide encoding a CD19-CAR (chimeric antigen receptor), and optionally one or more of a CD16 or a variant thereof that mediates ADCC when in combination with an monoclonal antibody, a CD38 knockout, and an IL7 signaling complex.

5. ADR Armed CAR-19 NK cells

[000129] Unwanted activation of T- and NK- cells often promotes allo-immune reactions leading to development of graft-versus-host disease (GvHD). Although some steps may be taken to reduce the reactivity of allogeneic cells in the recipient individual, such cells would still be targeted by the immune system of the recipient (primarily T- and NK-cells), which would recognize them as foreign leading to rejection and limiting therapeutic benefit. On the other hand, modulating a host immune system to reduce allo-immune reactions, for example, by lympho-conditioning using chemotherapy such as Cy/Flu (cyclophosphamide/fludarabine) often leads to associated hematologic toxicities, including increased susceptibility to severe infections, due to indiscriminate lymphodepletion and a severely compromised immune system as a result. To control pathogenic conditions due to unwanted activation of the immune system, in various embodiments, genetically engineered immune cells, iPSCs and derivative effector cells thereof provided herein comprise an allo-immune defense receptor (ADR), among other components. In some embodiments, the application provides immune cells, iPSCs, and iPSC-derived effector

cells that are genetically engineered to comprise, among other editing as contemplated and described herein, a 4-1BB or CD38 specific allo-immune defense receptor (ADR) for effector cell potentiation as well as selective depletion of alloreactive host NK cells and T cells with upregulated 4-1BB and/or CD38 expression, the latter of which include pathogenic T cells, and regulatory T cells, while sparing resting cells in the recipient.

[000130] In some embodiments of the ADR that is specific to 4-1BB (CD137, also referred to as “41BB”), the ADR comprises an extracellular domain that targets 4-1BB upregulated on host T or NK cells when they are activated, and a signaling domain promoting effector cell activation. For example, the 41BB-ADR extracellular domain may comprise any suitable ligand for 4-1BB, including 4-1BBL, an antibody (or functional fragment thereof) that targets 4-1BB, a fusion of Fc with 4-1BBL, or functional derivatives or fragments thereof. In some embodiments, the 41BB-ADR extracellular domain comprises 4-1BBL, or a fragment thereof effective to bind 4-1BB. In some embodiments, the 41BB-ADR extracellular domain comprises an amino acid sequence with at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% sequence identity to SEQ ID NO: 11. In some embodiments, the 41BB-ADR extracellular domain comprises an amino acid sequence with at least about 90% sequence identity to SEQ ID NO: 11. In some embodiments, the 41BB-ADR extracellular domain comprises an amino acid sequence with at least about 95% sequence identity to SEQ ID NO: 11. In some embodiments, the 41BB-ADR extracellular domain comprises the amino acid sequence of SEQ ID NO: 11.

SEQ ID NO: 11

GLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQLELRRV
VAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARA
RHAWQLTQGATVVLGLFRVTPEIPAGLPSPRSE

[000131] In particular embodiments, the ADR comprises CD3 ζ , represented by an amino acid sequence of at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% sequence identity to SEQ ID NO: 12 or a functional fragment thereof, or comprises a CD3 ζ derivative (for example, CD3 ζ 1XX, represented by an amino acid sequence of at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% sequence identity to SEQ ID NO: 13 or a functional fragment thereof). In some embodiments, the CD3 ζ comprises an amino acid sequence of at least about 90% sequence identity to SEQ ID NO: 12. In some embodiments, the CD3 ζ comprises an amino acid sequence of at least about 95% sequence identity to SEQ ID NO: 12. In some embodiments, the CD3 ζ comprises the amino acid sequence of SEQ ID NO: 12. In some embodiments, the CD3 ζ derivative comprises

an amino acid sequence of at least about 90% sequence identity to SEQ ID NO: 13. In some embodiments, the CD3 ζ derivative comprises an amino acid sequence of at least about 95% sequence identity to SEQ ID NO: 13. In some embodiments, the CD3 ζ derivative comprises the amino acid sequence of SEQ ID NO: 13. CD3 ζ mediates downstream ITAM-derived signaling during effector T or NK cell activation.

SEQ ID NO: 12

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
(CD3 ζ)

SEQ ID NO: 13

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLFNELQKDKMAEAFSEIGMKGERRRGKGHDGLFQGLSTATKDTYDALHMQALPPR
(CD3 ζ 1XX - containing 2 mutations in ITAM1)

[000132] In one embodiment of the 4-1BB specific ADR, the 41BB-ADR is represented by an amino acid sequence of at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% sequence identity to SEQ ID NO: 14 or SEQ ID NOs: 15-17. In some embodiments, the the 41BB-ADR comprises an amino acid sequence of at least about 90% identity to SEQ ID NO: 14 or SEQ ID NOs: 15-17. In some embodiments, the the 41BB-ADR comprises an amino acid sequence of at least about 95% identity to SEQ ID NO: 14 or SEQ ID NOs: 15-17. In some embodiments, the 41BB-ADR comprises the amino acid sequence of SEQ ID NO: 14. In some embodiments, the 41BB-ADR comprises the amino acid sequence of SEQ ID NO: 15. In some embodiments, the 41BB-ADR comprises the amino acid sequence of SEQ ID NO: 16. In some embodiments, the 41BB-ADR comprises the amino acid sequence of SEQ ID NO: 17.

SEQ ID NO: 14

MEFGLSWLFLVAILKGVQCGLLDLRQGMFAQLVAQNVLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGLPSRSEESKYGPPCPPCPGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNAYTQKSLSLSPGKKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

Signal peptide-41BBL-spacer-CD28 (TM)-CD3z (the signal peptide, spacer and TM/transmembrane domain may vary)

SEQ ID NO: 15

MEFGLSWLFLVAILKGVQCGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKEL
 VVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRRL
 LHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGLPSRSEESKYGPPCPPCPGQPREPQ
VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNAYTQKSLSLSPGKKDPKFWVLVVVGGVLAACYSLLVTVAFIIFWVRSKRSRLLH
 SDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKR
RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQA
LPPR

Signal peptide-41BBL-spacer-**CD28 (TM)**-CD28 (ICD)-CD3z (the signal peptide, spacer and TM/transmembrane domain may vary)

SEQ ID NO: 16

MEFGLSWLFLVAILKGVQCGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKEL
 VVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRRL
 LHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGLPSRSEESKYGPPCPPCPGQPREPQ
VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNAYTQKSLSLSPGKKDPKFWVLVVVGGVLAACYSLLVTVAFIIFWVRSRVKFSRS
 ADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLFNELQKDKMAEAFSEIGMK
GERRRGKGGHDGLFQGLSTATKDTFDALHMQALPPR

Signal peptide-41BBL-spacer-**CD28 (TM)**-CD3z1xx (the signal peptide, spacer and TM/transmembrane domain may vary)

SEQ ID NO: 17

MEFGLSWLFLVAILKGVQCGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKEL
 VVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRRL
 LHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGLPSRSEESKYGPPCPPCPGQPREPQ
VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNAYTQKSLSLSPGKKDPKFWVLVVVGGVLAACYSLLVTVAFIIFWVRSKRSRLLH
 SDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKR
RGRDPEMGGKPRRKNPQEGLFNELQKDKMAEAFSEIGMKGERRRGKGGHDGLFQGLSTATKDTFDALHMQA
LPPR

Signal peptide-41BBL-spacer-**CD28 (TM)**-CD28 (ICD)-CD3z1xx (the signal peptide, spacer and TM/transmembrane domain may vary)

6. Antibodies for immunotherapy

[000133] In some embodiments, in addition to the genomically engineered effector cells as provided herein, additional therapeutic agents comprising an antibody, or an antibody fragment that targets an antigen associated with a condition, a disease, or an indication may be used with these effector cells in a combinational therapy. In some embodiments, the antibody is used in combination with a population of the effector cells described herein by concurrent or consecutive administration to a subject. In other embodiments, such antibody or a fragment thereof may be expressed by the effector cells by genetically engineering an iPSC using an exogenous polynucleotide sequence encoding said antibody or fragment thereof, and directing differentiation of the engineered iPSC. In some embodiments, the effector cell expresses an

exogenous CD16 variant, wherein the cytotoxicity of the effector cell is enhanced by the antibody via ADCC. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a humanized antibody, a humanized monoclonal antibody, or a chimeric antibody. In some embodiments, the antibody, or antibody fragment, specifically binds to a viral antigen. In other embodiments, the antibody, or antibody fragment, specifically binds to a tumor antigen. In some embodiments, the antigen activates the administered iPSC-derived effector cells to enhance their killing ability. In some embodiments, the antibodies suitable for combinational treatment as an additional therapeutic agent to the administered iPSC-derived effector cells include, but are not limited to, anti-CD20 antibody (e.g. rituximab, veltuzumab, ofatumumab, ublituximab, ocaratuzumab, obinutuzumab), an anti-EGFR antibody (cetuximab, matuzumab, panitumumab and necitumumab), an anti-HER2 antibody (trastuzumab or biosimilars, pertuzumab, 4B5, ertumaxomab), an anti-PDL1 antibody (avelumab, durvalumab, pembrolizumab, nivolumab, or atezolizumab), a bi-specific antibody targeting EGFR and MET (amivantamab), and their humanized or Fc modified variants or fragments or their functional equivalents and biosimilars. Exemplary trastuzumab biosimilars include, but are not limited to, trastuzumab-anns (Kanjinti™), trastuzumab-dkst (Ogivri®), trastuzumab-qyyp (Trazimera™), trastuzumab-pkrb (Herzuma®), trastuzumab-dttb (Ontruzant®).

