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(54) Title: GENERATION AND USE IN ADOPTIVE IMMUNOTHERAPY OF STEM CELL-LIKE MEMORY T CELLS

(57) Abstract: Provided herein are methods of generating antigen-specific T cells for therapeutic administration to a human patient having or suspected of having a pathogen or cancer, utilizing stem cell-like memory T cells (T_{SCM} cells). Also disclosed are antigen-specific T cells generated by such methods, and methods of treating a human patient using such antigen-specific T cells.



GENERATION AND USE IN ADOPTIVE IMMUNOTHERAPY OF STEM CELL-LIKE MEMORY T CELLS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/399,311, filed on September 23, 2016, which is incorporated by reference herein in its entirety.

GOVERNMENT RIGHTS STATEMENT

[0002] This invention was made with government support under CA023766 awarded by National Institutes of Health. The government has certain rights in the invention.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0003] This application incorporates by reference a Sequence Listing submitted with this application as a text file entitled "Seqlisting_14259-034-228.txt" created on September 12, 2017 and having a size of 4.57 kilobytes.

1. FIELD

[0004] Provided herein are methods of generating antigen-specific T cells for therapeutic administration to a human patient having or suspected of having a pathogen or cancer, utilizing stem cell-like memory T cells (T_{SCM} cells). Also disclosed are antigen-specific T cells generated by such methods, and methods of treating a human patient using such antigen-specific T cells.

2. BACKGROUND

[0005] The characteristics of the T cells that are selected for expansion and adoptive transfer have been identified as a critical factor that determines the persistence of transferred cells. Antigen-specific T cells in the presence of infections or cancer can expand and differentiate into effector T cells devoted to rapidly clearing of the pathogens as well as memory T cells that can persist long-term and defend against recurrence of disease. The memory T cell compartment is heterogeneous and encompasses multiple subsets with distinctive properties. Current evidence indicates that central memory T cells (T_{CM} cells) that express high levels of CD62L and CCR7 are less differentiated, while $CD62L^-CCR7^-$ effector memory T cells (T_{EM} cells) represent

committed progenitor cells that undergo terminal differentiation (Berger *et al.*, 2008, J Clin Invest 118:294-305). Furthermore, studies in mice and nonhuman primates have shown that infused T cells that are derived from the T_{CM} population exhibit greater replicative potential in response to antigen and prolonged *in vivo* persistence compared with those derived from the T_{EM} population (Berger *et al.*, 2008, J Clin Invest 118:294-305; Wang *et al.*, 2011, Blood 117:1888-1898). Recently, the spectrum of immunological memory has been extended with the identification of stem cell-like memory T cells (T_{SCM} cells) that express CD45RA, CCR7 and CD62L, like naive T cells, but also express CD95. Human T_{SCM} cells have been expanded *in vitro* (Gattinoni *et al.*, 2011, Nat Med 17:1290-1297; Cieri *et al.*, 2013, Blood 121:573-584). When compared with other memory T cell populations, human T_{SCM} cells have exhibited increased proliferative capacity. T_{SCM} cells transduced to express a mesothelin-specific chimeric antigen receptor have also exhibited greater proliferation and superior antitumor responses following adoptive transfer in a humanized mouse model (Gattinoni *et al.*, 2011, Nat Med 17:1290-1297). While T_{SCM} cells can differentiate into T_{CM}, T_{EM} and effector T cells, they also have a marked potential for self-renewal as shown by serial transplantation experiments (Cieri *et al.*, 2013, Blood 121:573-584). Because of these attributes, T_{SCM} cells have attracted considerable interests as a potential critical reservoir of antigen-specific T cells for reconstituting immunity following human allogeneic hematopoietic cell transplants (allo HCT) (Cieri *et al.*, 2013, Blood 121:573-584; Xu *et al.*, 2015, J Hematol Oncol 8:113; Biasco *et al.*, 2015, Sci Transl Med 7:273ra213; Roberto *et al.*, 2015, Blood 125:2855-2864). Indeed, analyses of T cell receptor CDR3 sequences have shown that T_{SCM} cells undergo marked proliferation and clonal diversification early after allo HCT (Roberto *et al.*, 2015, Blood 125:2855-2864; Cieri *et al.*, 2015, Blood 125:2865-2874). Furthermore, T_{SCM} cell clones, distinguished by retroviral vector insertion sites, may resist in humans for up to 12 years post infusion (Biasco *et al.*, 2015, Sci Transl Med 7:273ra213). The low frequency of antigen-specific T_{SCM} cells has limited their detailed characterization (Schmueck-Henneresse *et al.*, 2015, J Immunol 194:5559-5567). Furthermore, the contribution of different memory subsets to the maintenance of the overall memory compartment of antigen-specific T cells has not been fully elucidated.

[0006] Citation of a reference herein shall not be construed as an admission that such is prior art to the present disclosure.

[0006a] Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each of the appended claims.

[0006b] Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

3. SUMMARY OF THE INVENTION

[0007] The present invention provides methods of generating antigen-specific T cells for therapeutic administration to a human patient having or suspected of having a pathogen or cancer, utilizing stem cell-like memory T cells (T_{SCM} cells). Also disclosed are antigen-specific T cells generated by such methods, and methods of treating a human patient using such antigen-specific T cells.

[0008] In one aspect, provided herein are methods of generating a population of cells comprising antigen-specific T cells for therapeutic administration to a human patient having or suspected of having a pathogen or cancer, comprising: (a) *ex vivo* sensitizing a population of human blood cells to one or more antigens of the pathogen or cancer over a period of time in culture, wherein at the initiation of said period of time, the population of human blood cells contains at least 50% stem cell-like memory T cells (T_{SCM} cells); and (b) cryopreserving (i) the *ex vivo* sensitized population of human blood cells, or (ii) cells derived therefrom that comprise antigen-specific T cells recognizing the one or more antigens of the pathogen or cancer; thereby producing said population of cells comprising antigen-specific T cells.

[0008a] In another aspect of the invention, provided herein is a method of generating a population of cells comprising antigen-specific T cells for therapeutic administration to a human patient having or suspected of having a pathogen or cancer, comprising: (a) *ex vivo* sensitizing a population of human blood cells to one or more antigens of the pathogen or cancer over a period of time in culture that is in the range of 9-21 days, wherein at the initiation of said period of time,

the population of human blood cells contains at least 50% stem cell-like memory T cells (TSCM cells); and (b) cryopreserving (i) the *ex vivo* sensitized population of human blood cells, or (ii) cells derived therefrom that comprise antigen-specific T cells recognizing the one or more antigens of the pathogen or cancer; thereby producing said population of cells comprising antigen-specific T cells.

[0009] In certain embodiments, the method of generating a population of cells comprising antigen-specific T cells further comprises, after the step of cryopreserving, steps of thawing and optionally expanding in culture the *ex vivo* sensitized population of human blood cells or cells derived therefrom.

[0010] In specific embodiments, the aforementioned period of time in culture (termed herein “the Sensitization Culture Time;” *i.e.*, the culture time period over which sensitization occurs) is in the range of 9-21 days. In a specific embodiment, the Sensitization Culture Time is in the range of 9-14 days. In another specific embodiment, the Sensitization Culture Time is 14 days.

[0011] In specific embodiments, the *ex vivo* sensitizing step comprises co-culturing the population of human blood cells with one or more immunogenic peptides or proteins derived from the one or more antigens. In specific embodiments, the *ex vivo* sensitizing step comprises co-culturing the population of human blood cells with antigen presenting cells that present the one or more antigens.

[0012] The antigen presenting cells used in the *ex vivo* sensitizing step can be any antigen presenting cells suitable for presenting the one or more antigens, such as dendritic cells, cytokine-activated monocytes, peripheral blood mononuclear cells (PBMCs), Epstein-Barr virus-transformed B-lymphoblastoid cell line cells (EBV-BLCL cells), or artificial antigen presenting cells (AAPCs). In a specific embodiment, the antigen presenting cells are AAPCs.

[0013] In some embodiments, the antigen presenting cells are loaded with one or more immunogenic peptides or proteins derived from the one or more antigens. In other embodiments, the antigen presenting cells are genetically engineered to recombinantly express one or more immunogenic peptides or proteins derived from the one or more antigens.

[0014] In some embodiments, the one or more immunogenic peptides or proteins are a pool of overlapping peptides derived from the one or more antigens. In specific embodiments, the pool of overlapping peptides is a pool of overlapping pentadecapeptides. In other embodiments,

the one or more immunogenic peptides or proteins are one or more proteins derived from the one or more antigens.

[0015] In some embodiments, the population of cells comprising antigen-specific T cells, as described herein, comprises antigen-specific T cells that endogenously express a public T cell receptor (TCR) recognizing the one or more antigens. In other embodiments, the population of cells comprising antigen-specific T cells, as described herein, comprises antigen-specific T cells that recombinantly express a public TCR recognizing the one or more antigens. In specific embodiments, the method of generating a population of cells comprising antigen-specific T cells, as described herein, further comprises transducing the population of human blood cells with a nucleic acid encoding a public TCR (*e.g.*, at a time when the population of human blood cells has been cultured for 3-5 days). In a specific embodiment wherein the one or more antigens is cytomegalovirus (CMV) pp65, the public TCR comprises a β -chain comprising a variable domain, which comprises a complementarity determining region (CDR)3 of CASSPQTGASYGYTF (SEQ ID NO:3). In another specific embodiment wherein the one or more antigens is CMV pp65, the public TCR comprises a β -chain comprising a variable domain, which comprises a CDR3 of CASSPKTGAVYGYTF (SEQ ID NO:4).

[0016] In certain embodiments, the population of cells comprising antigen-specific T cells, as described herein, comprises antigen-specific T cells that recombinantly express a chimeric antigen receptor (CAR) recognizing the one or more antigens. In specific embodiments, the method of generating a population of cells comprising antigen-specific T cells, as described herein, further comprises transducing the population of human blood cells with a nucleic acid encoding a CAR (*e.g.*, at a time when the population of human blood cells has been cultured for

3-5 days).

[0017] In another aspect, provided herein are methods of generating a population of cells comprising antigen-specific T cells for therapeutic administration to a human patient having or suspected of having a pathogen or cancer, comprising transducing a population of human blood cells with a nucleic acid encoding a public TCR recognizing one or more antigens of the pathogen or cancer at a time when the population of human blood cells has been cultured for 3-5 days, wherein the population of human blood cells contains at least 50% T_{SCM} cells; thereby producing said population of cells comprising antigen-specific T cells. In a specific embodiment wherein the one or more antigens is CMV pp65, the public TCR comprises a β -chain comprising a variable domain, which comprises a CDR3 of CASSPQTGASYGYTF (SEQ ID NO:3). In another specific embodiment wherein the one or more antigens is CMV pp65, the public TCR comprises a β -chain comprising a variable domain, which comprises a CDR3 of CASSPKTGAVYGYTF (SEQ ID NO:4). In specific embodiments, the method of generating a population of cells comprising antigen-specific T cells further comprises, after the step of transducing, a step of cryopreserving the transduced population of human blood cells or cells derived therefrom. In a specific embodiment, the method of generating a population of cells comprising antigen-specific T cells further comprises, after the step of cryopreserving, steps of thawing and optionally expanding in culture the transduced population of human blood cells or cells derived therefrom.

[0018] In another aspect, provided herein are methods of generating a population of cells comprising antigen-specific T cells for therapeutic administration to a human patient having or suspected of having a CMV infection, comprising transducing a population of human blood cells with a nucleic acid encoding a public TCR recognizing CMV pp65 at a time when the population of human blood cells has been cultured for 3-5 days, wherein the public TCR comprises a β -chain comprising a variable domain, which comprises a CDR3 of CASSPQTGASYGYTF (SEQ ID NO:3), and wherein the population of human blood cells contains at least 50% T_{SCM} cells; thereby producing said population of cells comprising antigen-specific T cells. In specific embodiments, the method of generating a population of cells comprising antigen-specific T cells further comprises, after the step of transducing, a step of cryopreserving the transduced population of human blood cells or cells derived therefrom. In a specific embodiment, the method of generating a population of cells comprising antigen-specific T cells further comprises,

after the step of cryopreserving, steps of thawing and optionally expanding in culture the transduced population of human blood cells or cells derived therefrom.

[0019] In another aspect, provided herein are methods of generating a population of cells comprising antigen-specific T cells for therapeutic administration to a human patient having or suspected of having a CMV infection, comprising transducing a population of human blood cells with a nucleic acid encoding a public TCR recognizing CMV pp65 at a time when the population of human blood cells has been cultured for 3-5 days, wherein the public TCR comprises a β -chain comprising a variable domain, which comprises a CDR3 of CASSPKTGAVYGYTF (SEQ ID NO:4), and wherein the population of human blood cells contains at least 50% T_{SCM} cells; thereby producing said population of cells comprising antigen-specific T cells. In specific embodiments, the method of generating a population of cells comprising antigen-specific T cells further comprises, after the step of transducing, a step of cryopreserving the transduced population of human blood cells or cells derived therefrom. In a specific embodiment, the method of generating a population of cells comprising antigen-specific T cells further comprises, after the step of cryopreserving, steps of thawing and optionally expanding in culture the transduced population of human blood cells or cells derived therefrom.

[0020] In another aspect, provided herein are methods of generating a population of cells comprising antigen-specific T cells for therapeutic administration to a human patient having or suspected of having a pathogen or cancer, comprising transducing a population of human blood cells with a nucleic acid encoding a CAR recognizing one or more antigens of the pathogen or cancer at a time when the population of human blood cells has been cultured for 3-5 days, wherein the population of human blood cells contains at least 50% T_{SCM} cells; thereby producing said population of cells comprising antigen-specific T cells. In specific embodiments, the method of generating a population of cells comprising antigen-specific T cells further comprises, after the step of transducing, a step of cryopreserving the transduced population of human blood cells or cells derived therefrom. In a specific embodiment, the method of generating a population of cells comprising antigen-specific T cells further comprises, after the step of cryopreserving, steps of thawing and optionally expanding in culture the transduced population of human blood cells or cells derived therefrom.

[0021] The population of human blood cells used in accordance with the methods of generating a population of cells comprising antigen-specific T cells described herein contains at

least 50% T_{SCM} cells. In a specific embodiment, the population of human blood cells contains at least 90% T_{SCM} cells. In another specific embodiment, the population of human blood cells contains at least 95% T_{SCM} cells. In another specific embodiment, the population of human blood cells contains at least 99% T_{SCM} cells. In another specific embodiment, the population of human blood cells contains 100% T_{SCM} cells.

[0022] In certain embodiments, the population of human blood cells contains less than 10% T_N cells. In a specific embodiment, the population of human blood cells contains less than 5% T_N cells. In another specific embodiment, the population of human blood cells contains less than 1% T_N cells. In another specific embodiment, the population of human blood cells contains no T_N cells.

[0023] In certain embodiments, the method of generating a population of cells comprising antigen-specific T cells described herein further comprises a step of deriving the population of human blood cells from a human cell sample. In specific embodiments, the deriving step comprises enriching for T_{SCM} cells from the human cell sample. In a specific embodiment, the enriching step comprises selecting for cells that are CD3⁺CD62L⁺CD45RO⁻CD95⁺. In some embodiments, the step of deriving the population of human blood cells from a human cell sample comprises sorting T_{SCM} cells from the human cell sample by fluorescence-activated cell sorting (FACS).

[0024] In preferred embodiments, the population of human blood cells is derived from a human donor that is seropositive for the one or more antigens.

[0025] In certain embodiments, the population of cells comprising antigen-specific T cells, as described herein, lacks substantial cytotoxicity *in vitro* toward antigen presenting cells that are not loaded with or genetically engineered to express one or more peptides or proteins derived from the one or more antigens.

[0026] In specific embodiments of the methods of generating a population of cells comprising antigen-specific cells described herein, the *ex vivo* sensitizing step comprises *ex vivo* sensitizing the population of human blood cells to one or more antigens of the pathogen. The pathogen can be a virus, bacterium, fungus, helminth or protist. In some embodiments, the pathogen is a virus. In a specific embodiment, the virus is cytomegalovirus (CMV).

[0027] In specific embodiments of the methods of generating a population of cells comprising antigen-specific cells described herein, the *ex vivo* sensitizing step comprises *ex vivo*

sensitizing the population of human blood cells to one or more antigens of Epstein-Barr virus (EBV).

[0028] In specific embodiments of the methods of generating a population of cells comprising antigen-specific cells described herein, the *ex vivo* sensitizing step comprises *ex vivo* sensitizing the population of human blood cells to one or more antigens of BK virus (BKV), John Cunningham virus (JCV), herpesvirus, adenovirus (ADV), human immunodeficiency virus (HIV), influenza virus, ebola virus, poxvirus, rhabdovirus, or paramyxovirus.

[0029] In specific embodiments of the methods of generating a population of cells comprising antigen-specific cells described herein, the *ex vivo* sensitizing step comprises *ex vivo* sensitizing the population of human blood cells to one or more antigens of the cancer. The cancer can be a blood cancer. In a specific embodiment, the cancer is multiple myeloma or plasma cell leukemia. In an aspect of the specific embodiment, the one or more antigens of the cancer is Wilms tumor 1 (WT1). The cancer can also be a solid tumor cancer, such as, but is not limited to: a cancer of the breast, lung, ovary, stomach, pancreas, larynx, esophagus, testes, liver, parotid, biliary tract, colon, rectum, cervix, uterus, endometrium, kidney, bladder, prostate, thyroid, brain, or skin.