[000134] In some embodiments, an initial dose of the monoclonal antibody is administered in an effective amount at a starting time prior to a first cycle of administering the iPSC-derived effector cells. In some embodiments, the starting time is about 2-6 days prior to the first cycle of administering the iPSC-derived effector cells. In some embodiments, the antibody for combination treatment is rituximab and wherein the initial dose is a single initial dose of about 300 mg/m² to about 450 mg/m² administered to the subject about 4 days prior to the first cycle of administering the effector cells.

II. Therapeutic Use of Derivative Immune Cells for Autoimmune Disease

[000135] Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease, with immunological abnormalities, including the presence of a plethora of pathogenic autoantibodies, hyperactive B cells, activated T cells, increased cytokines, and increased innate immune function. Hyperactive B cells may play a role both as the precursors to autoantibody-producing cells and as antigen-presenting cells to T cells, while immune complexes comprising autoantibodies complexed with their autoantigens stimulate plasmacytoid dendritic cells and neutrophils, with immune complex formation deposited in various tissues including the kidneys, skin, and joints.

[000136] SLE is a chronic heterogeneous systemic autoimmune disease that affects many organs. Around half of people with lupus experience high blood pressure, pericarditis (inflammation of the heart), atherosclerosis (hardening of the arteries), angina, Raynaud's Syndrome, and other cardiovascular symptoms. Cutaneous lupus is the skin complications unique to lupus. They are categorized into three types: chronic cutaneous lupus, subacute cutaneous lupus, and acute cutaneous lupus. Lupus poses a significant risk to the kidneys because the disease attacks the body's renal tissue. It is estimated that roughly 6 out of 10 people with lupus go on to develop lupus nephritis and around 1 in 10 of those will develop end-stage renal disease because of this complication. Those with lupus are also more likely to develop infections and infection-related complications than those without it. The most common infections include those that affect the respiratory tract, skin, and urinary system. Opportunistic infections are also common with lupus patients, such as herpes zoster, staphylococcus aureus, E.coli, salmonella, and Candida albicans. Metabolic syndrome that can lead to the development of type II diabetes is common in lupus. People with lupus are at an increased risk of many types of cancer, including bladder, cervix, esophagus, colon, anus, liver, pancreatic, hepatobiliary, lung, non-Hodgkin's lymphoma, Hodgkin's lymphoma, larynx, leukemia, multiple myeloma, non-melanoma skin, oropharynx, renal, thyroid, cervical, vagina/vulva, and skin cancer. Both hyperthyroidism and thyroid nodules, two types of thyroid disorders, are more common in people with lupus than in the general population, and the rate of thyroid cancer doubles in lupus than in the general population. In addition, around one-third of people with lupus develop fibromyalgia, a condition that causes fatigue and widespread muscle pain across the body. Moreover, neuropsychiatric lupus, a condition with a cluster of symptoms including depression, headaches, and cognitive fog, is experienced by 80-90% of people with lupus.

[000137] SLE is typically a disease of young women ages 15 through 45, with a 10-fold greater incidence in women than in men. There is greater prevalence and severity of disease in persons of African, Hispanic, Asian, and Native American descent. While the risk of mortality has decreased to 5% to 10% at 10 years (Yen et al., Ann Intern Med, 2017, Dec 5;167(11):777-85), patients still die of active disease, infection, cardiovascular causes, and treatment-associated effects. Despite improved survival, few patients have good to excellent disease control sustained for 1 year despite medication as the disease is one of remissions and flares.

[000138] While the goals of treatment are to reduce inflammation and damage to the organs and to prevent or reverse disease exacerbations, most of the agents that have typically been used are non-specific immunosuppressants. Combinations of topical or low dose oral steroids, hydroxychloroquine, non-steroidal anti-inflammatory drug (NSAIDs), and methotrexate are used for non-organ-threatening disease, and high-dose oral or intravenous

steroids, cyclophosphamide (CY), azathioprine (AZA), mycophenolate mofetil (MMF), voclosporin, anifrolumab, and belimumab for organ-threatening disease. Aggressive treatment is warranted when vital organs are involved to prevent organ damage or failure. Many of these therapeutics fail to reduce disease activity and flares are common.

[000139] Therefore, there is a need for more targeted agents to control the disease long-term. Because of the presumed role of B cells in SLE, a B-cell inhibitor might provide a longer-term solution to disease control. However, monoclonal antibodies directed to B-cell antigens may show marked decreases in circulating B cells but do not seem to affect the large number in affected tissue. In addition, a very recent finding suggests that the most pathogenic B cells are derived and activated extrafollicularly in the tissues (Jenks et al., *Immunity*, 2018, Oct 16;49(4):725-739.e6). In addition, some of the classical B-cell markers are not present on these subsets, although they all share the expression of the CD19 marker.

[000140] Provided herein is an iPSC-derived CD19-directed CAR T cell product to drive deep and durable responses by targeting and eliminating activated B cells in tissues in active, organ-threatening lupus. The Phase 1 study described herein is designed to evaluate the safety, pharmacokinetics, and anti B-cell activity of FT819 in participants with SLE.

[000141] Treatment using derived hematopoietic lineage cells of embodiments disclosed herein, or the compositions provided herein, could be carried out upon symptom presentation, or for relapse prevention. The terms “treating,” “treatment,” and the like are used herein to generally mean obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect or symptom associated with the disease. Prophylactic benefit includes reducing the incidence and/or worsening of one or more diseases, conditions, or symptoms under treatment (e.g. as between treated and untreated populations, or between treated and untreated states of a subject). “Treatment” as used herein covers any intervention of a disease in a subject and includes: preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; inhibiting the disease (e.g., arresting its development); or relieving the disease (e.g., causing regression of the disease, or reinduction of disease response to the therapy) or one or more symptoms associated with the disease. Treatment of ongoing disease, where the treatment stabilizes or reduces the undesirable clinical symptoms of the patient, is also of particular interest. In some embodiments, the subject in need of a treatment has a disease, a condition, and/or an injury that can be contained, ameliorated, and/or improved in at least one associated symptom by a cell therapy.

[000142] FT819 drug substance comprises allogeneic T cells, derived from a clonal, T cell receptor (TCR) knockout, human-induced pluripotent stem cell (iPSC) line (master cell bank; MCB), that encode a CD19 targeted CAR with expression regulated by the T cell receptor α constant (TRAC) locus promoter. In some embodiments, manufacture of drug substance from an iPSC master cell bank involves stages of iPSC expansion, followed by stages of differentiation to T cells and T cell expansion, which may include sequential co-culture with two separate irradiated feeder cell lines. Cells that are harvested after T-cell expansion may then be washed and designated as the drug substance. The manufacture of FT819 drug product from the MCB directly address many of the limitations associated with current patient and donor specific cell therapies. Notably, many doses of FT819 drug product can be uniformly produced in a single manufacturing campaign. These doses of drug product are homogeneous and can be (i) tested to assure compliance with a predefined quality specification, (ii) cryopreserved in an infusion media, and (iii) stored to maintain a sustainable inventory. As such, in the clinical setting, FT819 has off the-shelf availability for use in multidose regimens, which may prove critical for driving long-term durable responses in patients with aggressive disease. The off-the-shelf availability of FT819, which enables multidose administration of a homogeneous cell product, also allows for clinical investigation of alternative dose schedules that could potentially convey a benefit of FT819 compared to that of patient- and donor-specific CAR T cell immunotherapies.

[000143] FT819 comprises a CD19-targeted CAR that has been specifically developed to provide enhanced CAR T-cell efficacy against CD19+ B cells. The anti-CD19 single chain fragment variable (scFv) is linked to a CD28 transmembrane domain and signaling domains from CD28 and CD3 ζ . Tyrosine to phenylalanine point mutations within the immunoreceptor tyrosine-based activation motifs (ITAMs) of the CD3 ζ chain were introduced to generate a modified ITAM configuration, CD3 ζ -1XX. The 1XX ITAM configuration acts in combination with the CD28-signaling domain to initiate calibrated cellular-signaling pathways designed to enhance CAR T cell effector function and prevent CAR T cell exhaustion. The CAR expression in FT819 is controlled by the promoter of the TRAC locus. Disruption of the TRAC locus by the insertion of the CAR in a bi allelic manner prevents the expression of the endogenous TCR and is designed to minimize the risk of graft-versus-host (GvH) responses in the setting of allogeneic cell therapy.

[000144] The regimen and dosage design of the cell product takes into consideration drug efficacy and risk reduction. In some embodiments, the regimen and dosage design is towards a subpopulation of the patients. In some embodiments, the regimen and dosage design is towards a subset of an indication, a disease, or a condition.

[000145] Specific conditions potentially related to engineered cellular immunotherapy products in general include, but are not limited to, new malignancies, new or worsening neurologic disorders, new or worsening autoimmune or rheumatologic disorders, or new hematologic disorders. Subjects may undergo safety monitoring during treatment, including assessment of the nature, frequency, and severity of adverse events (AEs).

[000146] An adverse event (AE) is any untoward medical occurrence in a patient or clinical study subject temporally associated with the use of study treatment, whether or not considered related to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product, whether or not related to the medicinal product.

[000147] Events meeting the definition of AE include, but are not limited to, the following: (i) any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (i.e., not related to progression of underlying disease); (ii) exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition; (iii) new conditions detected or diagnosed after study treatment administration even though it may have been present before the start of the study; (iv) signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction; (v) signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment(s) or a concomitant medication. Overdose per se is not considered an AE/SAE (serious adverse event).

[000148] Acute allergic/infusion reactions may occur with any treatment, including with the use of CY, FLU, bendamustine, docetaxel, cisplatin, and mAbs. Subjects may be closely monitored for the occurrence of acute allergic/anaphylactoid infusion reactions such as rigors and chills, rash, urticaria, hypotension, dyspnea, and angioedema during and following completion of the infusion. Acute allergic/infusion reactions may also be a manifestation of immunogenicity of an allogeneic cell product.

[000149] Evidence of FT819 immunogenicity and its clinical impact is monitored, assessed, and managed over the course of treatment, given that potential FT819 induced immune response may manifest only clinically, e.g., as infusion-related reactions with varying degrees of severity. FT819

[000150] FT819 is formulated in DMSO to enable cryopreservation. DMSO side effects and symptoms are monitored, assessed, and managed over the course of treatment. DMSO related

side effects are generally associated with histamine release and include coughing, flushing, rash, chest tightness and wheezing, nausea and vomiting, and cardiovascular instability. In some embodiments, the method includes slowing the rate of infusion, and/or medicating with antihistamines.

[000151] FT819 is extensively tested during manufacturing to minimize the potential risk of disease transmission. However, while FT819 is a cell therapy of human origin, the cell products may be in contact with reagents of animal origin during processing. Therefore, transmission of infectious disease and/or disease agents by known or unknown agents during the treatment is closely monitored and managed.

[000152] FT819 has been engineered to express a CD19 CAR. CRS and ICANS are toxicities that have been observed in other CD19 CAR T-cell therapies. Low-grade CRS, but not ICANS, has been observed in patients with B-cell malignancies treated with FT819. Cytokine Release Syndrome (CRS) is defined as a supraphysiologic response following any immune therapy that results in the activation or engagement of endogenous or infused immune effector cells. CRS is a clearly defined syndrome following treatment with autologous and allogeneic CAR T cell therapies, which can be fatal or life-threatening (Breyanzi USPI; Kymriah USPI; Yescarta USPI). In addition to fever, hypoxia, and hypotension, other clinical manifestations of CRS may include cardiac, gastrointestinal, hepatic, coagulation, renal, respiratory, skin, and constitutional (fever, rigors, headaches, malaise, fatigue, arthralgia, nausea, and vomiting) signs and symptoms. To consistently characterize its severity, CRS are defined and graded according to the ASCO/ASTCT CRS consensus grading. Since the signs and symptoms of CRS are not unique to CRS, clinical symptoms, such as bacteremia and other severe infections, that are not considered related to FT819 are not reported as CRS.

[000153] Neurologic toxicities arising as a result of immune therapies has been termed Immune Effector Cell-Associated Neurotoxicity Syndromes, or ICANS, defined as a disorder characterized by a pathologic process involving the CNS following any immune therapy that results in the activation or engagement of endogenous or infused immune effector cells. ICANS has been reported with CAR T-cell therapy and bispecific antibodies such as blinatumomab (Blinicyto[®] USPI). The exact mechanism of toxicity in these settings is not known, but has generally improved with treatment discontinuations and corticosteroids. Central nervous system toxicities following CD19 CAR T-cell therapy are characterized by encephalopathy, confusion, delirium, aphasia, obtundation, and seizures (Kymriah[®] USPI; Yescarta[®] USPI). Cases of cerebral edema have also been reported in CAR-T therapies.

[000154] Because FT819 is an allogeneic immune effector cell product, there is a potential risk of Acute Graft-versus-Host Disease (GvHD). In some embodiments, acute GvHD

assessments are performed with assignment of the overall severity based on the CIBMTR acute GvHD grading scale, and the method may include management of GvHD, such as by methods known in the art.

[000155] In addition, FT819 may be considered a xenotransplantation product when it comes in contact with cells of animal origin (e.g., mouse cells) during the manufacturing process. In some embodiments, the cells of animal origin serve as an ancillary material in the manufacture of drug product but are not intended to be in the drug product. Risks of receiving a xenotransplantation product may include, but are not limited to, developing infections from agents that may be associated with cells of animal origin, transmitting these infectious agents to others, and the growth of tumors. Animal cells that come into contact with the FT819 cells during the manufacturing process may originate from a master cell bank that has been extensively tested to reduce these risks. In some embodiments, subjects are monitored for clinical manifestations of xenotransplantation products throughout the course of treatment, including long-term follow-up, in case of rare associated condition or illness develops.

[000156] In some embodiments, chemotherapy is administered prior to the administration of FT819 to create an immune environment amenable to FT819 persistence and expansion. This is accomplished by promoting homeostatic proliferation of FT819 as well as eliminating regulatory immune cells and other elements of the immune system that compete for homeostatic cytokines. However, chemotherapy medications, such as Cyclophosphamide (CY) and Fludarabine (FLU), have been reported to cause myelosuppression (neutropenia and/or thrombocytopenia), immunosuppression, infections, leukopenia, anemia, and in some cases, bone marrow failure. Hematologic cytopenias could be further compounded by other factors such as underlying disease, concurrent illnesses, and concomitant medications. Close monitoring of complete blood count for the development of cytopenias and infections may be undertaken. In some embodiments, the method includes management of cytopenias and infections, including transfusion support, antimicrobial prophylaxis, and use of growth factors, such as by methods known in the art.

[000157] Warnings and precautions related to cyclophosphamide (CY) include: myelosuppression, immunosuppression, bone marrow failure, and infections; urinary tract and renal toxicity including hemorrhagic cystitis, pyelitis, ureteritis, and hematuria; cardiotoxicity including myocarditis, myopericarditis, pericardial effusion, arrhythmias, and congestive heart failure; pulmonary toxicity including pneumonitis, pulmonary fibrosis, and pulmonary veno-occlusive disease leading to respiratory failure; secondary malignancies; veno-occlusive liver disease; and embryo-fetal toxicity. Adverse reactions reported most often include neutropenia, febrile neutropenia, fever, alopecia, nausea, vomiting, and diarrhea. In some

embodiments, dose modifications due to toxicity are taken into consideration during a course of treatment.

[000158] Warnings and precautions related to fludarabine (FLU) include severe bone marrow suppression, notably anemia, thrombocytopenia, and neutropenia; transfusion-associated GvHD; severe CNS toxicity; infections; renal insufficiency; TLS; and embryo-fetal toxicity. Severe CNS toxicity was observed in patients treated at FLU doses of 96 mg/m² for 5 to 7 days. This toxicity was observed in $\leq 0.2\%$ of patients treated at FLU doses of 25 mg/m². Adverse reactions occurring in $>30\%$ of participants treated with FLU include myelosuppression (neutropenia, thrombocytopenia, and anemia), fever, infection, nausea and vomiting, fatigue, anorexia, cough, and weakness.

[000159] In some embodiments, Bendamustine may be used as an alternative chemotherapy regimen to CY/FLU. Bendamustine may be administered, and potentially in multiple treatment cycles, in participants who experienced severe hemorrhagic cystitis with prior CY. Warnings and precautions related to bendamustine include myelosuppression, infections, infusion reactions and anaphylaxis TLS; skin reactions including rash, toxic skin reactions (such as Stevens-Johnson syndrome and toxic epidermal necrolysis), and bullous exanthema; other malignancies; and fetal harm. Compared with CY/FLU chemotherapy, bendamustine chemotherapy was associated with lower rates of CRS, neurotoxicity, hematological toxicities, infections, neutropenic fever, and post-infusion hospitalization while providing similar clinical efficacy.

[000160] In comparison to autologous CAR T-cell therapy in SLE, FT819 can be administered in an off-the-shelf/on-demand fashion without the need for leukapheresis. Since immunosuppression necessary for autologous CAR T-cell therapy can be omitted, or discontinued immediately, prior to pretreatment and infusion of FT819, the risk for SLE flare due to immunosuppression would reduce. In some embodiments, the SLE flare risk reduction in SLE treatment using FT819 is substantial.

[000161] In one aspect, of the present invention provides a method of treating lupus or a symptom thereof in a subject. In some embodiments, the method comprises (a) administering to the subject one or more doses of chemotherapy; and (b) after step (a), administering to the subject one or more doses of an adoptive cell therapy product in a first effective amount; wherein the adoptive cell therapy product comprises an engineered T lineage effector cell comprising (i) expression of a CD19-CAR (chimeric antigen receptor) at a T-cell receptor α constant (TRAC) locus, and (ii) a T cell receptor (TCR) knockout.

[000162] In some embodiments, the method further comprises (c) after step (b), administering to the subject one or more doses of an additional chemotherapy, wherein the additional chemotherapy is the same or different as the chemotherapy of step (a); and (d) after

step (c), administering to the subject one or more doses of an adoptive cell therapy product in a second effective amount that is the same or different as the first effective amount.

[000163] In some embodiments, the lupus or the symptom thereof comprises: (i) cutaneous lupus; (ii) lupus nephritis; (iii) neuropsychiatric lupus; or (iv) pericarditis, atherosclerosis, angina, Raynaud's syndrome, renal disease, metabolic syndrome, thyroid disorder, fibromyalgia, respiratory tract infection, skin infection, or urinary system infection.

[000164] In some embodiments, the chemotherapy comprises (a) one or more of cyclophosphamide (CY) or fludarabine (FLU); or (b) bendamustine. In some embodiments, CY is administered as a conditioning agent. In some embodiments FLU is administered as a conditioning agent. In some embodiments, both CY and FLU are administered for conditioning. In some embodiments, CY is not used for conditioning. In some embodiments, FLU is not used for conditioning. In some other embodiments, neither CY nor FLU are used for conditioning. In yet some other embodiments, bendamustine is used for conditioning. In some embodiments, the chemotherapy is administered one or more days prior to administering the adoptive cell therapy product; optionally wherein the chemotherapy is administered on one or more of (i) three days before the adoptive cell therapy product, (ii) four days before the adoptive cell therapy product, or (iii) five days before the adoptive cell therapy product. In some embodiments, the chemotherapy comprises cyclophosphamide administered at a daily dose of about 250 mg/m² to about 600 mg/m² and fludarabine administered at a daily dose of about 20 mg/m² to about 40 mg/m² for 3 consecutive days starting from about 4-6 days prior to day 1 of administering the adoptive cell therapy product. In some embodiments, the chemotherapy comprises bendamustine and is administered at a daily dose of about 30 mg/m² to about 150 mg/m² for 2 consecutive days starting from about 4-6 days prior to day 1 of administering the adoptive cell therapy product.