[0030] In another aspect, provided herein are methods of treating a human patient having a pathogen or cancer, comprising: (i) generating a population of cells comprising antigen-specific T cells according to a method described herein; and (ii) administering the population of cells comprising antigen-specific T cells to the human patient.

[0031] In another aspect, provided herein are methods of treating a human patient having a pathogen or cancer, comprising administering a population of cells comprising antigen-specific T cells to the human patient, wherein the population of cells comprising antigen-specific T cells is the product of a method comprising generating the population of cells comprising antigen-specific T cells according to a method described herein.

[0032] In some embodiments, the antigen-specific T cells contained in the population of cells comprising antigen-specific T cells are restricted by an HLA allele shared with the diseased cells in the human patient to be treated. In other embodiments, the antigen-specific T cells contained in the population of cells comprising antigen-specific T cells share at least 2 HLA alleles (for example, at least 2 out of 8 HLA alleles, such as two HLA-A alleles, two HLA-B alleles, two HLA-C alleles, and two HLA-DR alleles) with the diseased cells in the human patient to be

treated.

[0033] In specific embodiments, the population of human blood cells is derived from a human donor that is allogeneic to the human patient. In a specific embodiment, the human patient has been the recipient of a transplant from a transplant donor, and the human donor is a third-party donor that is different from the transplant donor.

[0034] In some embodiments, the administering step comprises administering the population of cells comprising antigen-specific T cells to the human patient, at a dose that is less than or equal to about 1×10^5 cells of the population of cells comprising antigen-specific T cells per kg of the human patient. In a specific embodiment, the administering step comprises administering the population of cells comprising antigen-specific T cells to the human patient, at a dose that is less than or equal to about 5×10^4 cells of the population of cells comprising antigen-specific T cells per kg of the human patient. In a preferred embodiment, the administering step comprises administering the population of cells comprising antigen-specific T cells to the human patient, at a dose that is less than or equal to about 1×10^4 cells of the population of cells comprising antigen-specific T cells per kg of the human patient. In another specific embodiment, the administering step comprises administering the population of cells comprising antigen-specific T cells to the human patient, at a dose that is less than or equal to about 5×10^3 cells of the population of cells comprising antigen-specific T cells per kg of the human patient. In another specific embodiment, the administering step comprises administering the population of cells comprising antigen-specific T cells to the human patient, at a dose that is less than or equal to about 1×10^3 cells of the population of cells comprising antigen-specific T cells per kg of the human patient.

[0035] In certain embodiments, the step of administering comprises administering the population of cells comprising antigen-specific T cells to the human patient at the dose described above weekly.

[0036] In certain embodiments, the step of administering is by bolus intravenous infusion.

[0037] In certain embodiments, the step of administering comprises administering at least 2 doses of the population of cells comprising antigen-specific T cells to the human patient. In specific embodiments, the step of administering comprises administering 2, 3, 4, 5, or 6 doses of the population of cells comprising antigen-specific T cells to the human patient.

[0038] In a specific embodiment, the step of administering comprises administering a first

cycle of one dose per week of the population of cells comprising antigen-specific T cells for 3 consecutive weeks followed by a washout period during which no dose of the population of cells comprising antigen-specific T cells is administered, followed by a second cycle of said one dose per week of the population of cells comprising antigen-specific T cells for 3 consecutive weeks.

[0039] In specific embodiments, the step of administering comprises administering two, three, four, five, or six cycles of one dose per week of the population of cells comprising antigen-specific T cells for three consecutive weeks, each cycle separated by a washout period during which no dose of the population of cells comprising antigen-specific T cells is administered.

[0040] In specific embodiments, the washout period is about 1, 2, 3, or 4 weeks. In a preferred embodiment, the washout period is about 3 weeks.

[0041] In specific embodiments, the step of administering of the population of cells comprising antigen-specific T cells does not result in any graft-versus-host disease (GvHD) in the human patient.

[0042] In another aspect, provided herein are populations of cells comprising antigen-specific T cells for therapeutic administration to a human patient having or suspected of having a pathogen or cancer, wherein the population of cells comprising antigen-specific T cells is the product of a method comprising generating the population of cells comprising antigen-specific T cells according to a method described herein. In specific embodiments, provided herein is a population of cells comprising antigen-specific T cells for therapeutic administration to a human patient having or suspected of having a pathogen or cancer, wherein the population of cells comprising antigen-specific T cells is the product of a method comprising generating the population of cells comprising antigen-specific T cells according to a method described herein, and wherein the population of cells comprising antigen-specific T cells is cryopreserved.

[0043] In another aspect, provided herein is a cell bank comprising a plurality of populations of cells comprising antigen-specific T cells described herein.

4. BRIEF DESCRIPTION OF FIGURES

[0044] **Figure 1. Isolation and characterization of T_N, T_{SCM}, T_{CM} and T_{EM} populations from human peripheral blood.** Flow cytometry was used to isolate the T_N, T_{SCM}, T_{CM} and T_{EM} subsets from peripheral blood mononuclear cell (PBMCs). Lymphocytes were first gated within PBMCs by forward scatter (FSC) and side scatter (SCC) and analyzed for CD45RO, CD95,

CD62L and CD3 expression. CD3⁺ lymphocytes were then gated for CD45RO⁺CD62L⁺ T_{CM} and CD45RO⁺CD62L⁻ T_{EM}, CD45RO⁻CD62L⁺CD95⁺ T_{SCM} cells and CD45RO⁻CD62L⁺CD95⁻ T_N cells.

[0045] **Figure 2. Phenotypic characterization of naive and memory (T_N, T_{SCM}, T_{CM} and T_{EM})-derived CMV-specific CD8⁺ T cells upon antigen stimulation.** (A), (B) and (C) Isolated T cells from T_N, T_{SCM}, T_{CM} and T_{EM} cell populations were sensitized with artificial antigen presenting cells (AAPCs) expressing CMVpp65 peptide and HLA-A:0201. CD3⁺ T lymphocytes derived from each cell population were evaluated at 7 (A), 14 (B) and 30 days (C) post-stimulation. CD8⁺ single, live T cells were gated from CD3⁺ T lymphocytes. HLA-A:0201-NLV Tetramer (Tet)⁺ and NLV-Tet⁻ T cells were then gated within CD8⁺ T cells (left panel). Expression frequencies of CD62L, CCR7 and CD45RA gated on NLV-Tet⁺ and NLV-Tet⁻ population are shown for a representative donor. (Regular font text: NLV-Tet⁺; Bold font text: NLV-Tet⁻.) (Figures 2A-2C were originally in color, so for purposes of this black and white reproduction of the figures, outlines of the former blue FACS plot contours were traced manually in order to make them distinguishable on black and white reproduction from the former red contours, the outlines of which were not traced.) Percentages of CCR7⁺ cells 14 days post-stimulation are shown in (D) for NLV-Tet⁺ and in (E) for NLV-Tet⁻ T cells (n=6; *P < 0.05; **P < 0.01; from Mann-Whitney test).

[0046] **Figure 3. Distinct expression of co-stimulatory and senescence markers within T_N, T_{SCM}, T_{CM} and T_{EM}.** Expression frequencies of CD27, CD57, CD127, CD28, KLRG1 and PD1 gated on NLV-Tet⁺ and NLV-Tet⁻ populations after *in-vitro* stimulation for 14-18 days are shown. (A) Percentage of CD27⁺ cells within NLV-Tet⁺ CD8⁺ T cells (n=6) derived from T_N, T_{SCM}, T_{CM} and T_{EM} populations, respectively. (B) Representative fluorescence-activated cell sorting (FACS) plots of the CD27 expression within CD8⁺ T cells. (C) Percentage of NLV-Tet⁺ CD8⁺ T cells expressing CD57 (n=8) derived from T_N, T_{SCM}, T_{CM} and T_{EM} populations, respectively. (D) Representative FACS plots of the CD57 expression in CD8⁺ T cells. (E) and (F) Percentage of NLV-Tet⁺ CD8⁺ T cells expressing CD 127(n=6) and CD28 (n=8) respectively, derived from T_N, T_{SCM}, T_{CM} and T_{EM} populations, respectively. (G) and (H) Percentage of CD8⁺ T cells expressing KLRG1 within NLV-Tet⁺ (G) and NLV-Tet⁻ T cells post-stimulation (n=9). (I) and (J) Percentage of CD8⁺ T cells expressing PD1 within NLV-Tet⁺ (I) and NLV-Tet⁻ (J) T cells post-stimulation (n=11). (*P < 0.05; **P < 0.01; ***P < 0.005; from

Mann-Whitney test.)

[0047] **Figure 4. Enrichment of CMV-specific CD8⁺ T cells results from rapid expansion of early memory T cells.**

(A) *In-vitro* proliferation of A2-NLV-Tet⁺ T cells was evaluated after *in-vitro* sensitization with CMVpp65 antigen by EdU as shown in a representative donor on Day 3, 5 and 7 post-stimulation. CD3⁺ T cells were sensitized with artificial antigen presenting cells (AAPCs) expressing CMVpp65 peptide and HLA-A*0201. The enrichment of NLV-Tet⁺ T cells after stimulation was evaluated as shown on the left panel. Percentage of EdU incorporation gated on single, live CD8⁺ T lymphocytes is shown in the middle panel. Percentage of EdU incorporation gated on NLV-Tet⁺ CD8⁺ T cells is shown on the right panel. (B) Phenotypic analysis of NLV-Tet⁺ T cells (right panel) in comparison to NLV-Tet⁺ T cells with EdU incorporation (left panel) is shown for a representative donor. (White: CD45RO⁺CD62L⁻ T_{EM}; Light Grey: CD45RO⁺CD62L⁺ T_{CM}; Dark Grey: CD45RO⁻CD62L⁺CD95⁺ T_{SCM}.) CD45RO⁻CD62L⁺CD95⁻ T_N cells were not detected post antigen stimulation. (C) Fold expansion of A2-NLV-Tet⁺ T cells after 14 days of antigen-specific T cell stimulation was evaluated within NLV-Tet⁺ CD8⁺ T cells within the T_N⁻, T_{SCM}⁻, T_{CM}⁻ and T_{EM}-derived cells. NLV-Tet⁺ CD8⁺ T cells were not detected before exposure to antigen in 4 out of 6 donors for the T_N population (n=6) (*P = 0.03; ns, not significant; from Mann-Whitney test).

[0048] **Figure 5. Functional cytokine profile and cytotoxic activity of *in vitro* expanded CMV-specific T cells derived from naive (T_N) and memory T cells (T_{SCM}, T_{CM} and T_{EM}).**

(A) Expanded naive and memory T cell populations were stimulated for 18 hours with NLV peptide loaded autologous B-lymphoblastoid cell line cells (BLCL cells) at a ratio of 5:1. T cells co-cultured with autologous BLCLs without peptide loading served as controls. CD8⁺ T cells secreting IFN- γ and TNF- α were evaluated by intracellular staining. The percentage of CD8⁺ T cells expressing CD137 are shown on the first 2 panels (on Figure 5A-1) with or without peptide stimulation. The proportion of CD8⁺ T cells expressing CD137 and secreting IFN- γ or TNF- α cytokine is shown in the next 2 panels (on Figure 5A-2). CD8⁺ T cells secreting both IFN- γ and TNF- α cytokine are shown in the last panel (on Figure 5A-2). (B) Cytotoxic activity was evaluated by CD107a degranulation assay. The percentage of CD8⁺ T cells expressing CD107a with (middle panel) or without (left panel) peptide stimulation is shown. The percentage of CD8⁺ T cells expressing both CD137 and CD107a is shown in the right panel. Data shown is a representative of three experiments (n=3).

[0049] **Figure 6. Clonal diversity and clonotype selection within CMV-specific T_N, T_{SCM}, T_{CM} and T_{EM} cells.** Next-generation sequencing was performed for TCRV β repertoire analysis. TCR clonality was analyzed using immun^oSEQ Analyzer 2.0 (A) before and (B) 15 days after T cell expansion, for NLV-Tet⁺ T cells contained within (A) sorted T_N, T_{SCM}, T_{CM} and T_{EM} subsets or (B) cells derived from these subsets, respectively. Heat map indicates similarity of sample profiles. The similarity accounts for overlap between unique nucleotide sequences within any two samples. (C) Sorted NLV-Tet⁺ T cells derived from Naive (T_N), and memory (T_{SCM}, T_{CM} and T_{EM}) subsets from the same donor after 30 days expansion were compared for their TCR sequences. TCR sequencing was performed on sorted NLV-Tet⁺ T cells derived from Naive (T_N), and memory (T_{SCM}, T_{CM} and T_{EM}) subsets from a separate donor (D) before, (E) after 15 days and, (F) after 30 days of *in vitro* stimulation, and compared for their similarities. Values shown are proportions of overlap. The TCR sequencing analysis for this donor at the same time points (pre-, day 15 and day 30 post-stimulation) is summarized in (G).

[0050] **Figure 7. Enhanced proliferation of T_{SCM} cells may not explain immunodominance.** (A) T cells from HLA-A:0201⁺ and A:2402⁺ CMV seropositive donors were sensitized with artificial antigen presenting cells (AAPCs) expressing HLA-A:0201 or HLA-A:2402 and CMVpp65 protein. T cells gated on single, live CD8⁺ T lymphocytes were gated for cells binding the HLA-A:0201-NLVPMVATV (SEQ ID NO:1) peptide tetrameric complexes (NLV-Tet⁺) or HLA-A:2402-QYDPVAALF (SEQ ID NO:2) peptide tetrameric complexes (QYD-Tet⁺) after 4, 5, 7 and 8 days post antigen specific stimulation (n=2). Results are shown for one representative donor. Phenotypic analysis of NLV-Tet⁺ or QYD-Tet⁺ T cells was performed to evaluate the proportion of T_{SCM}, T_{CM} and T_{EM}-derived cells after antigen specific stimulation (CD45RO⁺CD62L⁺ T_{CM}: Light Gray; CD45RO⁺CD62L⁻ T_{EM}: White; CD45RO⁻CD62L⁺CD95⁺ T_{SCM}: Dark Gray). There were no Tet⁺ T cells with T_N phenotype CD45RO⁻CD62L⁺CD95⁻ post-sensitization. (B) T cells labeled with EdU were used to evaluate and compare the proportion of proliferating T cells within NLV-Tet⁺ and the proportion of proliferating T cells within QYD-Tet⁺ T cells, within T_{SCM}-, T_{CM}- and T_{EM}-derived cells 4,5,7 and 8 days post stimulation (Figure 7B-1). The proportion of apoptotic T cells within EdU labeled NLV-Tet⁺ or EdU labeled QYD-Tet⁺ population was evaluated after T cell stimulation at 4,5,7 and 8 days using Annexin V labeling (Figure 7B-2).

5. DETAILED DESCRIPTION

[0051] The present invention provides methods of generating antigen-specific T cells for therapeutic administration to a human patient having or suspected of having a pathogen or cancer, antigen-specific T cells generated by such methods, and methods of treating a human patient using such antigen-specific T cells. According to the present invention, stem cell-like memory T cells (T_{SCM} cells) are a more suitable source of T cells for the generation of antigen-specific T cells for adoptive immunotherapy, relative to naive T cells (T_N cells), central memory T cells (T_{CM} cells) and effector memory T cells (T_{EM} cells), because they allow rapid, persistent and selective *in vitro* expansion of antigen-specific T cells that recognize dominant epitopes of antigens of pathogens found in human blood and are the principal and persistent reservoir for rapid repopulation of immunodominant T cells *in vivo*.

5.1. Methods of Generating Antigen-Specific T Cells for Adoptive Immunotherapy

5.1.1. Methods Using *Ex Vivo* Sensitization of T_{SCM} cells

[0052] In one aspect, provided herein are methods of generating a population of cells comprising antigen-specific T cells for therapeutic administration to a human patient having or suspected of having a pathogen or cancer, comprising: (a) *ex vivo* sensitizing a population of human blood cells to one or more antigens of the pathogen or cancer over a period of time in culture, wherein at the initiation of said period of time, the population of human blood cells contains at least 50% stem cell-like memory T cells (T_{SCM} cells); and (b) cryopreserving (i) the *ex vivo* sensitized population of human blood cells, or (ii) cells derived therefrom that comprise antigen-specific T cells recognizing the one or more antigens of the pathogen or cancer; thereby producing said population of cells comprising antigen-specific T cells.

[0053] *Ex vivo sensitization*

[0054] In specific embodiments, the aforementioned period of time in culture (termed herein “the Sensitization Culture Time;” *i.e.*, the culture time period over which sensitization occurs) is in the range of 9-21 days. As will be clear, this means that the period of time in culture for sensitization purposes starting with the first culturing in the presence of antigen until the end of culturing in the presence of antigen is only for 9-21 days and not longer. (The cells may optionally be cultured for a longer period of time, but not in the presence of antigen for sensitization.) In a specific embodiment, the Sensitization Culture Time is in the range of 9-14

days. In another specific embodiment, the Sensitization Culture Time is 9 days. In another specific embodiment, the Sensitization Culture Time is 10 days. In another specific embodiment, the Sensitization Culture Time is 11 days. In another specific embodiment, the Sensitization Culture Time is 12 days. In another specific embodiment, the Sensitization Culture Time is 13 days. In another specific embodiment, the Sensitization Culture Time is 14 days. In another specific embodiment, the Sensitization Culture Time is 15 days. In another specific embodiment, the Sensitization Culture Time is 16 days. In another specific embodiment, the Sensitization Culture Time is 17 days. In another specific embodiment, the Sensitization Culture Time is 18 days. In another specific embodiment, the Sensitization Culture Time is 19 days. In another specific embodiment, the Sensitization Culture Time is 20 days. In another specific embodiment, the Sensitization Culture Time is 21 days.

[0055] The *ex vivo* sensitizing step can be performed by any method known in the art to stimulate T cells to be antigen-specific *ex vivo*, such as a method as described in Koehne et al., 2000, Blood 96:109-117; Trivedi et al., 2005, Blood 105:2793-2801; Haque et al., 2007, Blood 110:1123-1131; Hasan et al., 2009, J Immunol 183: 2837-2850; Feuchtinger et al., 2010, Blood 116:4360-4367; Doubrovina et al., 2012, Blood 120:1633-1646; Leen et al., 2013, Blood 121:5113-5123; Papadopoulou et al., 2014, Sci Transl Med 6:242ra83; Sukdolak et al., 2013, Biol Blood Marrow Transplant 19:1480-1492; Koehne et al., 2015, Biol Blood Marrow Transplant 21: 1663-1678, or International Patent Application Publication No. WO 2016/073550.

[0056] In specific embodiments, the *ex vivo* sensitizing step comprises co-culturing the population of human blood cells with one or more immunogenic peptides or proteins derived from the one or more antigens (preferably also in the presence of antigen presenting cells). In specific embodiments, the *ex vivo* sensitizing step comprises co-culturing the population of human blood cells with antigen presenting cells that present the one or more antigens. The *ex vivo* sensitizing step preferably comprises first supplementing the culture with IL-15 and IL-7 (*e.g.*, starting from day 4 after the initiation of the Sensitization Culture Time, or earlier), and then supplementing the culture with IL-2 (*e.g.*, at least 7 days after the initiation of the Sensitization Culture Time) optionally together with IL-15 and IL-7. IL-15 and IL-7 help to maintain stem cell-like phenotype of the T_{SCM} cells, and are preferably added to the culture within the first seven days of culturing in the *ex vivo* sensitizing step and then again for multiple

times. IL-2 helps to boost expansion of the antigen-specific T cells, and is preferably added to the cell culture on a culture date that is later than the initial addition of IL-15 and IL-7 to the cell culture in the *ex vivo* sensitizing step (*e.g.*, IL-2 is added at least 7 days after the initiation of the Sensitization Culture Time). In a specific embodiment, the *ex vivo* sensitizing step comprises supplementing the culture with IL-15 and IL-7 on day 4 and day 7 after the initiation of the Sensitization Culture Time, and then supplementing the culture with IL-15, IL-7 and IL-2 every other day starting after day 12 after the initiation of the Sensitization Culture Time.

[0057] The antigen presenting cells used in the *ex vivo* sensitizing step can be any antigen presenting cells suitable for presenting the one or more antigens, such as dendritic cells, cytokine-activated monocytes, peripheral blood mononuclear cells (PBMCs), Epstein-Barr virus-transformed B-lymphoblastoid cell line cells (EBV-BLCL cells), or artificial antigen presenting cells (AAPCs). In a specific embodiment, the antigen presenting cells are dendritic cells. In another specific embodiment, the antigen presenting cells are PBMCs. In another specific embodiment, the antigen presenting cells are EBV-BLCL cells. In another specific embodiment, the antigen presenting cells are AAPCs. In certain embodiments, the antigen presenting cells are derived from the donor of the population of human blood cells. The antigen presenting cells can be obtained by any method known in the art, such as the method(s) described in Koehne et al., 2000, *Blood* 96:109-117; Koehne et al., 2002, *Blood* 99:1730-1740; Trivedi et al., 2005, *Blood* 105:2793-2801; O'Reilly et al., 2007, *Immunol Res* 38:237-250; Hasan et al., 2009, *J Immunol* 183: 2837-2850; Barker et al., 2010, *Blood* 116:5045-5049; O' Reilly et al., 2011, *Best Practice & Research Clinical Haematology* 24:381-391; Doubrovina et al., 2012, *Blood* 120:1633-1646; Koehne et al., 2015, *Biol Blood Marrow Transplant* 21: 1663-1678, or International Patent Application Publication No. WO 2016/073550.

[0058] In some embodiments, the antigen presenting cells are loaded with one or more immunogenic peptides or proteins derived from the one or more antigens. Non-limiting exemplary methods for loading antigen presenting cells with peptide(s) derived from antigen(s) can be found in Trivedi et al., 2005, *Blood* 105:2793-2801; Hasan et al., 2009, *J Immunol* 183: 2837-2850; and International Patent Application Publication No. WO 2016/073550. In other embodiments, the antigen presenting cells are genetically engineered to recombinantly express one or more immunogenic peptides or proteins derived from the one or more antigens. Any appropriate method known in the art for introducing nucleic acid vehicles into cells to express

proteins, such as transduction or transformation, can be used to genetically engineer the antigen presenting calls to recombinantly express the one or more immunogenic peptides or proteins derived from the one or more antigens.

[0059] In some embodiments, the one or more immunogenic peptides or proteins are a pool of overlapping peptides derived from the one or more antigens. In specific embodiments, the pool of overlapping peptides is a pool of overlapping pentadecapeptides. In other embodiments, the one or more immunogenic peptides or proteins are one or more proteins derived from the one or more antigens.

[0060] Cryopreservation

[0061] In preferred embodiments, the step of cryopreserving comprises steps of: (c) recovering from culture the *ex vivo* sensitized population of human blood cells or cells derived therefrom; (d) combining with a cryopreservative the *ex vivo* sensitized population of human blood cells or cells derived therefrom; and (e) freezing the *ex vivo* sensitized population of human blood cells, or cells derived therefrom, combined with the cryopreservative. Cells derived from the *ex vivo* sensitized population of human blood cells and comprising antigen-specific T cells which recognize the one or more antigens of the pathogen or cancer can be a fraction of the *ex vivo* sensitized population of human blood cells (*e.g.*, a CD3⁺ T cell population enriched from the *ex vivo* sensitized population of human blood cells, or a CD8⁺ cytotoxic T cell population enriched from the *ex vivo* sensitized population of human blood cells) or an expanded population of the *ex vivo* sensitized population of human blood cells.

[0062] Freezing of cells is ordinarily destructive. On cooling, water within the cell freezes. Injury then occurs by osmotic effects on the cell membrane, cell dehydration, solute concentration, and ice crystal formation. As ice forms outside the cell, available water is removed from solution and withdrawn from the cell, causing osmotic dehydration and raised solute concentration which eventually destroy the cell. (For a discussion, see Mazur, 1977, *Cryobiology* 14:251-272.) These injurious effects can be circumvented by (a) use of a cryopreservative, (b) control of the freezing rate, and (c) storage at a temperature sufficiently low to minimize degradative reactions.

[0063] Cryopreservative which can be used in accordance with the present invention can be, but is not limited to, dimethyl sulfoxide (DMSO), glycerol, polyvinylpyrrolidone, polyethylene glycol, albumin, dextran, sucrose, ethylene glycol, i-erythritol, D-ribitol, D-mannitol, D-sorbitol,

i-inositol, D-lactose, choline chloride, amino acids, methanol, acetamide, glycerol monoacetate, or inorganic salts. In a preferred embodiment, the cryopreservative which is used in accordance with the present invention is DMSO. Being a small molecule, DMSO freely permeates the cell and protects intracellular organelles by combining with water to modify its freezability and prevent damage from ice formation. Addition of plasma, fetal calf serum, or human albumin can augment the protective effect of DMSO. After addition of DMSO, cells should be kept at 0 °C until freezing, since DMSO concentrations of about 1% are toxic at temperatures above 4 °C.

[0064] A controlled slow cooling rate is also critical. Different cryopreservatives (Rapatz, G., et al., 1968, *Cryobiology* 5(1):18-25) and different cell types have different optimal cooling rates (*see, e.g.*, Rowe and Rinfret, 1962, *Blood* 20:636; Rowe, 1966, *Cryobiology* 3:12-18; Lewis *et al.*, 1967, *Transfusion* 7:17-32; and Mazur, 1970, *Science* 168:939-949). The heat of fusion phase where water turns to ice should be minimal. The cooling procedure can be carried out by use of, *e.g.*, a programmable freezing device or a methanol bath procedure. Any method for cryopreserving that preserves viability of the cells can be used. In specific embodiments, a controlled rate freezer is used in the cryopreserving step to bring the temperature of the vial of cells to ≤ -90 °C or less, at a rate ranging from -0.3 to -2 °C per minute. By way of example but not limitation, the following program can be used: 1) wait for chamber is 4 °C and sample is 6.0 °C; 2) ramp at 1.0 °C/min. until sample is -6.0 °C; 3) ramp at 25 °C/min. until chamber is -45 °C; 4) ramp at 10 °C/min. until chamber is -14 °C; 5) ramp at 1.0 °C/min. until chamber is -40 °C; 6) ramp at 10 °C/min. until chamber is -90 °C; and 7) transfer to liquid nitrogen. Alternatively, the cells can be placed in a Mr. Frosty™ or other alcohol/polystyrene insulated freezing chamber pre-conditioned at -20 °C and frozen overnight by transferring the chamber to a -80 °C freezer, prior to transfer to liquid nitrogen storage.

[0065] After thorough freezing, the *ex vivo* sensitized population of human blood cells or cells derived therefrom can be rapidly transferred to a long-term cryogenic storage vessel. In a preferred embodiment, the *ex vivo* sensitized population of human blood cells or cells derived therefrom can be cryogenically stored in liquid nitrogen (-196 °C) or its vapor (-165 °C). In a specific embodiment, the cells are stored at -80 °C for 2 days and then transferred to liquid nitrogen. In a specific embodiment, a plurality of the populations of cells comprising antigen-specific T cells are generated and stored as described herein, thereby producing a cell bank.

[0066] In a preferred embodiment, by way of example but not limitation, the cryopreserving

step is performed as follows: First, a freeze mix is prepared containing 90% fetal calf serum and 10% DMSO (in sterile, 15 ml tubes, 9 ml of heat inactivated and filtered fetal calf serum is mixed with sterile, filtered 1 ml of DMSO). Aliquots of this freeze mix are prepared under sterile conditions and then stored at -20 °C for use as a suspension medium for freezing cells. T cells suspended at 1×10^6 /ml in Tcell medium are centrifuged in 15 or 50 ml tubes at 1500 rpm for 5 mins. The supernatant is gently discarded, cells are washed a second time with phosphate buffered saline (PBS), and cell pellet is then suspended in the thawed freeze mix at 10×10^6 cells/ml.

[0067] Other known methods of cryopreservation of viable cells, or modifications thereof, are available and envisioned for use (*e.g.*, cold metal-mirror techniques; Livesey and Linner, 1987, Nature 327:255; Linner *et al.*, 1986, J Histochem Cytochem 34:1123-1135; *see also* U.S. Patent No. 4,199,022 by Senkan *et al.*, U.S. Patent No. 3,753,357 by Schwartz, U.S. Patent No. 4,559,298 by Fahy).

[0068] In certain embodiments, the method of generating a population of cells comprising antigen-specific T cells further comprises, after the step of cryopreserving, steps of thawing and optionally expanding in culture the *ex vivo* sensitized population of human blood cells or cells derived therefrom. Frozen cells are preferably thawed quickly (*e.g.*, in a water bath maintained at 37-41 °C) and chilled immediately upon thawing. In particular, the vial containing the frozen cells can be immersed up to its neck in a warm water bath; gentle rotation will ensure mixing of the cell suspension as it thaws and increase heat transfer from the warm water to the internal ice mass. As soon as the ice has completely melted, the vial can be immediately placed in ice.

[0069] The cryopreservative, if toxic in humans, can be removed prior to therapeutic administration, and the removal is preferably accomplished upon thawing. However, when the cryopreservative is DMSO, it is preferable to omit this step in order to avoid cell loss, since DMSO in a low concentration has no serious toxicity.

[0070] TCR- or CAR-Expression

[0071] In some embodiments, the population of cells comprising antigen-specific T cells comprises antigen-specific T cells that recombinantly express a protein of interest, for example, a T cell receptor (TCR) or a chimeric antigen receptor (CAR). This can be achieved by transducing the population of human blood cells during their time in culture with a nucleic acid encoding the protein of interest. The nucleic acid preferably is a vector in which a nucleic acid

sequence encoding the protein of interest is operably linked to a promoter. The transducing preferably occurs during days 3-5 in culture, since, as shown by the example section herein (*i.e.*, Section 6), this time is when the T_{SCM} cells exhibit the highest proliferative capacity.

[0072] In some embodiments, the population of cells comprising antigen-specific T cells, as described herein, comprises antigen-specific T cells that endogenously express a public T cell receptor (TCR) recognizing the one or more antigens. In other embodiments, the population of cells comprising antigen-specific T cells, as described herein, comprises antigen-specific T cells that recombinantly express a public TCR recognizing the one or more antigens. In specific embodiments, the method of generating a population of cells comprising antigen-specific T cells, as described herein, further comprises transducing the population of human blood cells with a nucleic acid encoding a public TCR (*e.g.*, at a time when the population of human blood cells has been cultured for 3-5 days), for example, using a transducing method as described in Section 5.1.2, *infra*. The transducing step can be performed before, during, or after the *ex vivo* sensitizing step, which reduces alloreactivity of the population of cells comprising antigen-specific T cells.

[0073] Public TCRs are peptide-specific TCRs with highly homologous sequences detected in multiple individuals (Li *et al.*, 2012, Cell Res 22:33-42). Public TCRs for a variety of human viruses have been described (Argaet *et al.*, 1994, J Exp Med 180:2335-2340; Wang *et al.*, 2012, Sci Transl Med 4:128ra142; Nguyen *et al.*, 2014, J Immunol 192:5039-5049; Trautmann *et al.*, 2005, J Immunol 175:6123-6132).

[0074] In a specific embodiment wherein the one or more antigens is cytomegalovirus (CMV) pp65, the public TCR comprises a β -chain comprising a variable domain, which comprises a complementarity determining region (CDR)3 of CASSPQTGASYGYTF (SEQ ID NO:3). In another specific embodiment wherein the one or more antigens is CMV pp65, the public TCR comprises a β -chain comprising a variable domain, which comprises a CDR3 of CASSPKTGAVYGYTF (SEQ ID NO:4). In another specific embodiment wherein the one or more antigens is CMV pp65, the public TCR comprises a β -chain comprising a variable domain, which comprises a CDR3 of S*_nTG*_nGY (SEQ ID NO:16; *n indicates any amino acid sequence of any length and any amino acid combination).

[0075] In certain embodiments, the population of cells comprising antigen-specific T cells, as described herein, comprises antigen-specific T cells that recombinantly express a chimeric

antigen receptor (CAR) recognizing the one or more antigens (*see* Section 5.1.3, *infra*, for more details regarding CAR). In specific embodiments, the method of generating a population of cells comprising antigen-specific T cells, as described herein, further comprises transducing the population of human blood cells with a nucleic acid encoding a CAR (*e.g.*, at a time when the population of human blood cells has been cultured for 3-5 days), for example, using a transducing method as described in Section 5.1.3, *infra*. The transducing step can be performed before, during, or after the *ex vivo* sensitizing step, which reduces alloreactivity of the population of cells comprising antigen-specific T cells.

5.1.2. Methods Using TCR-Transduced T_{SCM} cells

[0076] In another aspect, provided herein are methods of generating a population of cells comprising antigen-specific T cells for therapeutic administration to a human patient having or suspected of having a pathogen or cancer, comprising transducing a population of human blood cells with a nucleic acid encoding a public TCR recognizing one or more antigens of the pathogen or cancer (preferably at a time when the population of human blood cells has been cultured for 3-5 days), wherein the population of human blood cells contains at least 50% T_{SCM} cells; thereby producing said population of cells comprising antigen-specific T cells. The transducing preferably occurs during days 3-5 in culture, since, as shown by the example section herein (*i.e.*, Section 6), this time is when the T_{SCM} cells exhibit the highest proliferative capacity.

[0077] In a specific embodiment wherein the one or more antigens is CMV pp65, the public TCR comprises a β -chain comprising a variable domain, which comprises a CDR3 of CASSPQTGASYGYTF (SEQ ID NO:3). In another specific embodiment wherein the one or more antigens is CMV pp65, the public TCR comprises a β -chain comprising a variable domain, which comprises a CDR3 of CASSPKTGAVYGYTF (SEQ ID NO:4). In another specific embodiment wherein the one or more antigens is CMV pp65, the public TCR comprises a β -chain comprising a variable domain, which comprises a CDR3 of S*_nTG*_nGY (SEQ ID NO:16; *n indicates any amino acid sequence of any length and any amino acid combination).

[0078] In another aspect, provided herein are methods of generating a population of cells comprising antigen-specific T cells for therapeutic administration to a human patient having or suspected of having a CMV infection, comprising transducing a population of human blood cells with a nucleic acid encoding a public TCR recognizing CMV pp65 (preferably at a time when

the population of human blood cells has been cultured for 3-5 days), wherein the public TCR comprises a β -chain comprising a variable domain, which comprises a CDR3 of CASSPQTGASYGYTF (SEQ ID NO:3), and wherein the population of human blood cells contains at least 50% T_{SCM} cells; thereby producing said population of cells comprising antigen-specific T cells.