[000165] In some embodiments, the engineered T lineage effector cell is derived from an engineered induced pluripotent stem cell (iPSC) comprising a TCR knockout, and a polynucleotide encoding the CD19-CAR. In some embodiments, the first effective amount of the adoptive cell therapy product is about 1.8×10^8 cells to about 9×10^9 cells, and is optionally escalated. In some embodiments, the first effective amount of the adoptive cell therapy product is about 3.6×10^8 cells, about 1×10^9 cells, about 3×10^9 cells, or about 9×10^9 . In some embodiments, the adoptive cell therapy product is cryopreserved, and then thawed prior to administering. In some embodiments, the adoptive cell therapy product is FT819.

[000166] In some embodiments, the subject has had a lupus diagnosis. In some embodiments, the subject is positive in at least one of (a) an anti-nuclear antibody; (b) an anti-dsDNA antibody; or (c) an anti-Smith antibody. In some embodiments, treating lupus comprises

obtaining lupus remission or low activity in a subject. In some embodiments, remission or reduced disease activity is defined by DORIS (Definition of remission in systemic lupus erythematosus) complete remission, DORIS clinical remission on treatment, and/or LLDAS (lupus low disease activity state). In some embodiments, treating lupus comprises controlling lupus flare. In some embodiments, the treatment comprises longer disease-free intervals off immunosuppressive medications, decreases autoantibody production in the subject; and/or reconstitutes healthy B cell compartment in the subject as compared to B cells under active lupus. In some embodiments, the subject previously received one or more prior treatments comprising glucocorticoids, CY, mycophenolic acid or its derivatives, belimumab, methotrexate, azathioprine, anifrolumab, rituximab, obinutuzumab, cyclosporin, tacrolimus, or voclosporin. In some embodiments, the subject did not respond to the one or more prior treatments.

[000167] In one aspect, of the present invention provides a method of treating lupus or a symptom thereof in a subject. In some embodiments, the method comprises (a) administering to the subject one or more doses of chemotherapy; and (b) after step (a), administering to the subject one or more doses of an adoptive cell therapy product in a first effective amount; wherein the adoptive cell therapy product comprises an engineered T lineage effector cell comprising (i) expression of a CD19-CAR (chimeric antigen receptor) at a T-cell receptor α constant (TRAC) locus, and (ii) a T cell receptor (TCR) knockout; and wherein the subject previously received and failed to respond to one or more prior treatments comprising glucocorticoids, CY, mycophenolic acid or its derivatives, belimumab, methotrexate, azathioprine, anifrolumab, rituximab, obinutuzumab, cyclosporin, tacrolimus, or voclosporin.

[000168] Lupus typically involves periods of markedly increased disease activity (called “flares”) alternating with suppressed or decreased disease activity. A flare has been defined as a measurable increase in disease activity in one or more organ systems involving new or worse clinical signs and symptoms and/or laboratory measurements. Flares are typically taken as an indication to initiate or change treatment (e.g., escalating dose of treatment a subject is already taking, or introducing a new therapeutic intervention). In some embodiments, a flare may be characterized or defined by hospitalization due to SLE. In some embodiments, a flare may be or comprise a measurable increase in disease activity in one or more organ systems involving new or worse clinical signs and symptoms and/or laboratory measurements, for example, as compared to a previously taken measurement. Different clinical measurements have been used to determine whether a subject with lupus is having a clinic flare. One such measurement is the Systemic Lupus Erythematosus Disease Activity Index SLENA Modification. This scale uses a point system to calculate when the accumulated significance of recent changes in various indicators translates into a mild/moderate (SLENA-SLEDA Index of 3-11 point change) or a

severe (12 of more point change) flare. Other examples of disease metrics for categorizing lupus activity and flare identification include Physician Global Assessment (PGA), SLEDAI-2K, and BILAG-2004. In some embodiments, flare is characterized by or defined as a change of greater than or equal to 1.0 in the physician's global assessment of disease activity (measured on a 0-3) scale from the previous visit or from a visit within the last 200 days (e.g., last 100 days, e.g., last 93 days, e.g., last 75 days, e.g., last 50 days, e.g., last 25 days, e.g., last 10 days, e.g., last 5 days, e.g., last 1 day). In some embodiments, a lupus flare is defined as >1 new BILAG-2004 A or >2 new (worsening) BILAG-2004 B domain scores over a predetermined observation period (e.g., a month).

[000169] In one aspect, methods of treating lupus disclosed herein comprise controlling lupus flares. Controlling lupus flares may comprise shortening flare duration, reducing flare severity, and/or reducing the incidence of flares in a subject. Improvements achieved in controlling lupus flare may be by comparison to the treated subject prior to receiving treatment, or to a reference population of untreated subjects with lupus.

ILLUSTRATIVE EMBODIMENTS

[000170] The present disclosure provides the following illustrative embodiments.

[000171] Embodiment 1. A method of treating lupus or a symptom thereof in a subject, the method comprising:

- (a) administering to the subject one or more doses of chemotherapy; and
- (b) after step (a), administering to the subject one or more doses of an adoptive cell

therapy product in a first effective amount;

wherein the adoptive cell therapy product comprises an engineered T lineage effector cell comprising (i) expression of a CD19-CAR (chimeric antigen receptor) at a T-cell receptor α constant (TRAC) locus, and (ii) a T cell receptor (TCR) knockout.

[000172] Embodiment 2. The method of Embodiment 1, further comprising:

(c) after step (b), administering to the subject one or more doses of an additional chemotherapy, wherein the additional chemotherapy is the same or different as the chemotherapy of step (a); and

(d) after step (c), administering to the subject one or more doses of an adoptive cell therapy product in a second effective amount that is the same or different as the first effective amount.

[000173] Embodiment 3. The method of Embodiment 1, wherein the lupus or the symptom thereof comprises: (i) cutaneous lupus; (ii) lupus nephritis; (iii) neuropsychiatric lupus; or (iv) pericarditis, atherosclerosis, angina, Raynaud's syndrome, renal disease, metabolic

syndrome, thyroid disorder, fibromyalgia, respiratory tract infection, skin infection, or urinary system infection.

[000174] Embodiment 4. The method of any one of Embodiments 1-3, wherein the chemotherapy comprises (a) one or more of cyclophosphamide (CY) or fludarabine (FLU); or (b) bendamustine.

[000175] Embodiment 5. The method of any one of Embodiments 1-4, wherein the chemotherapy is administered one or more days prior to administering the adoptive cell therapy product; optionally wherein the chemotherapy is administered on one or more of (i) three days before the adoptive cell therapy product, (ii) four days before the adoptive cell therapy product, or (iii) five days before the adoptive cell therapy product.

[000176] Embodiment 6. The method of any one of Embodiments 1-5, wherein the chemotherapy comprises cyclophosphamide administered at a daily dose of about 250 mg/m² to about 600 mg/m² and fludarabine administered at a daily dose of about 20 mg/m² to about 40 mg/m² for 3 consecutive days starting from about 4-6 days prior to day 1 of administering the adoptive cell therapy product.

[000177] Embodiment 7. The method of any one of Embodiments 1-6, wherein the chemotherapy comprises bendamustine and is administered at a daily dose of about 30 mg/m² to about 150 mg/m² for 2 consecutive days starting from about 4-6 days prior to day 1 of administering the adoptive cell therapy product.

[000178] Embodiment 8. The method of any one of Embodiments 1-7, wherein the engineered T lineage effector cell is derived from an engineered induced pluripotent stem cell (iPSC) comprising a TCR knockout, and a polynucleotide encoding the CD19-CAR.

[000179] Embodiment 9. The method of any one of Embodiments 1-8, wherein the first effective amount of the adoptive cell therapy product is about 1.8×10^8 cells to about 9×10^9 cells, and is optionally escalated.

[000180] Embodiment 10. The method of Embodiment 9, wherein the first effective amount of the adoptive cell therapy product is about 3.6×10^8 cells, about 1×10^9 cells, about 3×10^9 cells, or about 9×10^9 .

[000181] Embodiment 11. The method of any one of Embodiments 1-10, wherein the adoptive cell therapy product is cryopreserved, and then thawed prior to administering.

[000182] Embodiment 12. The method of any one of Embodiments 1-11, wherein the adoptive cell therapy product is FT819.

[000183] Embodiment 13. The method of any one of Embodiments 1-12, wherein the subject has had a lupus diagnosis.

[000184] Embodiment 14. The method of any one of Embodiments 1-12, wherein the subject is positive in at least one of (a) an anti-nuclear antibody; (b) an anti-dsDNA antibody; or (c) an anti-Smith antibody.

[000185] Embodiment 15. A method of obtaining lupus remission or low activity in a subject according to any one of Embodiments 1-14.

[000186] Embodiment 16. The method of Embodiment 15, wherein the lupus remission or low activity is defined by DORIS (Definition of remission in systemic lupus erythematosus) complete remission, DORIS clinical remission on treatment, and/or LLDAS (lupus low disease activity state).

[000187] Embodiment 17. A method of controlling lupus flare according to any one of Embodiments 1-14.

[000188] Embodiment 18. The method according to any one of Embodiments 1-14, wherein the treating comprises longer disease-free intervals off immunosuppressive medications, decreases autoantibody production in the subject; and/or reconstitutes healthy B cell compartment in the subject as compared to B cells under active lupus.