[0079] In another aspect, provided herein are methods of generating a population of cells comprising antigen-specific T cells for therapeutic administration to a human patient having or suspected of having a CMV infection, comprising transducing a population of human blood cells with a nucleic acid encoding a public TCR recognizing CMV pp65 (preferably at a time when the population of human blood cells has been cultured for 3-5 days), wherein the public TCR comprises a β -chain comprising a variable domain, which comprises a CDR3 of CASSPKTGAVYGYTF (SEQ ID NO:4), and wherein the population of human blood cells contains at least 50% T_{SCM} cells; thereby producing said population of cells comprising antigen-specific T cells.

[0080] In another aspect, provided herein are methods of generating a population of cells comprising antigen-specific T cells for therapeutic administration to a human patient having or suspected of having a CMV infection, comprising transducing a population of human blood cells with a nucleic acid encoding a public TCR recognizing CMV pp65 (preferably at a time when the population of human blood cells has been cultured for 3-5 days), wherein the public TCR comprises a β -chain comprising a variable domain, which comprises a CDR3 of S*_nTG*_nGY (SEQ ID NO:16; *n indicates any amino acid sequence of any length and any amino acid combination), and wherein the population of human blood cells contains at least 50% T_{SCM} cells; thereby producing said population of cells comprising antigen-specific T cells.

[0081] In specific embodiments, the method of generating a population of cells comprising antigen-specific T cells further comprises, after the step of transducing, a step of cryopreserving the transduced population of human blood cells or cells derived therefrom. In a specific embodiment, the method of generating a population of cells comprising antigen-specific T cells further comprises, after the step of cryopreserving, steps of thawing and optionally expanding in culture the transduced population of human blood cells or cells derived therefrom. The cryopreserving and thawing steps can be performed as described in Section 5.1.1, *supra*.

[0082] In specific embodiments, the method of generating a population of cells comprising

antigen-specific T cells further comprises, after the step of transducing, a step of expanding in culture the transduced population of human blood cells or cells derived therefrom, wherein the transduced population of human blood cells or cells derived therefrom has not been cryopreserved.

[0083] TCR is a cell surface molecule on T cells that is responsible for recognizing antigen peptide-bound major histocompatibility complex (MHC) molecules. Public TCRs are peptide-specific TCRs with highly homologous sequences detected in multiple individuals (Li *et al.*, 2012, *Cell Res* 22:33-42). Public TCRs for a variety of human viruses have been described (Argaet *et al.*, 1994, *J Exp Med* 180:2335-2340; Wang *et al.*, 2012, *Sci Transl Med* 4:128ra142; Nguyen *et al.*, 2014, *J Immunol* 192:5039-5049; Trautmann *et al.*, 2005, *J Immunol* 175:6123-6132).

[0084] The population of human blood cells transduced with a nucleic acid encoding a TCR can be generated by any method known in the art, for example, as described in Stauss *et al.*, 2015, *Curr Opin Pharmacol* 24:113-118; Sharpe and Mount, 2015, *Dis Model Mech* 8:337-350; Kunert *et al.*, 2013, *Front Immunol* 4: 363; Stone *et al.*, 2012, *Methods Enzymol* 503:189-222; or Park *et al.*, 2011, *Trends Biotechnol* 29:550-557.

[0085] The nucleic acid encoding a TCR can be DNA, RNA, or a nucleic acid analog. In specific embodiments, such a nucleic acid may be part of a vector. In a specific embodiment, the vector is an expression vector that is capable of directing the expression of a nucleic acid encoding a polypeptide of the TCR described herein in T cells. Non-limiting examples of expression vectors suitable for directing the expression of a nucleic acid encoding a polypeptide of the TCR described herein include, but are not limited to, plasmids and viral vectors, such as synthetic vectors, lentiviral vectors, replication-defective retroviral vectors, autonomously replicating plasmids. In a specific embodiment, an expression vector used for directing the expression of a nucleic acid encoding a polypeptide of the TCR described herein includes one or more regulatory sequences operably linked to the nucleic acid to be expressed. "Operably linked" is intended to mean that a nucleic acid of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleic acid in T cells. Regulatory sequences include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals).

[0086] A nucleic acid encoding a polypeptide of the TCR described herein, for example, an

expression vector, can be transduced into host cells via conventional transformation or transfection (such as, transfection by a virus, *e.g.*, a retrovirus or lentivirus) techniques. Such techniques include, but are not limited to, calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, and electroporation. Cells containing a nucleic acid encoding a polynucleotide of the TCR described herein may be selected using one or more selectable markers known in the art.

5.1.3. Methods Using CAR-Transduced T_{SCM} cells

[0087] In another aspect, provided herein are methods of generating a population of cells comprising antigen-specific T cells for therapeutic administration to a human patient having or suspected of having a pathogen or cancer, comprising transducing a population of human blood cells with a nucleic acid encoding a CAR recognizing one or more antigens of the pathogen or cancer (preferably at a time when the population of human blood cells has been cultured for 3-5 days), wherein the population of human blood cells contains at least 50% T_{SCM} cells; thereby producing said population of cells comprising antigen-specific T cells. The transducing preferably occurs during days 3-5 in culture, since, as shown by the example section herein (*i.e.*, Section 6), this time is when the T_{SCM} cells exhibit the highest proliferative capacity.

[0088] In specific embodiments, the method of generating a population of cells comprising antigen-specific T cells further comprises, after the step of transducing, a step of cryopreserving the transduced population of human blood cells or cells derived therefrom. In a specific embodiment, the method of generating a population of cells comprising antigen-specific T cells further comprises, after the step of cryopreserving, steps of thawing and optionally expanding in culture the transduced population of human blood cells or cells derived therefrom. The cryopreserving and thawing steps can be performed as described in Section 5.1.1, *supra*.

[0089] In specific embodiments, the method of generating a population of cells comprising antigen-specific T cells further comprises, after the step of transducing, a step of expanding in culture the transduced population of human blood cells or cells derived therefrom, wherein the transduced population of human blood cells or cells derived therefrom has not been cryopreserved.

[0090] CARs are engineered receptors that provide both antigen binding and immune cell activation functions (Sadelain et al., 2013, *Cancer Discovery* 3:388-398). They usually comprise

an antigen-binding domain (*e.g.*, derived from a monoclonal antibody or the extracellular domain of a receptor), a transmembrane domain, an intracellular domain, and optionally a co-stimulatory domain. CARs can be used to graft the specificity of an antigen-binding domain onto an immune cell such as a T cell.

[0091] The population of human blood cells transduced with a nucleic acid encoding a CAR can be generated by any method known in the art, for example, as described in Stauss et al., 2015, *Curr Opin Pharmacol* 24:113-118; Sharpe and Mount, 2015, *Dis Model Mech* 8:337-350; or Park et al., 2011, *Trends Biotechnol* 29:550–557.

[0092] The nucleic acid encoding a CAR can be DNA, RNA, or a nucleic acid analog. In specific embodiments, such a nucleic acid may be part of a vector. In a specific embodiment, the vector is an expression vector that is capable of directing the expression of a nucleic acid encoding a polypeptide of the CAR described herein in T cells. Non-limiting examples of expression vectors suitable for directing the expression of a nucleic acid encoding a polypeptide of the CAR described herein include, but are not limited to, plasmids and viral vectors, such as synthetic vectors, lentiviral vectors, replication-defective retroviral vectors, autonomously replicating plasmids. In a specific embodiment, an expression vector used for directing the expression of a nucleic acid encoding a polypeptide of the CAR described herein includes one or more regulatory sequences operably linked to the nucleic acid to be expressed. “Operably linked” is intended to mean that a nucleic acid of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleic acid in T cells. Regulatory sequences include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals).

[0093] A nucleic acid encoding a polypeptide of the CAR described herein, for example, an expression vector, can be transduced into host cells via conventional transformation or transfection (such as, transfection by a virus, *e.g.*, a retrovirus or lentivirus) techniques. Such techniques include, but are not limited to, calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, and electroporation. Cells containing a nucleic acid encoding a polynucleotide of the CAR described herein may be selected using one or more selectable markers known in the art.

5.1.4. The population of human blood cells

[0094] The population of human blood cells used in accordance with the methods of generating a population of cells comprising antigen-specific T cells described herein contains at least 50% T_{SCM} cells. In a specific embodiment, the population of human blood cells contains at least 60% T_{SCM} cells. In another specific embodiment, the population of human blood cells contains at least 70% T_{SCM} cells. In another specific embodiment, the population of human blood cells contains at least 80% T_{SCM} cells. In another specific embodiment, the population of human blood cells contains at least 90% T_{SCM} cells. In another specific embodiment, the population of human blood cells contains at least 95% T_{SCM} cells. In another specific embodiment, the population of human blood cells contains at least 99% T_{SCM} cells. In another specific embodiment, the population of human blood cells contains 100% T_{SCM} cells. In another specific embodiment, the population of human blood cells contains about 50- 75% T_{SCM} cells. In another specific embodiment, the population of human blood cells contains about 75- 90% T_{SCM} cells. In another specific embodiment, the population of human blood cells contains about 90- 100% T_{SCM} cells. In another specific embodiment, the population of human blood cells contains about 95- 100% T_{SCM} cells.

[0095] In some embodiments, T_{SCM} cells are CD3⁺CD62L⁺CD45RO⁻CD95⁺. In other embodiments, T_{SCM} cells are CD3⁺CD62L⁺CD45RA⁺CD95⁺. In other embodiments, T_{SCM} cells are CD3⁺CCR7⁺CD45RA⁺CD95⁺. In other embodiments, T_{SCM} cells are CD3⁺CCR7⁺CD28⁺CD45RO⁻CD95⁺. In other embodiments, T_{SCM} cells are CD3⁺CD62L⁺CCR7⁺CD45RA⁺CD95⁺. In other embodiments, T_{SCM} cells are CD3⁺CCR7⁺CD45RA⁺CD28⁺CD95⁺. In other embodiments, T_{SCM} cells are CD3⁺CCR7⁺CD45RA⁺CD45RO⁻CD95⁺. In other embodiments, T_{SCM} cells are CD3⁺CCR7⁺CD45RA⁺CD127⁺CD95⁺. In other embodiments, T_{SCM} cells are CD3⁺CD62L⁺CCR7⁺CD45RA⁺CD27⁺CD127⁺CD45RO⁻CD95⁺. In other embodiments, T_{SCM} cells are CD3⁺CD62L⁺CCR7⁺CD28⁺CD45RA⁺CD27⁺CD127⁺CD103⁻CD45RO⁻CD95⁺. In various embodiments, the cell surface marker expression of T_{SCM} cells satisfies the following: (i) CD3⁺CD95⁺; (ii) CD45RO⁻ or CD45RA⁺, or a combination thereof; and (iii) CD62L⁺, or CCR7⁺, or CD127⁺, or a combination thereof; and optionally (iv) CD28⁺, or CD27⁺, or CD103⁻, or a combination thereof.

[0096] In various embodiments, the population of human blood cells contains less than 50% T_N cells. In a specific embodiment, the population of human blood cells contains less than 40%

T_N cells. In another specific embodiment, the population of human blood cells contains less than 30% T_N cells. In another specific embodiment, the population of human blood cells contains less than 20% T_N cells. In another specific embodiment, the population of human blood cells contains less than 10% T_N cells. In another specific embodiment, the population of human blood cells contains less than 5% T_N cells. In another specific embodiment, the population of human blood cells contains less than 2% T_N cells. In another specific embodiment, the population of human blood cells contains less than 1% T_N cells. In another specific embodiment, the population of human blood cells contains no T_N cells. In another specific embodiment, the population of human blood cells contains about 50-30% T_N cells. In another specific embodiment, the population of human blood cells contains about 30-20% T_N cells. In another specific embodiment, the population of human blood cells contains about 20-10% T_N cells. In another specific embodiment, the population of human blood cells contains about 10-0% T_N cells. In another specific embodiment, the population of human blood cells contains about 10-5% T_N cells. In another specific embodiment, the population of human blood cells contains about 5-0% T_N cells.

[0097] In some embodiments, T_N cells are $CD3^+CD62L^+CD45RO^-CD95^-$. In other embodiments, T_N cells are $CD3^+CD62L^+CD45RA^+CD95^-$. In other embodiments, T_N cells are $CD3^+CCR7^+CD45RA^+CD95^-$. In other embodiments, T_N cells are $CD3^+CCR7^+CD28^+CD45RO^-CD95^-$. In other embodiments, T_N cells are $CD3^+CD62L^+CCR7^+CD45RA^+CD95^-$. In other embodiments, T_N cells are $CD3^+CCR7^+CD45RA^+CD28^+CD95^-$. In other embodiments, T_N cells are $CD3^+CCR7^+CD45RA^+CD45RO^-CD95^-$. In other embodiments, T_N cells are $CD3^+CCR7^+CD45RA^+CD127^+CD95^-$. In other embodiments, T_N cells are $CD3^+CD62L^+CCR7^+CD45RA^+CD27^+CD127^+CD45RO^-CD95^-$. In other embodiments, T_N cells are $CD3^+CD62L^+CCR7^+CD28^+CD45RA^+CD27^+CD127^+CD103^-CD45RO^-CD95^-$. In various embodiments, the cell surface marker expression of T_N cells satisfies the following: (i) $CD3^+CD95^-$; (ii) $CD45RO^-$ or $CD45RA^+$, or a combination thereof; and (iii) $CD62L^+$, or $CCR7^+$, or $CD127^+$, or a combination thereof; and optionally (iv) $CD28^+$, or $CD27^+$, or $CD103^-$, or a combination thereof.

[0098] In certain embodiments, the method of generating a population of cells comprising antigen-specific T cells described herein further comprises a step of deriving the population of human blood cells from a human cell sample. The human cell sample can be any cell sample that

contains T_{SCM} cells or cells that can be induced in culture to become T_{SCM} cells, such as, but is not limited to, a hematopoietic cell sample, a blood cell sample, a fractionated or unfractionated whole blood sample, a fractionated or unfractionated apheresis collection (*e.g.*, a leukapheresis collection, such as leukopak), PBMCs, or a T cell population (*e.g.*, T cells enriched for from PBMCs). In a specific embodiment, the human cell sample is PBMCs. PBMCs can be isolated from the blood sample by any method known in the art to isolated PBMCs from a blood sample, such as by Ficoll-Hypaque centrifugation as described in Koehne et al., 2000, Blood 96:109-117; Trivedi et al., 2005, Blood 105:2793-2801; or as described in Section 6.2, *infra*. In another specific embodiment, the human cell sample is a population enriched in T cells from PBMCs. T cells can be enriched for from the PBMCs by any method known in the art to enrich for T cells from a blood sample or PBMCs. Non-limiting exemplary methods for enriching for T cells from PBMCs can be found in Koehne et al., 2000, Blood 96:109-117; Trivedi et al., 2005, Blood 105:2793-2801; Hasan et al., 2009, J Immunol 183: 2837-2850; and Koehne et al., 2015, Biol Blood Marrow Transplant 21: 1663-1678. For example, T cells can be enriched for from PBMCs by sorting the PBMCs using an anti-CD3 antibody and/or depleting from the PBMCs adherent monocytes and natural killer cells.

[0099] The step of deriving the population of human blood cells from a human cell sample can employ any known method in the art to produce a population of human blood cells that contains at least 50% T_{SCM} cells from the human cell sample, such as, but is not limited to, sorting the human cell sample to select for T_{SCM} cells or *in vitro* reprogramming cells in the human cell sample to turn them into T_{SCM} cells. In specific embodiments, the step of deriving the population of human blood cells from a human cell sample comprises affinity selection for cells that express cell surface markers of T_{SCM} cells (*e.g.*, using antibodies to the cell surface markers). In some embodiments, the step of deriving the population of human blood cells from a human cell sample comprises sorting T_{SCM} cells from the human cell sample by fluorescence-activated cell sorting (FACS). In other embodiments, the step of deriving the population of human blood cells from a human cell sample comprises sorting T_{SCM} cells from the human cell sample by magnetic separation.

[00100] In specific embodiments, the deriving step comprises enriching for T_{SCM} cells from the human cell sample. T_{SCM} cells exhibit a set of cell surface markers that can be used to distinguish them from other T cell subsets, thus the enriching step can comprise selecting for

T_{SCM} cells based on their markers. In a specific embodiment, the enriching step comprises selecting for T_{SCM} cells that are CD3⁺CD62L⁺CD45RO⁻CD95⁺. In another specific embodiment, the enriching step comprises selecting for T_{SCM} cells that are CD3⁺CD62L⁺CD45RA⁺CD95⁺. In another specific embodiment, the enriching step comprises selecting for T_{SCM} cells that are CD3⁺CCR7⁺CD45RA⁺CD95⁺. In another specific embodiment, the enriching step comprises selecting for T_{SCM} cells that are CD3⁺CCR7⁺CD28⁺CD45RO⁻CD95⁺. In another specific embodiment, the enriching step comprises selecting for T_{SCM} cells that are CD3⁺CD62L⁺CCR7⁺CD45RA⁺CD95⁺. In another specific embodiment, the enriching step comprises selecting for T_{SCM} cells that are CD3⁺CCR7⁺CD45RA⁺CD28⁺CD95⁺. In another specific embodiment, the enriching step comprises selecting for T_{SCM} cells that are CD3⁺CCR7⁺CD45RA⁺CD45RO⁻CD95⁺. In another specific embodiment, the enriching step comprises selecting for T_{SCM} cells that are CD3⁺CCR7⁺CD45RA⁺CD127⁺CD95⁺. In another specific embodiment, the enriching step comprises selecting for T_{SCM} cells that are CD3⁺CD62L⁺CCR7⁺CD45RA⁺CD27⁺CD127⁺CD45RO⁻CD95⁺. In another specific embodiment, the enriching step comprises selecting for T_{SCM} cells that are CD3⁺CD62L⁺CCR7⁺CD28⁺CD45RA⁺CD27⁺CD127⁺CD103⁻CD45RO⁻CD95⁺. In various embodiments, the enriching step comprises selecting for cells whose cell surface marker expression satisfies the following: (i) CD3⁺CD95⁺; (ii) CD45RO⁻ or CD45RA⁺, or a combination thereof; and (iii) CD62L⁺, or CCR7⁺, or CD127⁺, or a combination thereof; and optionally (iv) CD28⁺, or CD27⁺, or CD103⁻, or a combination thereof.