[000189] Embodiment 19. The method according to any one of Embodiments 1-18, wherein the subject previously received one or more prior treatments comprising glucocorticoids, CY, mycophenolic acid or its derivatives, belimumab, methotrexate, azathioprine, anifrolumab, rituximab, obinutuzumab, cyclosporin, tacrolimus, or voclosporin.

[000190] Embodiment 20. The method of Embodiment 19, wherein the subject did not respond to the one or more prior treatments.

[000191] Embodiment 21. A method of treating lupus or a symptom thereof in a subject, the method comprising:

(a) administering to the subject one or more doses of chemotherapy; and

(b) after step (a), administering to the subject one or more doses of an adoptive cell therapy product in a first effective amount;

wherein the adoptive cell therapy product comprises an engineered T lineage effector cell comprising (i) expression of a CD19-CAR (chimeric antigen receptor) at a T-cell receptor α constant (TRAC) locus, and (ii) a T cell receptor (TCR) knockout; and

wherein the subject previously received and failed to respond to one or more prior treatments comprising glucocorticoids, CY, mycophenolic acid or its derivatives, belimumab, methotrexate, azathioprine, anifrolumab, rituximab, obinutuzumab, cyclosporin, tacrolimus, or voclosporin.

[000192] Embodiment 22. A method of treating lupus or a symptom thereof in a subject, the method comprising:

administering to the subject one or more doses of an adoptive cell therapy product in a first effective amount;

wherein the adoptive cell therapy product comprises an engineered NK lineage effector cell comprising a CD38 knockout and expression of (i) a CD19-CAR (chimeric antigen receptor); (ii) an alloimmune defense receptor (ADR) targeting 4-1BB; (iii) a high-affinity, non-cleavable CD16 (hnCD16); and (iv) an interleukin-15 (IL15)/IL15 receptor fusion protein (IL15RF).

[000193] Embodiment 23. The method of Embodiment 22, wherein the method does not comprise administration of chemotherapy for lympho-conditioning.

[000194]

EXAMPLES

[000195] The following examples are offered by way of illustration and not by way of limitation.

EXAMPLE 1 – Activity and Biodistribution of FT819

[000196] Autoreactive B cells are key drivers of various autoimmune diseases such as systemic lupus erythematosus (SLE), lupus nephritis, rheumatoid arthritis (RA), and scleroderma. Management of autoimmune disease often includes prolonged use of immune suppressants and B cell directed agents. For example, rituximab, an anti-CD20 antibody, has demonstrated the ability to deplete peripheral blood B cells. However, the clinical efficacy of rituximab is limited and varies broadly among different autoimmune patient populations due to incomplete clearance of autoreactive B cells in secondary lymphoid tissues. FT819 co-cultured with PBMCs from five healthy donors demonstrated potent and specific CD19+ B cell killing in an in vitro cytotoxicity assay. Similarly, PBMCs derived from SLE and RA patients exhibited durable elimination of CD19+ B cells in the same assay.

[000197] Biodistribution studies in immunodeficient mice illustrated the ability of FT819 to be well distributed throughout the mouse, including in tertiary tissues that are implicated in autoimmune diseases, such as bone marrow and spleen. Blood and tumor samples obtained from lymphoma patients treated with FT819 were evaluated for FT819 mediated B cell effects by flow cytometry and immunohistochemistry. After FT819 infusion, peripheral blood B cells were nearly undetectable in a majority of the patients with sustained suppression up to 29 days. Importantly, B cell depletion was also observed in lymph node biopsies illustrating the ability of FT819 to reach secondary tissues. The recovery of B cells in the periphery occurred 1-3 months

after the treatment cycle, indicating an avoidance of prolonged B cell aplasia by B cell reconstitution. Collectively, FT819 demonstrates a unique potential to be a promising treatment paradigm for autoimmune disease. Its superior safety profile in oncology coupled with the renewable source of off-the-shelf product makes FT819 ideal for combination therapy with standard of care regimens (including, but not limited to, glucocorticoids, CY, mycophenolic acid or its derivatives, belimumab, methotrexate, azathioprine, anifrolumab, rituximab, obinutuzumab, cyclosporin, tacrolimus, and voclosporin) in autoimmune diseases.

EXAMPLE 2 – FT819 Study Design

[000198] Lupus patients with organ-threatening disease often go through cycles of remission and flares, yet the disease activity persists despite the most current medications available. Disease persistence causes end-organ damage that results in, for example, the need for a kidney transplant. A therapy that could reset the immune system is needed for autoimmune therapies. This study was designed to assess the safety, tolerability, and clinical activity of FT819 in a lupus population with organ threatening disease activity. It is to explore whether FT819 can provide longer disease-free intervals off all immunosuppressive medications, and/or decrease autoantibody production in patients, and/or reconstitute a healthy B cell compartment as compared to B cells in active lupus.

[000199] FT819 drug substance utilized in the present example comprises allogeneic T cells, derived from a T-cell receptor (TCR) knockout iPSC line, and express a CD19 targeted CAR. Manufacture of drug substance from the iPSC master cell bank involved stages of iPSC expansion, followed by stages of differentiation to T cells and T cell expansion. Cells that were harvested after T-cell expansion were then washed and designated as the drug substance. FT819 is uniform in composition. The study as described herein was designed to evaluate the safety, pharmacokinetics (PK), and anti-B cell activity of FT819 in patients with SLE. Subjects are enrolled in 2 stages: a dose-escalation stage and a dose expansion stage. After the safety and tolerability have been assessed to define the maximum tolerated dose (MTD) in the dose-escalation stage, the dose expansion stage further evaluates the safety and activity of FT819.

[000200] The human clinical study includes a screening period, followed by 2 or 3 consecutive days of chemotherapy, depending on the chemotherapy regimen (including those standard of care regimens for autoimmune diseases), prior to infusion of FT819 on Day 1. The DLT (dose limited toxicity) evaluation period is from the time of FT819 administration on Day 1 to Day 29, followed by post-treatment follow-up (Fig. 1). As noted in FIG. 1, bendamustine may be used instead of CY/FLU. In the timeline of FIG. 1, there is no Day 0; rather, days within a cycle progress from Day -5 to Day -1, followed by Day 1, when FT819 is infused. The

FT819 treatment cycle proceeds through Day 29. Following completion of FT819 treatment cycle, defined as the treatment cycle completion/early treatment discontinuation visit, participants enter the post-treatment follow-up (PTFU) phase, and, maybe additionally, the long-term follow-up (LTFU), of the study and are regularly monitored for new and/or resolving treatment-emergent adverse events (TEAEs) and exploratory assessments. Participants with progressive disease (PD) during the PTFU may be eligible to receive additional cycle(s) of retreatment.

[000201] Standard lupus assessments including the Hybrid SLEDAI and Physician Global Assessment (PGA) are performed throughout the treatment. In addition, the Functional Assessment of Chronic Illness Therapy (FACIT) scale is included to assess fatigue. Standard laboratory measurements as well as immune markers and autoantibodies are obtained, and exploratory markers collected for assay development.

EXAMPLE 3 – FT819 Dose Escalation and Dose Expansion

[000202] The optimal dose of CAR T-cell therapies in lupus and other autoimmune diseases is not known, and to date has largely been based on doses and schedules that have been investigated in oncology indications. The objective of the dose-escalation stage in the present study is to determine dose levels of FT819 not exceeding the MTD appropriate for further assessment in dose expansion to determine the recommended Phase 2 dose (RP2D). Assessment of successive dose levels to determine continuation of dose escalation is conducted in accordance with the dose-escalation rules as provided herein.

[000203] Dosing is based on CD19 CAR expression, where $\geq 90.0\%$ of administered viable FT819 cells express CD19 CAR. Dose escalation commences at DL1. As shown in Fig. 2, the initial FT819 dose level in dose escalation (Dose Level 1 [DL1]) starts with 3.6×10^8 VC/dose (viable cells per dose). When the DL1 dose-escalation cohort clears DLT assessment then dose escalation proceeds to the next dose level (DL2). When DL1 does not exceed the MTD in the lupus setting, successive dose levels would not exceed 3 times ($\pm 15\%$ variance) the preceding highest dose level that has been cleared. If DL1 shows evidence of clinical activity (SLEDAI of 0 within 3 months), lower FT819 doses is explored. In some embodiments, DL1 is not tolerable or exceeds the MTD (maximum tolerated dose), then DL0, 1.8×10^8 VC/dose, is assessed; and when DL0 does not exceed the MTD, dose expansion at DL0 proceeds under the same expansion scheme. In the absence of dose-limiting toxicities (DLTs), dose levels may be explored further based on observed safety, tolerability, activity, and PK and pharmacodynamic data.

[000204] The FT819 dose for each regimen to be administered in dose expansion is determined based on the clinical and available PK and pharmacodynamic data from the respective dose-escalation stage and does not exceed the MTD. One or more dose levels that have cleared DLT assessment are evaluated in dose expansion to further define clinical activity. Combined safety and efficacy analyses from both dose escalation and dose expansion are used to determine the RP2D for each regimen.

EXAMPLE 4 – FT819 Treatment Regimen

[000205] The purpose of chemotherapy prior to the administration of FT819 is creating an immune environment amenable to FT819 persistence and expansion. While the mechanism is not fully understood, the combination of CY and FLU may induce cytokines that promote homeostatic proliferation and eliminate regulatory immune cells and other elements of the immune system that compete for homeostatic cytokines, thereby promoting homeostatic proliferation of FT819.

[000206] In some embodiment, bendamustine is an alternative to CY/FLU treatment before administration of the cell product in subjects who experienced severe hemorrhagic cystitis with prior CY. Bendamustine is an alkylating agent with a well-characterized and favorable safety profile as a component of immunochemotherapy regimens. The planned doses of CY/FLU and bendamustine are identical to those used for approved CAR T cell therapies (Breyanzi® USPI; Kymriah USPI; Yescarta USPI).