[00101] In specific embodiments, the deriving step comprises depleting T_N cells from the human cell sample. Naive T cells (T_N cells) are distinguished from T_{SCM} cells by the expression of cell surface marker CD95. In a specific embodiment, the depleting step comprises selecting against (*i.e.*, depleting, or excluding) cells that are CD3⁺CD62L⁺CD45RO⁻CD95⁻. In another specific embodiment, the depleting step comprises selecting against (*i.e.*, depleting, or excluding) cells that are CD3⁺CD62L⁺CD45RA⁺CD95⁻. In another specific embodiment, the depleting step comprises selecting against (*i.e.*, depleting, or excluding) cells that are CD3⁺CCR7⁺CD45RA⁺CD95⁻. In another specific embodiment, the depleting step comprises selecting against (*i.e.*, depleting, or excluding) cells that are CD3⁺CCR7⁺CD28⁺CD45RO⁻CD95⁻. In another specific embodiment, the depleting step comprises selecting against (*i.e.*, depleting, or excluding) cells that are CD3⁺CD62L⁺CCR7⁺CD45RA⁺CD95⁻. In another specific embodiment,

the depleting step comprises selecting against (*i.e.*, depleting, or excluding) cells that are CD3⁺CCR7⁺CD45RA⁺CD28⁺CD95⁻. In another specific embodiment, the depleting step comprises selecting against (*i.e.*, depleting, or excluding) cells that are CD3⁺CCR7⁺CD45RA⁺CD45RO⁻CD95⁻. In another specific embodiment, the depleting step comprises selecting against (*i.e.*, depleting, or excluding) cells that are CD3⁺CCR7⁺CD45RA⁺CD127⁺CD95⁻. In another specific embodiment, the depleting step comprises selecting against (*i.e.*, depleting, or excluding) cells that are CD3⁺CD62L⁺CCR7⁺CD45RA⁺CD27⁺CD127⁺CD45RO⁻CD95⁻. In another specific embodiment, the depleting step comprises selecting against (*i.e.*, depleting, or excluding) cells that are CD3⁺CD62L⁺CCR7⁺CD28⁺CD45RA⁺CD27⁺CD127⁺CD103⁻CD45RO⁻CD95⁻. In various embodiments, the depleting step comprises selecting against (*i.e.*, depleting, or excluding) cells whose cell surface marker expression level satisfies the following: (i) CD3⁺CD95⁻; (ii) CD45RO⁻ or CD45RA⁺, or a combination thereof; and (iii) CD62L⁺, or CCR7⁺, or CD127⁺, or a combination thereof; and optionally (iv) CD28⁺, or CD27⁺, or CD103⁻, or a combination thereof.

[00102] In preferred embodiments, the population of human blood cells is derived from a human donor that is seropositive for the one or more antigens. In certain embodiments, the population of human blood cells is derived from a human donor that is seronegative for the one or more antigens.

[00103] The human donor can be an adult (at least age 16), an adolescent (age 12-15), a child (under age 12), or a fetus. In a specific embodiment, the human donor is an adult.

[00104] The term “about” shall be construed so as to allow normal variation.

5.2. Methods of Treating Patients Using the Generated Antigen-Specific T Cells

[00105] In another aspect, provided herein are methods of treating a human patient having a pathogen or cancer, comprising: (i) generating a population of cells comprising antigen-specific T cells according to a method described in Section 5.1, *supra*; and (ii) administering the population of cells comprising antigen-specific T cells to the human patient.

[00106] In another aspect, provided herein are methods of treating a human patient having a pathogen or cancer, comprising administering a population of cells comprising antigen-specific T cells to the human patient, wherein the population of cells comprising antigen-specific T cells is

the product of a method comprising generating the population of cells comprising antigen-specific T cells according to a method described in Section 5.1, *supra*.

[00107] In specific embodiments, the population of human blood cells is derived from a human donor that is allogeneic to the human patient. In a specific embodiment, the human patient has been the recipient of a transplant from a transplant donor, and the human donor is a third-party donor that is different from the transplant donor. In another specific embodiment, the human patient has been the recipient of a transplant from a transplant donor, and the human donor is the transplant donor. In some embodiments, the transplant is a hematopoietic stem cell transplantation (HSCT), such as a peripheral blood stem cell transplantation, a bone marrow transplantation, or a cord blood transplantation. In other embodiments, the transplant is a solid organ transplant, such as a kidney transplant, a liver transplant, a heart transplant, an intestinal transplant, a pancreas transplant, a lung transplant, or a small bowel transplant.

[00108] In specific embodiments, the step of administering of the population of cells comprising antigen-specific T cells does not result in any graft-versus-host disease (GvHD) in the human patient.

5.2.1. Administration and Dosage

[00109] The route of administration of the population of cells comprising antigen-specific T cells and the amount to be administered to the human patient can be determined based on the condition of the human patient and the knowledge of the physician. Generally, the administration is intravenous. In certain embodiments, the administering step is by infusion of the population of cells comprising antigen-specific T cells. In specific embodiments, the infusion is bolus intravenous infusion.

[00110] In some embodiments, the administering step comprises administering the population of cells comprising antigen-specific T cells to the human patient, at a dose that is less than or equal to about 1×10^5 cells of the population of cells comprising antigen-specific T cells per kg of the human patient. In a specific embodiment, the administering step comprises administering the population of cells comprising antigen-specific T cells to the human patient, at a dose that is less than or equal to about 5×10^4 cells of the population of cells comprising antigen-specific T cells per kg of the human patient. In a preferred embodiment, the administering step comprises administering the population of cells comprising antigen-specific T cells to the human patient, at

a dose that is less than or equal to about 1×10^4 cells of the population of cells comprising antigen-specific T cells per kg of the human patient. In another specific embodiment, the administering step comprises administering the population of cells comprising antigen-specific T cells to the human patient, at a dose that is less than or equal to about 5×10^3 cells of the population of cells comprising antigen-specific T cells per kg of the human patient. In another specific embodiment, the administering step comprises administering the population of cells comprising antigen-specific T cells to the human patient, at a dose that is less than or equal to about 1×10^3 cells of the population of cells comprising antigen-specific T cells per kg of the human patient. In another specific embodiment, the administering step comprises administering the population of cells comprising antigen-specific T cells to the human patient, at a dose of about 1×10^3 to 5×10^3 cells of the population of cells comprising antigen-specific T cells per kg of the human patient. In another specific embodiment, the administering step comprises administering the population of cells comprising antigen-specific T cells to the human patient, at a dose of about 5×10^3 to 1×10^4 cells of the population of cells comprising antigen-specific T cells per kg of the human patient. In another specific embodiment, the administering step comprises administering the population of cells comprising antigen-specific T cells to the human patient, at a dose of about 1×10^4 to 5×10^4 cells of the population of cells comprising antigen-specific T cells per kg of the human patient. In another specific embodiment, the administering step comprises administering the population of cells comprising antigen-specific T cells to the human patient, at a dose of about 5×10^4 to 1×10^5 cells of the population of cells comprising antigen-specific T cells per kg of the human patient.

[00111] In other embodiments, the administering step comprises administering the population of cells comprising antigen-specific T cells to the human patient, at a dose that is at least 1×10^5 cells of the population of cells comprising antigen-specific T cells per kg of the human patient. In a specific embodiment, the administering step comprises administering the population of cells comprising antigen-specific T cells to the human patient, at a dose that is about 5×10^5 cells of the population of cells comprising antigen-specific T cells per kg of the human patient. In another specific embodiment, the administering step comprises administering the population of cells comprising antigen-specific T cells to the human patient, at a dose that is about 1×10^6 cells of the population of cells comprising antigen-specific T cells per kg of the human patient. In another specific embodiment, the administering step comprises administering the population of

cells comprising antigen-specific T cells to the human patient, at a dose that is about 2×10^6 cells of the population of cells comprising antigen-specific T cells per kg of the human patient. In another specific embodiment, the administering step comprises administering the population of cells comprising antigen-specific T cells to the human patient, at a dose that is about 3×10^6 cells of the population of cells comprising antigen-specific T cells per kg of the human patient. In another specific embodiment, the administering step comprises administering the population of cells comprising antigen-specific T cells to the human patient, at a dose that is about 4×10^6 cells of the population of cells comprising antigen-specific T cells per kg of the human patient. In another specific embodiment, the administering step comprises administering the population of cells comprising antigen-specific T cells to the human patient, at a dose that is about 5×10^6 cells of the population of cells comprising antigen-specific T cells per kg of the human patient. In another specific embodiment, the administering step comprises administering the population of cells comprising antigen-specific T cells to the human patient, at a dose that is about 6×10^6 cells of the population of cells comprising antigen-specific T cells per kg of the human patient. In another specific embodiment, the administering step comprises administering the population of cells comprising antigen-specific T cells to the human patient, at a dose that is about 1×10^7 cells of the population of cells comprising antigen-specific T cells per kg of the human patient. In another specific embodiment, the administering step comprises administering the population of cells comprising antigen-specific T cells to the human patient, at a dose that is about 1×10^5 to 5×10^5 cells of the population of cells comprising antigen-specific T cells per kg of the human patient. In another specific embodiment, the administering step comprises administering the population of cells comprising antigen-specific T cells to the human patient, at a dose that is about 5×10^5 to 1×10^6 cells of the population of cells comprising antigen-specific T cells per kg of the human patient. In another specific embodiment, the administering step comprises administering the population of cells comprising antigen-specific T cells to the human patient, at a dose that is about 1×10^6 to 2×10^6 cells of the population of cells comprising antigen-specific T cells per kg of the human patient. In another specific embodiment, the administering step comprises administering the population of cells comprising antigen-specific T cells to the human patient, at a dose that is about 2×10^6 to 5×10^6 cells of the population of cells comprising antigen-specific T cells per kg of the human patient. In another specific embodiment, the administering step comprises administering the population of cells comprising antigen-specific T

cells to the human patient, at a dose that is about 5×10^6 to 1×10^7 cells of the population of cells comprising antigen-specific T cells per kg of the human patient.

[00112] In certain embodiments, the step of administering comprises administering the population of cells comprising antigen-specific T cells to the human patient at the dose described above weekly. In certain embodiments, the step of administering comprises administering the population of cells comprising antigen-specific T cells to the human patient at the dose described above twice weekly. In certain embodiments, the step of administering comprises administering the population of cells comprising antigen-specific T cells to the human patient at the dose described above biweekly. In certain embodiments, the step of administering comprises administering the population of cells comprising antigen-specific T cells to the human patient at the dose described above every three weeks.

[00113] In certain embodiments, the step of administering comprises administering at least 2 doses of the population of cells comprising antigen-specific T cells to the human patient. In specific embodiments, the step of administering comprises administering 2, 3, 4, 5, or 6 doses of the population of cells comprising antigen-specific T cells to the human patient. In a specific embodiment, the step of administering comprises administering 2 doses of the population of cells comprising antigen-specific T cells to the human patient. In another specific embodiment, the step of administering comprises administering 3 doses of the population of cells comprising antigen-specific T cells to the human patient. In another specific embodiment, the step of administering comprises administering 4 doses of the population of cells comprising antigen-specific T cells to the human patient.

[00114] In specific embodiments, the step of administering comprises administering at least two cycles (*e.g.*, 2, 3, 4, 5, or 6 cycles) of one dose per week of the population of cells comprising antigen-specific T cells for at least two consecutive weeks (*e.g.*, 2, 3, 4, 5, or 6 consecutive weeks), each cycle separated by a washout period during which no dose of the population of cells comprising antigen-specific T cells is administered. In a specific embodiment, the at least two consecutive weeks are 2 consecutive weeks. In a preferred embodiment, the at least two consecutive weeks are 3 consecutive weeks. In another specific embodiment, the at least two consecutive weeks are 4 consecutive weeks. In another specific embodiment, the step of administering comprises administering two, three, four, five, or six cycles of one dose per week of the population of cells comprising antigen-specific T cells for

three consecutive weeks, each cycle separated by a washout period during which no dose of the population of cells comprising antigen-specific T cells is administered. In another specific embodiment, the step of administering comprises administering a first cycle of one dose per week of the population of cells comprising antigen-specific T cells for 3 consecutive weeks followed by a washout period during which no dose of the population of cells comprising antigen-specific T cells is administered, followed by a second cycle of said one dose per week of the population of cells comprising antigen-specific T cells for 3 consecutive weeks. In specific embodiments, the washout period is at least about 1 week (*e.g.*, about 1-6 weeks). In specific embodiments, the washout period is about 1, 2, 3, or 4 weeks. In a specific embodiment, the washout period is about 2 weeks. In a preferred embodiment, the washout period is about 3 weeks. In another specific embodiment, the washout period is about 4 weeks. Preferably, an additional cycle is administered only when the previous cycle has not exhibited toxicity (for example, no grade 3-5 serious adverse events, graded according to NCI CTCAE 4.0).

[00115] In specific embodiments, the step of administering of the population of cells comprising antigen-specific T cells comprises continuously administering the population of cells comprising antigen-specific T cells at a dose described herein weekly (*i.e.*, there is no week during which the population of cells comprising antigen-specific T cells is not administered, and thus there is no washout period).

[00116] In certain embodiments, a first dosage regimen described herein is carried out for a first period of time, followed by a second and different dosage regimen described herein that is carried out for a second period of time, wherein the first period of time and the second period of time are optionally separated by a washout period. In specific embodiments, the washout period is at least about 1 week (*e.g.*, about 1-6 weeks). In specific embodiments, the washout period is about 1, 2, 3, or 4 weeks. In a specific embodiment, the washout period is about 2 weeks. In a preferred embodiment, the washout period is about 3 weeks. In another specific embodiment, the washout period is about 4 weeks. Preferably, the second dosage regimen is carried out only when the first dosage regimen has not exhibited toxicity (for example, no grade 3-5 serious adverse events, graded according to NCI CTCAE 4.0).

[00117] The term “about” shall be construed so as to allow normal variation.

5.2.2. Serial Treatment with Different Cell Populations

[00118] In certain embodiments, the method of treating a human patient having a pathogen or cancer as described above further comprises, after administering to the human patient a first population of cells comprising antigen-specific T cells generated according to a method described in Section 5.1, *supra*, administering to the human patient a second population of cells comprising antigen-specific T cells generated according to a method described in Section 5.1, *supra*, wherein the antigen-specific T cells in the second population of cells comprising antigen-specific T cells are restricted by a different HLA allele (different from the HLA allele by which antigen-specific cells contained in the first population of cells comprising antigen-specific T cells are restricted) shared with the diseased cells in the human patient. In a specific embodiment, the method of treating a human patient having a pathogen or cancer comprises administering a first cycle of one dose per week of the first population of cells comprising antigen-specific T cells, for at least two consecutive weeks (*e.g.*, 2, 3, 4, 5, or 6 consecutive weeks), optionally followed by a washout period during which no dose of any population of cells comprising antigen-specific T cells is administered, and followed by a second cycle of one dose per week of the second population of cells comprising antigen-specific T cells for at least two consecutive weeks (*e.g.*, 2, 3, 4, 5, or 6 consecutive weeks). In specific embodiments, the washout period is at least about 1 week (*e.g.*, about 1-6 weeks). In specific embodiments, the washout period is about 1, 2, 3, or 4 weeks. In a specific embodiment, the washout period is about 2 weeks. In a preferred embodiment, the washout period is about 3 weeks. In certain embodiments, the human patient has no response, an incomplete response, or a suboptimal response (*i.e.*, the human patient may still have a substantial benefit from continuing treatment, but has reduced chances of optimal long-term outcomes) after administering the first population of cells comprising antigen-specific T cells and prior to administering the second population of cells comprising antigen-specific T cells.

[00119] The first and second populations of cells comprising antigen-specific T cells can each be administered by any route and any dosage regimen as described in Section 5.2.1, *supra*.

[00120] In specific embodiments, two populations of cells comprising antigen-specific T cells that are each restricted (*i.e.*, antigen-specific T cells in the two populations of cells are each restricted) by a different HLA allele shared with the diseased cells in the human patient are administered serially. In specific embodiments, three populations of cells comprising antigen-specific T cells that are each restricted (*i.e.*, antigen-specific T cells in the three populations of

cells are each restricted) by a different HLA allele shared with the diseased cells in the human patient are administered serially. In specific embodiments, four populations of cells comprising antigen-specific T cells that are each restricted (*i.e.*, antigen-specific T cells in the four populations of cells are each restricted) by a different HLA allele shared with the diseased cells in the human patient are administered serially. In specific embodiments, more than four populations of cells comprising antigen-specific T cells that are each restricted (*i.e.*, antigen-specific T cells in the more than four populations of cells are each restricted) by a different HLA allele shared with the diseased cells in the human patient are administered serially.

5.2.3. Additional Therapies

[00121] In specific embodiments, the method of treating a human patient having a pathogen or cancer further comprises concurrently treating the human patient with a second therapy for the pathogen or cancer, which second therapy is not treatment with a population of cells comprising antigen-specific T cells according to the invention, for example, at about the same time, the same day, or same week, or same treatment period (treatment cycle) during which the population of cells comprising antigen-specific T cells is administered, or on similar dosing schedules, or on different but overlapping dosing schedules. In specific embodiments, no second therapy for the pathogen or cancer is concurrently administered to the human patient over a period of time over which the population of cells is repeatedly administered to the human patient. In specific embodiments, the method of treating a human patient having a pathogen or cancer further comprises, before the administering step, a step of treating the human patient with a second therapy for the pathogen or cancer, which is not treatment with a population of cells comprising antigen-specific T cells according to the invention.

5.3. The Population of Cells Comprising Antigen-Specific T Cells and Their Characterization

[00122] In another aspect, provided herein are isolated populations of cells comprising antigen-specific T cells for therapeutic administration to a human patient having or suspected of having a pathogen or cancer, wherein the isolated population of cells comprising antigen-specific T cells is the product of a method comprising generating the population of cells comprising antigen-specific T cells according to a method described in Section 5.1, *supra*. In specific embodiments, provided herein is an isolated population of cells comprising antigen-specific T

cells for therapeutic administration to a human patient having or suspected of having a pathogen or cancer, wherein the isolated population of cells comprising antigen-specific T cells is the product of a method comprising generating the population of cells comprising antigen-specific T cells according to a method described in Section 5.1, *supra*, and wherein the population of cells comprising antigen-specific T cells is cryopreserved.

[00123] In specific embodiments, the isolated population of cells comprising antigen-specific T cells comprises CD8⁺ T cells. In specific embodiments, the isolated population of cells comprising antigen-specific T cells comprises CD4⁺ T cells. In specific embodiments, the isolated population of cells comprising antigen-specific T cells comprises both CD8⁺ and CD4⁺ T cells.

[00124] To be suitable for therapeutic administration to a human patient in adoptive immunotherapy, the population of cells comprising antigen-specific T cells generated by a method described in Section 5.1, *supra*, preferably (1) exhibits substantial cytotoxicity toward fully or partially HLA-matched (relative to the human donor of the population of human blood cells) antigen presenting cells that are loaded with or genetically engineered to express one or more peptides or proteins derived from the one or more antigens of the pathogen or cancer; (2) lacks substantial alloreactivity; and/or (3) is restricted (*i.e.*, the antigen-specific T cells contained in the population of cells comprising antigen-specific T cells are restricted) by an HLA allele shared with the diseased cells in the human patient, and/or shares (*i.e.*, the antigen-specific T cells contained in the population of cells comprising antigen-specific T cells share) at least 2 HLA alleles (*e.g.*, at least 2 out of 8 HLA alleles) with the diseased cells in the human patient. Thus, preferably, cytotoxicity, alloreactivity, information as to which HLA allele(s) the population of cells comprising antigen-specific T cells is restricted (*i.e.*, to which HLA allele(s) the antigen-specific T cells contained in the population of cells comprising antigen-specific T cells are restricted), and/or the HLA assignment of the population of cells comprising antigen-specific T cells (*i.e.*, the HLA assignment of the antigen-specific T cells contained in the population of cells comprising antigen-specific T cells) are measured by a method known in the art before administration to a human patient (for example, such a method as described in Koehne et al., 2000, *Blood* 96:109-117; Trivedi et al., 2005, *Blood* 105:2793-2801; Haque et al., 2007, *Blood* 110:1123-1131; Hasan et al., 2009, *J Immunol* 183: 2837-2850; Feuchtinger et al., 2010, *Blood* 116:4360-4367; Doubrovina et al., 2012, *Blood* 120:1633-1646; Leen et al., 2013, *Blood*

121:5113-5123; Papadopoulou et al., 2014, Sci Transl Med 6:242ra83; Sukdolak et al., 2013, Biol Blood Marrow Transplant 19:1480-1492; Koehne et al., 2015, Biol Blood Marrow Transplant 21: 1663-1678; or International Patent Application Publication No. WO 2016/073550).

[00125] Also provided herein is a cell bank comprising a plurality of isolated populations of cells comprising antigen-specific T cells described herein. Preferably, information as to cytotoxicity, alloreactivity, and/or HLA restriction and and/or assignment, as described herein, is ascertained for each of the plurality of isolated populations of cells comprising antigen-specific T cells contained in the cell bank, and linked to the identifier of the corresponding population of cells comprising antigen-specific T cells, so as to facilitate the selection of a suitable population of cells comprising antigen-specific T cells from the plurality for therapeutic administration to a human patient.

[00126] It is contemplated that, in specific embodiments of the foregoing characterization of the population of cells comprising antigen-specific T cells described herein, a different method of measuring the potency of the population of cells comprising antigen-specific T cells (such as an assay that instead of measuring cell lysis, measures, for example, CD107 degranulation or release of a cytokine such as IFN- γ), in lieu of a cytotoxicity assay, is used.

5.3.1. Cytotoxicity

[00127] The cytotoxicity of a population of cells comprising antigen-specific T cells generated by a method described in Section 5.1, *supra*, toward fully or partially HLA-matched (relative to the human donor of the population of human blood cells) antigen presenting cells can be determined by any assay known in the art to measure T cell mediated cytotoxicity. The assay can be performed using the population of cells comprising antigen-specific T cells directly, an aliquot thereof, or a precursor cell population that indicates the cytotoxicity of the population of cells comprising antigen-specific T cells. In a specific embodiment, the cytotoxicity is determined by a standard ^{51}Cr release assay as described in Trivedi et al., 2005, Blood 105:2793-2801 or Hasan et al., 2009, J Immunol 183: 2837-2850.

[00128] In certain embodiments, the population of cells comprising antigen-specific T cells generated by a method described in Section 5.1, *supra*, exhibits substantial cytotoxicity *in vitro* toward (*e.g.*, exhibits substantial lysis of) fully or partially HLA matched antigen presenting cells

that are loaded with or genetically engineered to express one or more peptides or proteins derived from the one or more antigens of the pathogen or cancer. Preferably, the fully or partially HLA-matched antigen presenting cells are fully HLA-matched antigen presenting cells (*e.g.*, antigen presenting cells derived from the human donor). In specific embodiments, the population of cells comprising antigen-specific T cells generated by a method described in Section 5.1, *supra*, exhibits lysis of greater than or equal to 20%, 25%, 30%, 35%, or 40% of the fully or partially HLA-matched antigen presenting cells that are loaded with or genetically engineered to express one or more peptides or proteins derived from the one or more antigens of the pathogen or cancer. In a specific embodiment, the population of cells comprising antigen-specific T cells generated by a method described in Section 5.1, *supra*, exhibits lysis of greater than or equal to 20% of the fully or partially HLA-matched antigen presenting cells that are loaded with or genetically engineered to express one or more peptides or proteins derived from the one or more antigens of the pathogen or cancer.

[00129] Antigen presenting cells that can be used in the cytotoxicity assay include, but are not limited to, dendritic cells, phytohemagglutinin (PHA)-lymphoblasts, macrophages, B-cells that generate antibodies, EBV-BLCL cells, and artificial antigen presenting cells (AAPCs).

[00130] In specific embodiments, the fully or partially HLA-matched antigen presenting cells used in the cytotoxicity assay are loaded with a pool of peptides derived from the one or more antigens of the pathogen or cancer. The pool of peptides, can be, for example, a pool of overlapping peptides (*e.g.*, pentadecapeptides) spanning the sequence of the one or more antigens of the pathogen or cancer.

5.3.2. Alloreactivity

[00131] Alloreactivity of a population of cells comprising antigen-specific T cells generated by a method described in Section 5.1, *supra*, can be measured using a cytotoxicity assay known in the art to measure T cell mediated cytotoxicity, such as a standard ⁵¹Cr release assay, as described in Section 5.3.1, *supra*, but with antigen presenting cells that are not loaded with or genetically engineered to express one or more peptides or proteins derived from the one or more antigens of the pathogen or cancer, and/or HLA-mismatched (relative to the human donor of the population of human cells) antigen presenting cells. The assay can be performed using the population of cells comprising antigen-specific T cells directly, an aliquot thereof, or a precursor

cell population that indicates the alloreactivity of the population of cells comprising antigen-specific T cells. A population of cells comprising antigen-specific T cells that lacks substantial alloreactivity results generally in the absence of graft-versus-host disease (GvHD) when administered to a human patient.

[00132] In certain embodiments, the population of cells comprising antigen-specific T cells generated by a method described in Section 5.1, *supra*, lacks substantial cytotoxicity *in vitro* toward antigen presenting cells that are not loaded with or genetically engineered to express one or more peptides or proteins derived from the one or more antigens of the pathogen or cancer. In preferred embodiments, such antigen-presenting cells are fully or partially HLA-matched antigen presenting cells (relative to the human donor of the population of human blood cells) (*e.g.*, antigen presenting cells derived from the human donor of the population of human blood cells). In specific embodiments, the population of cells comprising antigen-specific T cells generated by a method described in Section 5.1, *supra*, lyses less than or equal to 15%, 10%, 5%, 2%, or 1% of antigen presenting cells that are not loaded with or genetically engineered to express one or more peptides or proteins derived from the one or more antigens of the pathogen or cancer. In a specific embodiment, the population of cells comprising antigen-specific T cells generated by a method described in Section 5.1, *supra*, lyses less than or equal to 10% of antigen presenting cells that are not loaded with or genetically engineered to express one or more peptides or proteins derived from the one or more antigens of the pathogen or cancer. In another specific embodiment, the population of cells comprising antigen-specific T cells generated by a method described in Section 5.1, *supra*, lyses less than or equal to 5% of antigen presenting cells that are not loaded with or genetically engineered to express one or more peptides or proteins derived from the one or more antigens of the pathogen or cancer.

[00133] In certain embodiments, the population of cells comprising antigen-specific T cells generated by a method described in Section 5.1, *supra*, lacks substantial cytotoxicity *in vitro* toward HLA-mismatched (relative to the human donor of the population of human blood cells) antigen presenting cells. In some embodiments, such antigen-presenting cells are loaded with or genetically engineered to express one or more peptides or proteins derived from the one or more antigens of the pathogen or cancer. In other embodiments, such antigen-presenting cells are not loaded with or genetically engineered to express one or more peptides or proteins derived from the one or more antigens of the pathogen or cancer. In specific embodiments, the population of

cells comprising antigen-specific T cells generated by a method described in Section 5.1, *supra*, lyses less than or equal to 15%, 10%, 5%, 2%, or 1% of HLA-mismatched (relative to the human donor of the population of human blood cells) antigen presenting cells. In a specific embodiment, the population of cells comprising antigen-specific T cells generated by a method described in Section 5.1, *supra*, lyses less than or equal to 10% of HLA-mismatched (relative to the human donor of the population of human blood cells) antigen presenting cells. In another specific embodiment, the population of cells comprising antigen-specific T cells generated by a method described in Section 5.1, *supra*, lyses less than or equal to 5% of HLA-mismatched (relative to the human donor of the population of human blood cells) antigen presenting cells.

[00134] In certain embodiments, the population of cells comprising antigen-specific T cells generated by a method described in Section 5.1, *supra*, lacks substantial cytotoxicity *in vitro* toward antigen presenting cells that are not loaded with or genetically engineered to express one or more peptides or proteins derived from the one or more antigens of the pathogen or cancer, as described above, and lacks substantial cytotoxicity *in vitro* toward HLA-mismatched antigen presenting cells as described above.

[00135] Antigen presenting cells that can be used in the alloreactivity assay include, but are not limited to, dendritic cells, phytohemagglutinin (PHA)-lymphoblasts, macrophages, B-cells that generate antibodies, EBV-BLCL cells, and artificial antigen presenting cells (AAPCs).

5.3.3. HLA Type

[00136] The HLA assignment (*i.e.*, the HLA loci type) of a population of cells comprising antigen-specific T cells generated by a method described in Section 5.1, *supra*, (*i.e.*, the HLA assignment of the antigen-specific T cells contained in the population of cells comprising antigen-specific T cells) and/or the HLA assignment of the diseased cells in the human patient to be treated can be ascertained (*i.e.*, typed) by any method known in the art for typing HLA alleles. The assignment can be performed using the population of cells comprising antigen-specific T cells directly, an aliquot thereof, or a precursor cell population that indicates the HLA assignment of the population of cells comprising antigen-specific T cells. Non-limiting exemplary methods for ascertaining the HLA assignment can be found in ASHI Laboratory Manual, Edition 4.2 (2003), American Society for Histocompatibility and Immunogenetics; ASHI Laboratory Manual, Supplements 1 (2006) and 2 (2007), American Society for

Histocompatibility and Immunogenetics; Hurley, “DNA-based typing of HLA for transplantation.” in Leffell et al., eds., 1997, Handbook of Human Immunology, Boca Raton: CRC Press; Dunn, 2011, Int J Immunogenet 38:463-473; Erlich, 2012, Tissue Antigens, 80:1-11; Bontadini, 2012, Methods, 56:471-476; and Lange et al., 2014, BMC Genomics 15: 63. In specific embodiments, at least 4 HLA loci (preferably HLA-A, HLA-B, HLA-C, and HLA-DR) are typed. In a specific embodiment, 4 HLA loci (preferably HLA-A, HLA-B, HLA-C, and HLA-DR) are typed. In another specific embodiment, 6 HLA loci are typed. In another specific embodiment, 8 HLA loci are typed.

[00137] In general, high-resolution typing is preferable for HLA typing. The high-resolution typing can be performed by any method known in the art, for example, as described in ASHI Laboratory Manual, Edition 4.2 (2003), American Society for Histocompatibility and Immunogenetics; ASHI Laboratory Manual, Supplements 1 (2006) and 2 (2007), American Society for Histocompatibility and Immunogenetics; Flomenberg et al., Blood, 104:1923-1930; Kögler et al., 2005, Bone Marrow Transplant, 36:1033-1041; Lee et al., 2007, Blood 110:4576-4583; Erlich, 2012, Tissue Antigens, 80:1-11; Lank et al., 2012, BMC Genomics 13:378; or Gabriel et al., 2014, Tissue Antigens, 83:65-75.

[00138] In specific embodiments, the HLA assignment of the diseased cells in the human patient to be treated is ascertained by typing the origin of the diseased cells (*e.g.*, the human patient or a transplant donor for the human patient, as the case may be). The origin of the diseased cells can be determined by any method known in the art, for example, by analyzing variable tandem repeats (VTRs) (which is a method that uses unique DNA signature of small DNA sequences of different people to distinguish between the recipient and the donor of a transplant), or by looking for the presence or absence of chromosome Y if the donor and the recipient of a transplant are of different sexes (which is done by cytogenetics or by FISH (fluorescence in situ hybridization)).

[00139] The HLA allele by which the population of cells comprising antigen-specific T cells generated by a method described in Section 5.1, *supra*, is restricted (*i.e.*, the HLA allele by which the antigen-specific T cells contained in the population of cells comprising antigen-specific T cells are restricted) can be determined by any method known in the art, for example, as described in Trivedi et al., 2005, Blood 105:2793-2801; Barker et al., 2010, Blood 116:5045-5049; Hasan et al., 2009, J Immunol, 183:2837-2850; Doubrovina et al., 2012, Blood 120:1633-

1646; or International Patent Application Publication No. WO 2016/073550. The determination can be performed using the population of cells comprising antigen-specific T cells directly, an aliquot thereof, or a precursor cell population that indicates the HLA allele by which the population of cells comprising antigen-specific T cells is restricted (*i.e.*, the HLA allele by which the antigen-specific T cells contained in the population of cells comprising antigen-specific T cells are restricted).

[00140] In some embodiments, the antigen-specific T cells contained in the population of cells comprising antigen-specific T cells are restricted by an HLA allele shared with the diseased cells in the human patient to be treated. In other embodiments, the antigen-specific T cells contained in the population of cells comprising antigen-specific T cells share at least 2 HLA alleles (for example, at least 2 out of 8 HLA alleles, such as two HLA-A alleles, two HLA-B alleles, two HLA-C alleles, and two HLA-DR alleles) with the diseased cells in the human patient to be treated. In other embodiments, the antigen-specific T cells contained in the population of cells comprising antigen-specific T cells are restricted by an HLA allele shared with diseased cells in the human patient to be treated, and share at least 2 HLA alleles (for example, at least 2 out of 8 HLA alleles, such as two HLA-A alleles, two HLA-B alleles, two HLA-C alleles, and two HLA-DR alleles) with the diseased cells in the human patient to be treated.

5.3.4. Composition and Kits

[00141] In another aspect, provided herein are pharmaceutical compositions comprising a therapeutically effective amount of an isolated population of cells comprising antigen-specific T cells described herein, and a pharmaceutically acceptable carrier. In a preferred embodiment, the pharmaceutical composition is in a cryopreserved form.

[00142] The pharmaceutically acceptable carrier can be any physiologically-acceptable solution suitable for the storage and/or therapeutic administration of T cells, for example, a saline solution, a buffered saline solution, or a bio-compatible solution comprising one or more cryopreservatives (*e.g.*, phosphate-buffered saline containing 7% DMSO, 5% dextrose and 1% dextran; hypothermosol containing 5% DMSO and 5% human serum albumin; normal saline containing 10% DMSO and 16% human serum albumin; or normal saline containing 10% DMSO and 15% human serum albumin).