[000207] In treating patients with lupus, the starting dose of FT819 is about 3.6×10^8 VC/dose following a course of chemotherapy consisting of CY/FLU or bendamustine (Figure 1). Dose escalation proceeds by relative dose level increases, starting with DL2, consisting of intervals no greater than 3-times the previously cleared dose level. The relative dose interval is designed to allow flexibility to dose escalate by a smaller increment in the case of any non-DLT safety observations such that a smaller escalation increment is more beneficial, or is associated with lower safety risk. Multiple dose levels that have cleared DLT assessment in dose escalation may be evaluated in dose expansion to further define the risk-benefit profile at each dose level to determine the dose of FT819 that confers maximal clinical benefit in lupus. In some embodiments, the dose is an amount described herein. In some examples, where a first dose of FT819 to patients exhibits safety and disease remission, a lower dose of FT819 may be explored. A lower dose of FT819 for lupus treatment is possible in comparison to for B cell malignancy treatment because the B-cell burden in SLE could be much lower than that in B-cell malignancy.

EXAMPLE 5 –Patient Population for FT819 Treatment of Lupus in Each Study Cohort

[000208] Patients suitable for FT819 treatment include those who were diagnosed with SLE by the European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) classification criteria and are positive for at least one of the autoantibodies including, but not limited to, (1) antinuclear antibody positive by immunofluorescence $\geq 1:160$, homogeneous or ring pattern characteristic of lupus; (2) anti-dsDNA positive with confirmatory positive Crithidia test; and (3) anti-Smith positive. In some embodiments, the patients have active SLE at the time of screening.

[000209] Active SLE is defined as having SLEDAI of 8 or more (with a clinical SLEDAI of 4 or greater excluding alopecia, mucosal ulcers, and fever); and having one or more major organ systems with British Isles Lupus Assessment Group (BILAG) A score, excluding musculoskeletal, mucocutaneous, and/or constitutional organ systems. In some embodiments, the SLE patients suitable for FT819 treatment were not treated previously. In some embodiments, the SLE patients suitable for FT819 treatment have previously failed to respond to glucocorticoids (e.g., disease symptoms were not significantly improved, or worsened despite treatment). In some embodiments, the SLE patients suitable for FT819 treatment have previously failed at least one of treatments including, but not limited to: CY, mycophenolic acid or its derivatives, belimumab, methotrexate, azathioprine, anifrolumab, rituximab, obinutuzumab, cyclosporin, tacrolimus, and voclosporin. In some embodiments, the SLE patients suitable for FT819 treatment have failed at least two of the above treatments.

EXAMPLE 6 – Study Interventions and Concomitant Therapy

[000210] Study interventions are all pre-specified investigational and non-investigational medicinal products intended to be administered to the study participants during the study, which include FT819 (designated as Investigational Medicinal Product (IMP)), and authorized auxiliary medicinal product (AxMP) that are non-IMP, such as cyclophosphamide (CY), fludarabine (FLU), and bendamustine. Study interventions are according to Table 1 below:

Table 1: Study Interventions

Study Intervention	Route of Administration	Dose and Schedule
Chemotherapy^a		
Cyclophosphamide	IV infusion	500 mg/m ² ; Days -5, -4, and -3
Fludarabine	IV infusion	30 mg/m ² ; Days -5, -4, and -3

Study Intervention	Route of Administration	Dose and Schedule
<i>OR</i>		
Bendamustine	IV infusion	90 mg/m ² ; Days -5 and -4
FT819	IV infusion	Dose to be determined per dose escalation cohort; Day 1 FT819 should be administered 3-4 calendar days after the last dose of CY/FLU, or 4-5 days after the last dose of bendamustine.

[000211] FT819 drug product comprises allogeneic T cells derived from a clonal, TCR knockout iPSC line and express a CD19 targeted CAR regulated by the TRAC locus promoter. FT819 cells are suspended in infusion medium comprising albumin (human) and DMSO. In some embodiments, the FT819 cells are comprised in a cryopreserved bag and thawed at the site of administration. In some embodiments, FT819 is administered using an IV (intravenous) administration set with an in-line filter.

[000212] Prior to administration of FT819, participants are pre-medicated with acetaminophen 650 mg orally (PO) and diphenhydramine 25 to 50 mg PO or IV before and 4 to 6 hours after administration. Corticosteroids are not used as pre-medication for FT819.

[000213] Immediately prior to CY administration, participants receive 500 mL of IV normal saline per institutional standards. Participants may receive additional IV normal saline following CY administration following assessment of hydration status. Dose adjustments for weight/creatinine may also be made. CY is administered as an IV infusion at a dose of about 500 mg/m² for 3 consecutive days on Day -5, Day -4, and Day -3 of the treatment cycle. CY dosing is calculated based on actual body weight (ABW). If ABW is >150% of the ideal body weight (IBW) then the dose is computed using adjusted body weight as follows:

$$\text{Adjusted body weight} = \text{IBW} + 0.5(\text{ABW} - \text{IBW})$$

[000214] FLU is administered as an IV infusion at a dose of about 30 mg/m² for 3 consecutive days on Day -5, Day -4, and Day -3 of the treatment cycle. Dose adjustments for weight and/or renal function (e.g., as assessed by creatinine clearance) may be made. Bendamustine is administered as an IV infusion at a dose of 90 mg/m² for 2 consecutive days (Days -5 and -4).

[000215] In some embodiments, dose and schedule modifications may be made for CY/FLU. For participants with moderate impairment of renal function (creatinine clearance of

30-70 mL/min/1.73 m²), the FLU dose is reduced by 20%. FLU is not administered to participants with severely impaired renal function (creatinine clearance <30 mL/min/1.73 m²).

[000216] In some embodiments, dose and schedule modifications may be made for bendamustine. Bendamustine is not administered to participants who have renal or hepatic impairment as defined by: creatinine clearance <40 mL/min; AST or ALT that is 2.5 to 10 × ULN, and total bilirubin that is 1.5 to 3 × ULN; or total bilirubin >3 × ULN.

[000217] In some embodiments of the FT819 treatment of SLE, concomitant therapies are given for intervention. Concomitant therapy includes any medication (e.g., prescription drugs, over-the-counter drugs, vaccines, herbal or homeopathic remedies, nutritional supplements) used by a patient in addition to protocol-mandated intervention. In some embodiments, the concomitant medication is for SLE. In some embodiments, the concomitant medication is for non-SLE. In some embodiments, the concomitant medication comprises those for lupus flare. In some embodiments, the concomitant medication comprises those for SAEs.

[000218] In some embodiments, the lupus patients under the FT819 treatment may also be under a therapy with prednisone and/or hydroxychloroquine. In some embodiment the prednisone is at a low dose up to 10 mg or equivalent PO per day. In some embodiments, systemic corticosteroids given by intramuscular or IV routes is avoided.

EXAMPLE 7 – Disease Response Assessment and Efficacy Analyses

[000219] Disease response is assessed using Hybrid SLEDAI (systemic lupus erythematosus disease activity index), FACIT (Functional Assessment of Chronic Illness Therapy), and PGA.

[000220] The Hybrid SLEDAI detect amounts of lupus disease activity and identify improvements and worsening of disease and evaluates dozens of lupus manifestations. Each parameter/manifestation is scored if present, and each parameter's score is weighted from 1 to 8. Parameters with weights of 8 include seizure, psychosis, organic brain syndrome, visual disturbance, cranial nerve disorder, lupus headache, cerebrovascular accident, and vasculitis. Parameters with weights of 4 include arthritis, myositis, urinary casts, hematuria, proteinuria, and pyuria. Parameters with weights of 2 include rash, alopecia, mucosal ulcers, pleurisy, pericarditis, low complement, and increased DNA binding. Parameters with weights of 1 include fever, thrombocytopenia, and leukopenia. Scores of 1 to 5 reflect mild disease, 6 to 12 indicate moderate disease, and 13 to 20 indicate severe disease. Study inclusion criteria requires a Hybrid SLEDAI score of 8 or more, with a clinical SLEDAI of 4 or greater excluding alopecia, mucosal ulcers, and fever; AND one or more major organ systems with BILAG A score, excluding musculoskeletal, mucocutaneous, and/or constitutional organ systems

(involvement of renal, peri/myocarditis, pleuritis/lung involvement and/or non-CNS vasculitis must be moderate to severe and likely to be reversible in the opinion of the investigator).

[000221] FACIT-Fatigue is an assessment scale that measures an individual's level of fatigue during their daily activities over the past week. The scale measures over a dozen of items, and each item is answered on a 0 (not at all) to 4 (very much) scale. Lower total scores reflect more fatigue.

[000222] The PGA is a physician-rated, visual analog scale ranging from 0 ("no disease activity") to 3 ("most severe disease activity") for measuring disease activity in patients with SLE in the preceding month. The scale incorporates values 1 and 2 as inner markers to categorize disease activity as mild (≥ 0.5 to 1), moderate (>1 to ≤ 2), and severe (>2 to 3). The PGA is intended to rate the overall disease activity taking into account the severity of active manifestation and clinical laboratory results, but excluding organ damage, serology, and subjective findings unrelated to disease activity.

[000223] Efficacy analyses are performed based on the efficacy-evaluable population. SLE remission is defined in three manners: DORIS (Definition of remission in systemic lupus erythematosus) complete remission, DORIS clinical remission on treatment, and LLDAS (lupus low disease activity state).