[00143] The population of cells comprising antigen-specific T cells can be stored in the

pharmaceutical composition at any concentration desirable for its long-term storage and convenience of storage and handling. In a specific embodiment, the population of cells comprising antigen-specific T cells is stored in the pharmaceutical composition at a concentration of about 5×10^6 cells/mL. In another specific embodiment, the population of cells comprising antigen-specific T cells is stored in the pharmaceutical composition at a concentration of about 10×10^6 cells/mL. In another specific embodiment, the population of cells comprising antigen-specific T cells is stored in the pharmaceutical composition at a concentration of about 20×10^6 cells/mL. In another specific embodiment, the population of cells comprising antigen-specific T cells is stored in the pharmaceutical composition at a concentration of about 50×10^6 cells/mL. In another specific embodiment, the population of cells comprising antigen-specific T cells is stored in the pharmaceutical composition at a concentration of about 100×10^6 cells/mL. In another specific embodiment, the population of cells comprising antigen-specific T cells is stored in the pharmaceutical composition at a concentration of about 200×10^6 cells/mL. In another specific embodiment, the population of cells comprising antigen-specific T cells is stored in the pharmaceutical composition at a concentration of about 500×10^6 cells/mL. In another specific embodiment, the population of cells comprising antigen-specific T cells is stored in the pharmaceutical composition at a concentration of about 1 to 10×10^6 cells/mL. In another specific embodiment, the population of cells comprising antigen-specific T cells is stored in the pharmaceutical composition at a concentration of about 10 to 100×10^6 cells/mL. In another specific embodiment, the population of cells comprising antigen-specific T cells is stored in the pharmaceutical composition at a concentration of about 100 to 1000×10^6 cells/mL.

[00144] Also provided herein are kits comprising in one or more containers the pharmaceutical composition described herein. In specific embodiments, the kits further comprise a second pharmaceutical composition comprising a second compound or biological product for treating the pathogen infection or cancer.

[00145] Optionally associated with such one or more containers can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[00146] The pharmaceutical compositions and kits encompassed herein can be used in

accordance with the methods of treating a human patient as provided in this disclosure.

5.4. Antigen Specificity and Patients

[00147] The one or more antigens of a pathogen, as disclosed above, can be one or more peptides or proteins whose expressions are unique to the pathogen.

[00148] The pathogen can be a virus, bacterium, fungus, helminth or protist. In some embodiments, the pathogen is a virus (such as a virus that has a latency). In a specific embodiment, the virus is cytomegalovirus (CMV). In an aspect of the specific embodiment, the one or more antigens of CMV is CMV pp65, CMV IE1, or a combination thereof. In another aspect of the specific embodiment, the one or more antigens of CMV is CMV pp65.

[00149] In another specific embodiment, the virus is Epstein-Barr virus (EBV). In an aspect of the specific embodiment, the one or more antigens of EBV is EBNA1, EBNA2, EBNA3A, EBNA3B, EBNA3C, LMP1, LMP2, or a combination thereof. In another aspect of the specific embodiment, the one or more antigens of EBV is EBNA1, LMP1, LMP2, or a combination thereof.

[00150] In another specific embodiment, the virus is BK virus (BKV), John Cunningham virus (JCV), herpesvirus (such as human herpesvirus-6 or human herpesvirus-8), adenovirus (ADV), human immunodeficiency virus (HIV), influenza virus, ebola virus, poxvirus, rhabdovirus, or paramyxovirus. In another embodiment, the virus is hepatitis B virus (HBV), hepatitis C virus (HCV), herpes simplex virus (HSV), varicella zoster virus (VZV), human papillomavirus (HPV), or Merkel cell polyomavirus (MCV).

[00151] In specific embodiments of the methods of generating a population of cells comprising antigen-specific cells described herein, the *ex vivo* sensitizing step comprises *ex vivo* sensitizing the population of human blood cells to one or more antigens of the pathogen described herein. In a specific embodiment of the methods of generating a population of cells comprising antigen-specific cells described herein, the *ex vivo* sensitizing step comprises *ex vivo* sensitizing the population of human blood cells to one or more antigens of CMV. In another specific embodiment of the methods of generating a population of cells comprising antigen-specific cells described herein, the *ex vivo* sensitizing step comprises *ex vivo* sensitizing the population of human blood cells to one or more antigens of EBV. In another specific embodiment of the methods of generating a population of cells comprising antigen-specific cells

described herein, the *ex vivo* sensitizing step comprises *ex vivo* sensitizing the population of human blood cells to one or more antigens of BKV, JCV, herpesvirus (such as human herpesvirus-6 or human herpesvirus-8), adenovirus, human immunodeficiency virus, influenza virus, ebola virus, poxvirus, rhabdovirus, or paramyxovirus.

[00152] In specific embodiments of the methods of treating a human patient described herein, the human patient has an infection of the pathogen. In some embodiments of the methods of treating a human patient described herein, the human patient has a CMV infection (*e.g.*, CMV viremia, CMV retinitis, CMV pneumonia, CMV hepatitis, CMV colitis, CMV encephalitis, CMV meningoencephalitis, CMV-positive meningoma, or CMV-positive glioblastoma multiforme). In other embodiments of the methods of treating a human patient described herein, the human patient has an EBV-positive lymphoproliferative disorder (EBV-LPD) (for example, an EBV-positive post-transplant lymphoproliferative disorder) resulting from EBV infection, such as B-cell hyperplasia, lymphoma (such as, B-cell lymphoma, non-Hodgkin lymphoma (*e.g.*, diffuse large B-cell lymphoma, for example in the elderly), T-cell lymphoma, EBV-positive Hodgkin's lymphoma, Burkitt lymphoma), polymorphic or monomorphic EBV-LPD, autoimmune lymphoproliferative syndrome, or mixed PTLD (post-transplant lymphoproliferative disorder). In other embodiments of the methods of treating a human patient described herein, the human patient has an EBV-positive nasopharyngeal carcinoma. In other embodiments of the methods of treating a human patient described herein, the human patient has an EBV-positive gastric cancer. In other embodiments of the methods of treating a human patient described herein, the human patient has an EBV+ leiomyosarcoma. In other embodiments of the methods of treating a human patient described herein, the human patient has an EBV-positive NK/T lymphoma.

[00153] In other embodiments, the pathogen is a bacterium, such as *mycobacterium tuberculosis*.

[00154] The one or more antigens of a cancer, as disclosed above, can be one or more peptides or proteins whose expressions are higher in cancerous cells (of the corresponding type of cancer) relative to non-cancerous cells, or one or more peptides or proteins which are uniquely expressed in cancerous cells (of the corresponding type of cancer) relative to non-cancerous cells.

[00155] The cancer can be a blood cancer, such as, but is not limited to: acute lymphoblastic

leukemia, chronic lymphocytic leukemia, acute myelogenous leukemia, chronic myelogenous leukemia, hairy cell leukemia, T-cell prolymphocytic leukemia, Large granular lymphocytic leukemia, adult T-cell leukemia, plasma cell leukemia, Hodgkin lymphoma, Non-Hodgkin lymphoma, or multiple myeloma. In a specific embodiment, the cancer is multiple myeloma or plasma cell leukemia. In an aspect of the specific embodiment, the one or more antigens of the cancer is Wilms tumor 1 (WT1).

[00156] The cancer can also be a solid tumor cancer, including, but is not limited to, a sarcoma, a carcinoma, a lymphoma, a germ cell tumor, or a blastoma. The solid tumor cancer that can be, such as, but is not limited to: a cancer of the breast, lung, ovary, stomach, pancreas, larynx, esophagus, testes, liver, parotid, biliary tract, colon, rectum, cervix, uterus, endometrium, kidney, bladder, prostate, thyroid, brain, or skin.

[00157] In specific embodiments of the methods of generating a population of cells comprising antigen-specific cells described herein, the *ex vivo* sensitizing step comprises *ex vivo* sensitizing the population of human blood cells to one or more antigens of the cancer described herein. In a specific embodiment of the methods of generating a population of cells comprising antigen-specific cells described herein, the *ex vivo* sensitizing step comprises *ex vivo* sensitizing the population of human blood cells to WT1.

[00158] In specific embodiments of the methods of treating a human patient described herein, the human patient has a cancer described herein. In some embodiments of the methods of treating a human patient described herein, the human patient has multiple myeloma or plasma cell leukemia (*e.g.*, a WT1-positive multiple myeloma or plasma cell leukemia).

[00159] In a specific embodiment, the human patient is an adult (at least age 16). In another specific embodiment, the human patient is an adolescent (age 12-15). In another specific embodiment, the patient is a child (under age 12).

[00160] In a specific embodiment, the human patient treated with a method described herein has failed a previous therapy for the pathogen or cancer, which previous therapy is not treatment with a population of cells comprising antigen-specific T cells according to the invention, due to resistance to or intolerance of the previous therapy. A disease is considered resistant to a therapy, if it has no response, or has an incomplete response (a response that is less than a complete remission), or progresses, or relapses after the therapy. The previous therapy could be an antiviral agent known in the art (*e.g.*, an antiviral drug or antibody), or an anti-cancer therapy

known in the art (*e.g.*, a chemotherapy or a radiotherapy), as the case may be.

6. EXAMPLE

[00161] Certain embodiments provided herein are illustrated by the following non-limiting example, which demonstrates that stem cell-like memory T cells (T_{SCM} cells) are an advantageous source of T cells to generate antigen-specific T cells for adoptive immunotherapy. The results described herein suggest that Tet^+ T_{SCM} cells rather than Tet^+ T_N cells are the principal reservoir for rapid repopulation of immunodominant T cells in the circulation.

6.1. Summary

[00162] Latent CMV infection is controlled by a limited repertoire of immunodominant T cells specific for viral peptides. Antigen-specific T cell subsets responsible for maintaining memory T cells and repopulating them in response to periodic viral reactivations remain unclear. In this example described herein, T cells specific for CMVpp65 were generated from naive T cell (T_N cell), stem cell-like memory T cell (T_{SCM} cell), central memory T cell (T_{CM} cell) and effector memory T cell (T_{EM} cell) subsets isolated from the blood of HLA-A*0201 seropositive human donors, and NLV-HLA-A*0201 Tetramer (Tet) $^+$ T cells from each of these subsets were comparatively characterized. Following *in vitro* sensitization, Tet^+ T cells were regularly generated from $CD62L^+CD45RO^-CD95^-$ T_N cells and from $CD62L^+CD45RO^-CD95^+$ T_{SCM} cells, as well as T_{CM} cells and T_{EM} cells. Tet^+ T cells derived from each of the T_N , T_{SCM} , T_{CM} and T_{EM} subsets generated IFN- γ , TNF- α and granzyme B. Tet^+ T cells derived from each subset also expressed similar levels of PD-1 and KLRG-1. However, Tet^+ T cells derived from the T_N subset and those derived from the T_{SCM} subset expressed higher levels of CD27 and lower levels of CD57 than those derived from the T_{CM} or T_{EM} subset. Tet^+ T cells derived from the T_{SCM} subset were distinguished from those derived from the T_N , T_{CM} and T_{EM} subsets by a significantly greater level of proliferation and by their rapid and selective expansion of NLV-specific T cells bearing TCRs identical in sequence to those expressed by T_{EM} and T_{CM} in the blood. The example described herein suggests that Tet^+ T_{SCM} cells rather than Tet^+ T_N cells are the principal reservoir for rapid repopulation of immunodominant T cells in the circulation.

6.2. Materials and Methods

6.2.1. Donors

[00163] Blood samples were obtained from 12 healthy volunteer HLA-A*0201⁺ CMV-seropositive donors. High resolution HLA typing was performed by analysis of HLA allele-specific nucleotide sequences using standard high-resolution typing techniques. CMV serostatus was determined by standard serologic techniques in the clinical microbiology laboratory at Memorial Sloan Kettering Cancer Center.

6.2.2. Generation of CMV-specific T cells

[00164] Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by Ficoll-Hypaque density gradient separation (Accurate Chemical & Scientific Corporation, Westbury, NY USA), from which T cells were enriched by depletion of CD19⁺, CD14⁺, and CD56⁺ cells, using mAb-coated immunomagnetic beads (Pan T-Cell Isolation Kit II, Miltenyi Biotec Inc, Auburn, CA USA). Enriched T cells were labeled with fluorescent Abs: anti-CD3 PerCP, anti-CD45RO PE, anti-CD95 APC, anti-CD62L FITC (all purchased from BD Biosciences), and anti-CD45RA (eBiosciences, CA, USA). T cell populations representing T_N, T_{SCM}, T_{CM} and T_{EM} cells were gated and sorted on a BD FACS Aria-II SORT (BD Biosciences) based on the following markers: T_{SCM} population as CD3⁺CD45RO⁻CD62L⁺CD95⁺, T_N population as CD3⁺CD45RO⁻CD62L⁺CD95⁻, and the T_{CM} and T_{EM} populations as CD3⁺CD45RO⁺CD62L⁺ and CD3⁺CD45RO⁺CD62L⁻ respectively. Sorted T cell subsets (1x10⁶) were then sensitized with irradiated HLA-A0201⁺ and CMVpp65⁺ artificial antigen presenting cells (AAPCs) (0.1x10⁶) as previously described (Hasan *et al.*, 2009, J Immunol 183:2837-2850) and were additionally cultured in the presence of irradiated autologous PBMCs (2x10⁶) as feeder cells in culture medium X-VIVO™ 15 with gentamicin (Lonza, Allendale NJ, USA) and 15% heat inactivated human serum (Gemini, CA, USA) in humidified incubators at 37°C and 5% CO₂. T cells were supplemented with IL-7 (5 ng/ml), and IL-15 (5 ng/ml) on day 4 and day 7 after culture initiation and were re-stimulated every 10 days with AAPCs and autologous PBMC feeders. After day 12, IL-7 (5 ng/ml), IL-15 (5 ng/ml), and IL-2 (20U/ml) were supplemented to T cell cultures every other day.

6.2.3. Quantitation of antigen-specific CD8⁺ T cells by tetramer analysis

[00165] CMVpp65 specific T cells responsive to the NLV peptide within the cultured T cells were enumerated by Tetramer analysis as previously described (Hasan *et al.*, 2009, J Immunol 183:2837-2850). Commercially available CMVpp65 MHC-peptide tetramers for HLA-A*0201 and A*2402-bearing peptide sequences NLVPMVATV (SEQ ID NO:1) and QYDPVAALF (SEQ ID NO:2) (Beckman Coulter) were used. Briefly, T cells were incubated with CD3 FITC, CD8 PE, CD4 PerCP (BD Biosciences) and an APC-conjugated tetrameric complex for 20 mins on ice, washed and analyzed by FACS using a FACSCalibur flow cytometer with dual laser for four-color capability. Data were analyzed using FlowJo software (Tree Star). T cells were gated on CD3- and CD8-positive cells to determine the percentage of tetramer-positive CD8⁺ T lymphocytes.

6.2.4. Phenotypic analysis of T cell subsets

[00166] T cell subsets were further characterized by flow cytometry using specific T cell memory and co-stimulatory markers. T cells were labeled with fluorescent antibodies against CCR7 PE (BD Biosciences), CD27 FITC (Miltenyi Biotec), CD57 FITC (Miltenyi Biotec), CD127 PE (Miltenyi Biotec), CD28 PECy7 (BD Biosciences), KLRG1 PE (Miltenyi Biotec) and PD1 PECy7 (eBioscience), and analyzed by FACS. Doublet exclusion for lymphocytes was achieved by gating on forward scatter (FSC) vs side scatter (SSC) followed by FSC (height) versus FSC (area).

6.2.5. Functional characterization of antigen-specific T cells

[00167] Functional activity of T cells was evaluated after short secondary stimulation using several parameters including secretion of intracellular cytokines (IFN- γ and TNF- α), activation marker expression (CD137), and cytotoxicity (CD107a) by intracellular fluorescence staining. All antibodies were purchased from BD bioscience. Irradiated autologous B-lymphoblastoid cell line cells (BLCL cells) loaded with NLVPMVATV (SEQ ID NO:1) peptide were co-incubated with T cells in an effector to target ratio of 1:5 for 16 hours in the presence of 1 μ g/ml brefeldin A (Sigma-Aldrich). Co-cultured T cells were then labeled with anti-CD3 APC and anti CD8 PE for 15 mins at room temp, washed and then permeabilized with Perm solution (BD Biosciences) and then co-incubated with anti-IFN- γ PECy7 (BD Biosciences) and anti-TNF- α APC (Miltenyi

Biotec), or anti-CD137 PE (BD Biosciences), or anti-CD107a FITC (BD Biosciences).

6.2.6. Analysis of T cell proliferation and apoptosis

[00168] EdU labeling (ThermoFisher) was used to evaluate T cell proliferation. 10 μ M EdU was added to the culture media for 1h at 37 °C. Labeled cells were washed with PBS and resuspended in T cell culture media. T cells were then analyzed by flow cytometry, and the proportion of proliferating T cells was determined by the percentage of EdU⁺ gated T cells using the FlowJo software (Treestar). Apoptotic T cells were defined by Annexin V staining (BD Biosciences).