[000224] DORIS Clinical Remission on Treatment is described as:

- Clinical SLE disease activity index (SLEDAI) = 0, exclusive of measures of autoantibodies and C3/C4 levels
- Serological activity allowed
- SELENA-SLEDAI Physician Global Assessment (PGA) ≤ 0.5 (scale 0-3)
- Low-dose glucocorticoids (e.g., prednisone ≤ 5 mg/day) allowed
- Maintenance antimalarials, immunosuppressives, and/or stable (maintenance) biologics allowed

[000225] DORIS Complete Remission is described as:

- Clinical SLEDAI = 0, exclusive of measures of autoantibodies and C3/C4 levels
- No serological activity
- SELENA-SLEDAI PGA ≤ 0.5 (scale 0-3)
- No glucocorticoids
- Maintenance antimalarials allowed, but no immunosuppressives and/or biologics

[000226] The operational definition of a lupus low disease activity state (LLDAS) is fulfilled when all the following criteria are met:

- Hybrid systemic lupus erythematosus disease activity index (SLEDAI) ≤ 4 , with no activity in major organ systems;

- No new features of lupus disease activity compared to the previous assessment;
- Serological activity allowed (as long as total Hybrid SLEDAI ≤ 4);
- SELENA-SLEDIA Physician Global Assessment (PGA) ≤ 1 (scale 0-3);
- Current prednisolone (or equivalent) dose ≤ 7.5 mg; and
- Standard maintenance doses of immunosuppressive drugs and approved biological agents, excluding investigational drugs.

[000227] The percentage of participants reaching these criteria are computed at Months 3, 6, 9, and 12 as well as overall. The denominators for the percentage of participants achieving DORIS or LLDAS are based on the number of participants completing the necessary assessments at that visit.

[000228] As a secondary endpoint, the PK of FT819 is assessed by the detection of FT819 in peripheral blood (PB) following FT819 administration. To characterize the PK of FT819, PB samples are collected before and after FT819 infusion on days when FT819 is administered. Descriptive summaries including sample size (n), mean, standard deviation, standard error, median, Q1, Q3, and minimum and maximum values are provided for maximum observed concentration (C_{max}) and area under the curve (AUC).

[000229] Assessment of the pharmacodynamics of one cycle of FT819 is measured by the changes in immunoglobulin serum levels (i.e., IgG, IgM, IgA), serum complement components (i.e., C3, C4), and blood lymphocyte subsets (e.g., T, B, NK) from baseline at each scheduled assessment. Exploratory analyses are descriptive and include assessments of potential predictive and prognostic biomarkers in PB, serum or urine. The association between the PK of FT819 and other pharmacodynamic markers with safety and clinical activity will be assessed descriptively.

[000230] The association between baseline clinical and disease characteristics with the safety and clinical activity of FT819 is also explored. Blood samples are collected for detection of alloimmunization to the FT819 product human leukocyte antigen (HLA) by panel-reactive antibody. Additional blood, serum, and urine samples are collected for exploratory biomarker analyses that include, but are not limited to:

- Measurement of PB cytokine levels
- Measurement of autoantibody and complement levels
- Measurement of protein biomarkers of disease activity exploratory immune monitoring
- PBMC functional characterization and immunophenotyping, e.g., T-cell subset analysis
- Cellular immunogenicity

- Evaluation of urine protein biomarkers of disease activity

[000231] In some embodiments, assays and other exploratory analyses include, but are not limited to, analysis of lymphocytes, T-cell activation, TCR repertoire, cytokines associated with inflammation, complement activation markers, and B-cell subsets and activation.

EXAMPLE 8 – FT819 demonstrates rapid and deep depletion of CD19+ B cells from the peripheral blood of unmatched SLE patients

[000232] Rapid and deep depletion of CD19+ B cells was observed in a 72-hour killing assay against PBMCS (E:T 1:1) derived from an unmatched blood sample of a patient with SLE (FIG. 3). Diseased CD19+ B cell population in the periphery and tissue is estimated to be up to 300 million cells in autoimmune disease patients. As shown in FIG. 4, FT819 displayed improved targeting of CD19+ B cells from unmatched blood samples of SLE patients as compared to autologous-manufactured CAR T cells or allogeneic manufactured CAR T cells. In one E:T ratio analysis for this particular test, results suggest that at a dose of 100-300 million CAR T cells, FT819 can sufficiently deplete fully the CD19+ B cell burden of patients with SLE. The autologous-manufactured CAR T cells, in this analysis, were primary T cells transduced with lentivirus containing CAR transgene and cultured for 4 additional days for recovery and expansion; and the allogeneic-manufactured CAR T cells were primary T cells engineered with CRISPR targeting TRAC locus with CAR transgene and cultured for 11 additional days for recovery and expansion.

EXAMPLE 9 – FT819 demonstrates key therapeutic mechanisms of dose-dependent PK, rapid and deep B cell depletion, and naïve B cell reconstitution

[000233] In vivo CAR T-cell expansion and persistence were measured by a ddPCR assay according to the number of CAR transgene copies per microgram of genomic DNA in blood samples. The starting cell dose for the FT819 clinical trial in patients with SLE is 360 million cells (NCT06308978). FIG. 5 shows FT819 PK (mean $\pm$ SEM) at 90 and 360 million cells in a patient treatment setting of B-cell lymphoma (BCM). The profile of FT819 shows an expansion kinetics of reaching C_{max} at approximately Day 8, a dose-dependent PK increase, and its controlled and defined persistence in peripheral blood, which may enable more rapid reconstitution of the B-cell compartment and reduce complications associated with B-cell aplasia.

[000234] The data shown in FIG. 6 includes 23 patients with baseline B-cell counts in the range of normal (up to 1,000 cells/ μ L; mean $\pm$ SEM). Baseline represents B-cell counts in the peripheral blood prior to treatment with standard conditioning chemotherapy and single-dose

FT819. During a single dose of FT819 treatment cycle in these patients, a rapid and deep B-cell depletion in peripheral blood with sustained suppression of B cells was observed using flow cytometry plot (FIG. 6). A case study of two patients whose B-cell depletion was resistant to Cy / Flu conditioning chemotherapy also had rapid and deep B-cell depletion as illustrated in FIG. 7, following a single dose of FT819, showing FT819's capability of B-cell depletion independent of Cy/Flu conditioning. Further, FIG. 8 represents 4 patients treated with bendamustine as an alternative to Cy / Flu conditioning chemotherapy and a single-dose FT819. The baseline B-cell counts of these patients were in the range of normal (up to 1,000 cells/ μ L), and the depleted B-cell counts in the peripheral blood of these patients were either rapid and deep, and/or sustained. Together, FIGs 7 and 8 show that FT819 is capable of rapid, deep, and sustained depletion of CD19+ B cells without the use of one or both of cyclophosphamide and fludarabine as a conditioning agent(s), and that bendamustine can be used as an alternative conditioning agent with FT819.

[000235] FIG. 9 demonstrates a case study of plasma cell depletion and B-cell reconstitution in three patients with diverse B-cell repertoire at baseline. As shown, the reconstituted cell population in 6-10 weeks following single-dose FT819 was comprised of naïve B cells.

EXAMPLE 10 – FT819 demonstrates key therapeutic mechanisms in secondary and tertiary tissue to enable immune reset in autoimmune diseases

[000236] In a case study of a patient treated with conditioning chemotherapy followed by a single dose of FT819 at 90M cells, FT819 was detected and persisted in the bone marrow at Day 9, with complete elimination of CD19+ cells in the bone marrow and peripheral blood at the end of treatment cycle (FIG. 10).

[000237] FIG. 11 shows a case study of a patient treated with conditioning chemotherapy followed by a single dose of FT819 at 180M cells. The patient had three prior lines of therapy including autologous CD19-targeted CAR T-cell therapy. Baseline lymph node biopsy showed DLBCL cells with high CD19 expression. Single dose of FT819 resulted in rapid, deep, and sustained depletion of CD19+ B cells in the peripheral blood, and in >50% decrease in CD19+ tumor cells in secondary lymphoid tissue, after 1 cycle. Re-treatment with a single dose of FT819 resulted in complete metabolic response by FDG-PET with ~80% reduction of target lesion 1 and complete elimination of target lesion 2 at the end of cycle 2.

[000238] FIG. 12 shows a case study of a patient treated with conditioning chemotherapy followed by a single dose of FT819 at 90M cells. The patient had six prior lines of therapy and was refractory to autologous CD19-targeted CAR T-cell therapy. Single dose of FT819 resulted

in sustained depletion of CD19+ B cells in the peripheral blood and complete elimination of CD19+ masses in the liver indicating potential of FT819 to traffic, infiltrate, and function in secondary and tertiary tissue.

EXAMPLE 11 –SLE patient peripheral blood sample pre- and post- treatment analyses

[000239] In a Phase 1 autoimmunity study with single-dose administration of FT819, a pre-treatment sample of a patient's peripheral blood obtained prior to clinical administration of conditioning chemotherapy and FT819 was used for a 24-hour co-culture assay. The results in FIG. 13 showed a deep depletion of the patient's CD19+ B cells upon exposure to FT819 at 360 million cells in this ex vivo translational assessment, indicating potent ex vivo CD19+ B-cell depletion with single dose FT819 treatment.

[000240] The patient from the FT819 Phase 1 autoimmunity study received bendamustine as the conditioning chemotherapy followed by a single dose of FT819. As shown in FIG. 14, the post-treatment analysis of peripheral blood from the first SLE patient shows rapid, deep and sustained depletion of peripheral blood B cells through Day 8, coupled with initial recovery of the T-cell compartment following bendamustine conditioning.

EXAMPLE 12 – FT522 Disease Response Assessment and Efficacy Analyses

[000241] "FT522" refers to an engineered NK cell therapy generated from a clonal master engineered iPSC line, and is engineered with multiple modalities for enhanced innate immunity: (1) anti-CD19-CAR; (2) alloimmune defense receptor (ADR) targeting 4-1BB; (3) high-affinity, non-cleavable CD16 (hnCD16); (4) interleukin-15 (IL15)/IL15 receptor fusion protein (IL15RF), and CD38 knockout. The potential for FT522 to induce CD19+ B-cell depletion across a range of autoimmune diseases, including without administration to patients of intense chemotherapy conditioning to patients has been assessed. In a disseminated Nalm6 leukemia model comprised of CD19+ target cells resistant to T-cell killing, the ADR-armed, CD19-targeted CAR NK cells exhibited robust *in vivo* killing of CD19+ target cells in the presence of alloreactive T cells, suggesting that FT522 has the potential to deplete CD19+ B cells and drive clinical responses without administration of intense chemotherapy conditioning to patients. Studies were also conducted to assess FT522 in combination with monoclonal antibody therapy for its potential in depletion of both CD19+ B-cell and CD38+ plasma-cell autoantibody-producing lineages.

[000242] FT522 demonstrated better CAR-mediated CD19+ B cell killing compared to TCR-less, allogeneic-like, primary CAR-T (pCAR-T) cells in a 24hr cytotoxicity assay with multiple E:T ratios and in two different SLE PBMC donors, as shown in FIG. 15.

[000243] In an in vitro allogeneic reaction rechallenge assay was performed, in which SLE donor PBMCs were co-cultured with pCAR-T cells (primary CAR-T cells), FT596 (ADR-negative) or FT522 (ADR-armed) for 8 days, and re-dosed with effector cells and re-challenged with SLE donor PBMCs and allowed to co-culture for another 6 days (FIG. 16A). Flow cytometry of CD3⁺ SLE donor PBMC T cells on days 4 and 6 for CD25 and 4-1BB demonstrated T cell activation and allo-response to pCAR-T and FT596 cells, but not to FT522 cells (FIG. 16B). Total SLE donor PBMC-derived T cells expanded in response to the allogeneic challenge on day 6, and the CD19⁺ cell counts prior to re-challenge normalized to PBMC alone were shown (FIG. 16C). pCAR-T and FT596 cells were depleted upon completion of the allo-rechallenge assay, with FT522 cells demonstrating persistence and effective control of CD19⁺ cell counts at the end of the assay (FIG. 16D; normalized to PBMC alone). In summary, FT522 exhibited rapid and deep CD19⁺ B cell depletion and eliminated alloreactive 4-1BB⁺ T cells, while maintaining functional persistence, against SLE donor PBMCs.

[000244] FT522 elicited a stronger anti-tumor effect compared to FT596 across tested E:T ratio in in vitro killing assay of WIL2S cell line, as shown in FIG. 17A. In a Phase 1 study in a B-cell lymphoma (BCL) setting, FT522 demonstrated a higher PK at day 4 in comparison to that of FT596 at an equivalent dose of 3e8 cells given on day 1 and a higher overall AUC during treatment cycle (FT596 single dose of 3e8 or FT596 three doses at 1.8e9 / dose), as shown in FIG. 17B. In FIGS. 18 A and 18B, the baseline represents B-cell counts in the peripheral blood prior to treatment with standard conditioning chemotherapy. 30-day treatment cycle was broken into stages. The clinical activity of FT596 monotherapy (n=9) is shown in FIG. 18A. The clinical activity of FT522 in combination with rituximab (n=2) is shown in FIG. 18B, demonstrating rapid, deep, and sustained depletion of CD19⁺ B cells by FT522.

[000245] One skilled in the art would readily appreciate that the methods, compositions, and products described herein are representative of exemplary embodiments, and not intended as limitations on the scope of the invention. It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the present disclosure disclosed herein without departing from the scope and spirit of the invention.

[000246] All patents and publications mentioned in the specification are indicative of the levels of those skilled in the art to which the present disclosure pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated as incorporated by reference. The present disclosure illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations that are not specifically disclosed herein. Thus,

for example, in each instance herein any of the terms “comprising,” “consisting essentially of,” and “consisting of” may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the present disclosure claimed. Thus, it should be understood that although the present disclosure has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

CLAIMS

What is claimed is:

1. A method of treating lupus or a symptom thereof in a subject, the method comprising:
 - (a) administering to the subject one or more doses of chemotherapy; and
 - (b) after step (a), administering to the subject one or more doses of an adoptive cell therapy product in a first effective amount;wherein the adoptive cell therapy product comprises an engineered T lineage effector cell comprising (i) expression of a CD19-CAR (chimeric antigen receptor) at a T-cell receptor α constant (TRAC) locus, and (ii) a T cell receptor (TCR) knockout.
2. The method of claim 1, further comprising:
 - (c) after step (b), administering to the subject one or more doses of an additional chemotherapy, wherein the additional chemotherapy is the same or different as the chemotherapy of step (a); and
 - (d) after step (c), administering to the subject one or more doses of an adoptive cell therapy product in a second effective amount that is the same or different as the first effective amount.
3. The method of claim 1, wherein the lupus or the symptom thereof comprises: (i) cutaneous lupus; (ii) lupus nephritis; (iii) neuropsychiatric lupus; or (iv) pericarditis, atherosclerosis, angina, Raynaud's syndrome, renal disease, metabolic syndrome, thyroid disorder, fibromyalgia, respiratory tract infection, skin infection, or urinary system infection.
4. The method of claim 1, wherein the chemotherapy comprises (a) one or more of cyclophosphamide (CY) or fludarabine (FLU); or (b) bendamustine.
5. The method of claim 1, wherein the chemotherapy is administered one or more days prior to administering the adoptive cell therapy product; optionally wherein the chemotherapy is administered on one or more of (i) three days before the adoptive cell therapy product, (ii) four days before the adoptive cell therapy product, or (iii) five days before the adoptive cell therapy product.
6. The method of claim 1, wherein the chemotherapy comprises cyclophosphamide administered at a daily dose of about 250 mg/m² to about 600 mg/m² and fludarabine administered at a daily dose of about 20 mg/m² to about 40 mg/m² for 3 consecutive days starting from about 4-6 days prior to day 1 of administering the adoptive cell therapy product.

7. The method of claim 1, wherein the chemotherapy comprises bendamustine and is administered at a daily dose of about 30 mg/m² to about 150 mg/m² for 2 consecutive days starting from about 4-6 days prior to day 1 of administering the adoptive cell therapy product.
8. The method of claim 1, wherein the engineered T lineage effector cell is derived from an engineered induced pluripotent stem cell (iPSC) comprising a TCR knockout, and a polynucleotide encoding the CD19-CAR.
9. The method of claim 1, wherein the first effective amount of the adoptive cell therapy product is about 1.8×10^8 cells to about 9×10^9 cells, and is optionally escalated.
10. The method of claim 9, wherein the first effective amount of the adoptive cell therapy product is about 3.6×10^8 cells, about 1×10^9 cells, about 3×10^9 cells, or about 9×10^9 .
11. The method of claim 1, wherein the adoptive cell therapy product is cryopreserved, and then thawed prior to administering.
12. The method of claim 1, wherein the adoptive cell therapy product is FT819.
13. The method of claim 1, wherein the subject has had a lupus diagnosis.
14. The method of claim 1, wherein the subject is positive in at least one of (a) an anti-nuclear antibody; (b) an anti-dsDNA antibody; or (c) an anti-Smith antibody.
15. A method of obtaining lupus remission or low activity in a subject according to any one of claims 1-14.
16. The method of claim 15, wherein the lupus remission or low activity is defined by DORIS (Definition of remission in systemic lupus erythematosus) complete remission, DORIS clinical remission on treatment, and/or LLDAS (lupus low disease activity state).
17. A method of controlling lupus flare according to any one of claims 1-14.
18. The method according to any one of claims 1-14, wherein the treating comprises longer disease-free intervals off immunosuppressive medications, decreases autoantibody production in the subject; and/or reconstitutes healthy B cell compartment in the subject as compared to B cells under active lupus.

19. The method according to any one of claims 1-14, wherein the subject previously received one or more prior treatments comprising glucocorticoids, CY, mycophenolic acid or its derivatives, belimumab, methotrexate, azathioprine, anifrolumab, rituximab, obinutuzumab, cyclosporin, tacrolimus, or voclosporin.
20. The method of claim 19, wherein the subject did not respond to the one or more prior treatments.
21. A method of treating lupus or a symptom thereof in a subject, the method comprising:
(a) administering to the subject one or more doses of chemotherapy; and
(b) after step (a), administering to the subject one or more doses of an adoptive cell therapy product in a first effective amount;
wherein the adoptive cell therapy product comprises an engineered T lineage effector cell comprising (i) expression of a CD19-CAR (chimeric antigen receptor) at a T-cell receptor α constant (TRAC) locus, and (ii) a T cell receptor (TCR) knockout; and
wherein the subject previously received and failed to respond to one or more prior treatments comprising glucocorticoids, CY, mycophenolic acid or its derivatives, belimumab, methotrexate, azathioprine, anifrolumab, rituximab, obinutuzumab, cyclosporin, tacrolimus, or voclosporin.
22. A method of treating lupus or a symptom thereof in a subject, the method comprising:
administering to the subject one or more doses of an adoptive cell therapy product in a first effective amount;
wherein the adoptive cell therapy product comprises an engineered NK lineage effector cell comprising a CD38 knockout and expression of (i) a CD19-CAR (chimeric antigen receptor); (ii) an alloimmune defense receptor (ADR) targeting 4-1BB; (iii) a high-affinity, non-cleavable CD16 (hnCD16); and (iv) an interleukin-15 (IL15)/IL15 receptor fusion protein (IL15RF).
23. The method of claim 22, wherein the method does not comprise administration of chemotherapy for lympho-conditioning.