6.2.7. TCR next-generation sequencing

[00169] T cell receptor V β (TCRV β) chain hypervariable complementarity-determining region 3 (CDR3) was amplified and sequenced from DNA extracted from NLV-Tet⁺ T cell subsets (T_N, T_{SCM}, T_{CM}, and T_{EM}) isolated by fluorescence-activated cell sorting (FACS) (purity > 95%) or NLV-Tet⁺ T cells derived from these subsets, from days 0, 15 or 30 post-stimulation using the immunoSEQ platform at Adaptive Biotechnologies. Rearranged CDR3 sequences were classified as nonproductive if they included insertions or deletions resulting in frameshift or premature stop codons, and were excluded from subsequent analyses, according to the immunoSEQ validated algorithm. TCR clonality and sample overlap were determined using the immunoSEQ Analyzer 2.0. within a range of 0 to 1, where a low number indicates higher diversity, while a high number indicates higher clonality within the sample. Sample overlap indicates the percent of similar clones within a pair of sample types.

6.2.8. Statistical analysis

[00170] Statistical analyses were performed using Prism (GraphPad Software). For most of the comparisons a nonparametric Mann-Whitney test was used to compare two groups. One-way ANOVA was used to compare three or more groups. Significance of differences between two groups was calculated using the t-test ($\alpha = 0.05$). For all comparisons, two-sided P values were used; where indicated, *P < 0.05; **P < 0.01; ***P < 0.005.

6.3. Results

6.3.1. Detection and Isolation of CMVpp65-specific T_{SCM} cells within PBMCs from Healthy Seropositive Donors

[00171] Gattinoni *et al.* (2011, Nat Med 17:1290-1297), first described T_{SCM} cells within human PBMCs as a specific subset of memory T cells with high proliferative potential that when modified to express a tumor-specific chimeric antigen receptor, also exhibits superior functional activity. This example described herein was designed to test whether *in vivo* primed virus-specific human T_{SCM} cells also exhibit these attributes. T cells were isolated from PBMCs of 12 healthy HLA A0201⁺ CMV seropositive donors. In each of these CMV-seropositive donors, T_{SCM} population was identified as CD3⁺CD45RO⁻CD62L⁺CD95⁺ cells, T_N population as CD3⁺CD45RO⁻CD62L⁺CD95⁻ cells, and T_{CM} and T_{EM} populations as CD3⁺CD45RO⁺CD62L⁺ and CD3⁺CD45RO⁺CD62L⁻ cells respectively (Figure 1). The proportion of T_{SCM} cells ranged from 1.2%-10.8% within the T cell populations in the peripheral blood in the absence of any *in vitro* stimulation. Despite low proportions within the PBMCs, the T_{SCM} cells could consistently be identified in all donors tested (n=12). In comparison, the proportion of T_N, T_{CM} and T_{EM} cells were 3.8%-28.9%, 1.7%-32.4% and 15.4%-34.9% respectively (Table 1).

[00172] Table 1. Percentage of naive and memory T_N, T_{SCM}, T_{CM} and T_{EM} CD8⁺ T cells in healthy CMV seropositive individuals (ND= not detectable; NA= not applicable)

Donor	Within T cell population					Within NLV-Tet ⁺ population			
	T _{EM}	T _{CM}	T _{SCM}	T _N	Tet ⁺	T _{EM}	T _{CM}	T _{SCM}	T _N
1	31.9	8.7	4.2	8.7	2.5	35.4	1.1	0.6	ND
2	31.7	7.0	2.5	28.9	4.4	15.1	0.9	2.0	0.2
3	27.4	20.3	5.8	16.0	0.9	47.9	8.3	3.7	ND
4	22.2	13.8	3.8	22.0	0.4	59.7	9.7	2.4	ND
5	34.9	11.0	2.1	5.0	0.5	42.0	1.4	1.4	ND
6	33.0	12.8	3.1	6.0	0.3	79.7	3.4	1.7	ND
7	18.1	32.4	1.2	17.3	2.0	60.2	31.0	6.3	ND
8	21.0	13.0	1.9	28.6	0.1	41.7	2.1	8.3	ND
9	28.4	8.3	10.8	23.5	1.5	70.6	5.9	ND	ND
10	15.4	3.6	2.6	21.8	NA				
11	25.6	1.7	2.0	6.5	1.0	27.8	3.5	3.0	0.1
12	28.9	3.5	2.4	3.8	0.3	35.8	5.3	2.0	ND

[00173] Using CMVpp65 as a model antigen, the memory phenotype of the antigen-specific T cells was then examined. Accordingly, the CMVpp65-specific T cell populations in the blood of 11 of 12 donors were evaluated. In each of the donors tested, a discrete population of antigen-specific T cells could be identified using HLA peptide tetramers that were responsive to the well known CMVpp65 epitope NLVPMVATV (SEQ ID NO:1) presented by HLA-A0201. Further analysis of these tetramer positive T cells demonstrated that the majority of memory T cells in peripheral blood that recognize NLV epitope bear either a T_{CM} or T_{EM} phenotype (0.9%-31% and 15.1%-70.6% respectively) and the remainder were effector T cells (Table 1). Then it was determined whether there was a population of T_{SCM} cells circulating in the peripheral blood of healthy donors that were also antigen-specific. Of the 11 donors total, 10 had a distinct population of T_{SCM} cells within the CD95⁺ naive T cell precursors (CD45RO⁻CD62L⁺), that could bind to the A2-NLV tetramer (0.6% - 8.3%) (Table 1).

[00174] Other studies have demonstrated the feasibility of generating CMV specific T cells from cord blood donors from the naive T cell compartment (Hanley *et al.*, 2015, Sci Transl Med 7:285ra263; Szabolcs, 2011, Immunol Res 49:56-69). Therefore the proportion of NLV-Tet⁺ T cells within the CD95 negative naive T cells was examined. However, T_N cells within the NLV-Tet⁺ population in the peripheral blood could only be detected in 2 of 11 donors. Taken together, the data suggest that a minor proportion of NLV-tetramer binding CD8⁺ T cells are within the CD45RO⁻CD62L⁺ compartment and they consist of both the CD95⁺ T_{SCM} and CD95⁻ T_N cells.

6.3.2. Epitope specific T_{SCM} cells can be successfully expanded *in vitro* and maintain a less differentiated memory phenotype during antigen-specific stimulation

[00175] Next, it was evaluated which of the memory T cell populations detected in these healthy latently unrelated seropositive donors served as the reservoir of T cell immunity from which antigen-specific T cells were generated upon secondary challenge. It was particularly desirable to comparatively assess the potential of CMV-specific T_{SCM} cells to expand and act as a durable T cell reservoir. For these studies, an *in vitro* system was developed for expansion of CMVpp65 specific T cells from all memory T cell compartments from human PBMCs. T_{SCM}, T_N, T_{CM} and T_{EM} cell populations were FACS sorted from HLA-A*0201 CMV-seropositive donors as described above using the expression markers CD62L, CD45RO and CD95. Then the sorted T cell subsets were sensitized with artificial antigen presenting cells (AAPCs) exclusively

expressing HLA-A*0201, CMVpp65 and T cell costimulatory molecules as previously described (Hasan *et al.*, 2009, J Immunol 183:2837-2850). Then this approach was modified to include supplementation of the cultures with IL-7 and IL-15 every 2 days beginning at day 4 as previously described (Cieri *et al.*, 2013, Blood 121:573-584).

[00176] Using this method for *in vitro* sensitization of T_{SCM} cells, HLA-A*0201 NLV-tetramer⁺ CD8⁺ T cells could be enriched up to 5.5 % by 7 days from 0.6 % at day 0 as shown for one representative donor (Figure 2A). A similar enrichment of HLA-A*0201 NLV-tetramer⁺ T cells was also observed within the T_{CM}- and T_{EM}-derived CD8⁺ T cells (6.1% and 4.1% respectively) (Figure 2A). Over a period of 4 weeks after antigen-specific *in vitro* sensitization, T_{SCM}-derived cells gradually acquired T_{CM} and T_{EM} phenotype within both the NLV-Tet⁺ as well as NLV-Tet⁻ T cell populations (Figures 2B and 2C). Strikingly, within the NLV-Tet⁺ T cells derived from the T_{SCM} subset, a proportion of cells with a CD45RA⁺ and CD62L⁺ T_{SCM} phenotype could be detected for up to 14 days in culture. By this time, both T_{CM}- and T_{EM}-derived cells had converted to T_{EM} phenotype (Figure 2B). Higher CCR7 expression was also detected within both the NLV-Tet⁺ as well as NLV-Tet⁻ T cells derived from T_{SCM} cells after 14 days of antigen-specific stimulation in comparison to T_{CM}- and T_{EM}-derived cells (Figures 2D and 2E). CCR7 was expressed in 7.5% of T_{SCM}-derived NLV-Tet⁺ T cells (Figure 2D). These data suggest that the T_{SCM} cells follow a differentiation trajectory progressing to T_{CM}, and then T_{EM} cells during *in vitro* expansion. These results also suggest that T_{SCM}-derived cells have the ability to maintain a less differentiated phenotype than T_{CM}-derived cells.

[00177] Using the same approach, CMV-specific T cells derived from CD95⁻ T_N cells also were generated. Regardless of the detection of NLV-Tet⁺ T cells in the sorted T_N cells within the peripheral blood before antigen stimulation, NLV-Tet⁺ T cells could be generated from 6 of 6 different donors by this method of sensitization from sorted T_N cells. Upon stimulation, T_N cells upregulated the CD95 expression within 2 days and converted to a T_{SCM} phenotype. T_N cells also maintained a less differentiated memory phenotype, with expression of CD62L and CCR7 within a proportion of NLV-Tet⁺ T cells for a longer duration during antigenic stimulation than the T_{CM}- and T_{EM}-derived cells. In a representative example (Figure 2D), T_N-derived cells demonstrated CCR7 expression in 12% of NLV-Tet⁺ T cells after 14 days of continuous antigenic stimulation, in comparison to 7.5%, 0.8% and 0.5% CCR7 expressing NLV-Tet⁺ T cells derived from T_{SCM}, T_{CM} and T_{EM} subsets respectively. Within a total of 6 donors tested, the differential expression of

CCR7 in T_N-derived NLV-Tet⁺ T cells was significantly higher than that observed in T_{CM}- and T_{EM}-derived NLV-Tet⁺ T cells ($p < 0.01$) (Figure 2D). These studies demonstrate a less differentiated phenotype within antigen-experienced T cells derived from T_N and T_{SCM} cells. However, the enrichment of NLV-Tet⁺ populations was less pronounced within the T_N-derived cells (0.4%, 0.4% and 3.6% at Day 7, 14 and 30 as shown in Figures 2A, 2B and 2C) than within T cells derived from the memory T cell populations (T_{SCM}, T_{CM} and T_{EM} cells). The slow enrichment of CMV-specific cells might result from the extremely low precursor frequencies in the T_N subset in the peripheral blood.

6.3.3. Characterization of the co-stimulatory and senescence markers within memory T cell populations

[00178] Next, other characteristics within the naive and different memory T cell subsets were evaluated, to identify a particular subset with higher potential for proliferation and persistence. The expression of a panel of markers was evaluated, including the co-stimulatory markers CD27 and CD28 as well as IL-7R α (CD127), the activation marker PD-1 and the senescence marker CD57. The expression of each of these markers was compared in cells that recognize the same antigen to rule out differences occurring as a result of differential enrichment of antigen-specific T cells within each subset.

[00179] CD27 is constitutively expressed as a co-stimulatory molecule on naive and memory T cells. Its expression increases upon T cell activation, and is lost at the fully differentiated effector phase (Hintzen *et al.*, 1993, J Immunol 151:2426-2435). Consistent with its role in T cell activation and proliferation, a recent study also demonstrated that CD27 co-stimulation improves the function of chimeric antigen receptor (CAR) modified T cells (Song *et al.*, 2012, Blood 119:696-706). In the example herein, upon antigen stimulation, a significantly higher proportion of cells expressing CD27 was found, within NLV-Tet⁺ T cells derived from T_N cells compared to those derived from T_{CM} and T_{EM} cells, respectively, and within NLV-Tet⁺ T cells derived from T_{SCM} cells compared to those derived from T_{EM} cells, in 6 donors tested (** $p \leq 0.005$ and * $p \leq 0.05$) (Figure 3A). In the representative example shown (Figure 3B), high expression of CD27 is also demonstrated in almost all of the NLV-Tet⁺ CD8⁺ T cells derived from T_N cells (Figure 3B).

[00180] Then the expression of CD57, which has been described to be indicative of replicative senescence and antigen-induced apoptosis in HIV-specific CD8⁺ T cells (Brenchley *et al.*, 2003, Blood 101:2711-2720), was evaluated. After antigen-specific stimulation, a significantly lower

proportion of CD57 expressing cells was found within T_N-derived NLV-Tet⁺ T cells, compared to T_{CM}⁻ and T_{EM}-derived NLV-Tet⁺ T cells as shown for 6 donors tested (**p ≤ 0.005 and *p ≤ 0.05) (Figures 3C and 3D). T_{SCM}-derived cells expressed lower levels of CD57 than T_{CM}⁻ and T_{EM}-derived cells, though this difference was not statistically significant for the donors tested. Nonetheless, this data demonstrate a trend towards increasing levels of CD57 expression with increasing T cell differentiation (T_N < T_{SCM} < T_{CM} < T_{EM}).

[00181] Next, the expression of IL-7Rα (CD127), which has been shown to facilitate T cell engraftment and persistence in mouse models (Kaech *et al.*, 2003, Nat Immunol 4:1191-1198), was examined. In the example herein, a slightly higher level of CD127 expression was observed within T_{SCM}-derived NLV-Tet⁺ T cells compared to T_{CM}⁻ and T_{EM}-derived T cells though this was not statistically significant (T_{SCM} vs T_{EM} p = 0.11 and T_{SCM} vs T_{CM} p = 0.03) (Figure 3E). In contrast, moderate to high levels of CD28 expression were observed within NLV-Tet⁺ T cells derived from all subsets, suggesting adequate activation and co-stimulatory capacity within these activated antigen-specific T cells (Figure 3F). After antigenic stimulation, variable levels of PD-1 expression were detected within the NLV-Tet⁺ T cells derived from all T cell subsets tested, with no apparent trend (Figure 3I). However, the expression of PD-1 within NLV-Tet⁻ CD8⁺ T cells was lower than the NLV-Tet⁺ counterparts (Figure 3J). The expression of PD-1 in these T cells is possibly indicative of an activated state. High levels of KLRG-1 expression were also detected within the NLV-Tet⁺ T cells derived from all T cell subsets tested, suggesting the late differentiation stage in antigen-experienced cells (Figure 3G). In contrast, expression of KLRG1 in the T_N-derived NLV-Tet⁻ T cells was significantly lower than that expressed in T_{CM}⁻ and T_{EM}-derived NLV-Tet⁻ T cells, respectively (Figure 3H). Taken together, these data suggest that antigen-experienced cells derived from the T_{SCM} memory subset demonstrate a phenotype with CD27^{hi}, CD57^{low} and CD127^{int}, which is not observed in T_{CM}⁻ and T_{EM}-derived antigen-specific T cells.

6.3.4. T_{SCM}-derived T cells demonstrate higher proliferative capacity leading to superior expansion of antigen-specific T cells

[00182] The data above show that T_{SCM} cells maintain a less differentiated memory phenotype during antigen-specific activation, and a higher level of CD27 expression which sustains a greater proliferative potential. These data suggest that T_{SCM} cells might serve as a durable reservoir of antigen-experienced T cells for memory immune response upon antigen re-

challenge. To address this, the *in vitro* proliferative capacity of different memory T cell subsets upon antigen-specific stimulation with AAPCs expressing CMVpp65 was evaluated, and the phenotype of proliferating T cells was examined using EdU labeling (*see* Section 6.2, *supra*). Within total T cell populations stimulated, vigorous T cell proliferation was observed starting at 3 days after stimulation. Within the NLV-Tet⁺ T cells 10.2% proliferating cells were observed in a representative donor at day 3, reaching a peak of 52.2% at day 5 post-stimulation (Figure 4A). The less differentiated T_{SCM} and T_{CM} cells constituted 32% and 23% respectively, of the proliferating memory NLV-Tet⁺ T cells at day 3 (Figure 4B). At this time the total NLV-Tet⁺ T cells contained 22% of T_{SCM} cells and 32% T_{CM} cells (Figure 4B). By day 5, the T_{SCM} and T_{CM} subsets contributed to 21% of the proliferating memory NLV-Tet⁺ T cells, while they represented 13% of the NLV-Tet⁺ memory T cell compartment (Figure 4B). These data suggest that there is an early preferential proliferation within the T_{SCM} and T_{CM} subsets upon antigen-specific stimulation (Figure 4B). By day 7 post-stimulation, the majority of the NLV-Tet⁺ T cells differentiated to the T_{EM} phenotype in this donor, and at this time T_{EM} is the predominant subset constituting the proliferating NLV-Tet⁺ T cells (Figure 4B). Taken together, T_{SCM} cells appear to reach their maximum proliferative capacity on days 3-5 in culture, suggesting that this is the optimal time to carry out transduction of the T_{SCM} cells, if desired, particularly transduction with a vector (*e.g.*, a retroviral vector) that requires cell proliferation for integration. To compare the proliferative potential of each memory subset, the fold expansion of NLV-Tet⁺ T cells derived from each of these naive and memory subsets was examined. The yield of antigen-specific T cells was calculated and normalized to the number of Tet⁺ T cells present in peripheral blood. After two rounds of antigen exposure, a significantly higher fold expansion of antigen-specific T cells was observed within T_{SCM}-derived cells compared to T_{EM} derived cells ($p=0.03$) (Figure 4C). Although the overall fold expansion of T_{CM}-derived NLV-Tet⁺ T cells was lower than T_{SCM}-derived cells, this difference was not statistically significant. The same analysis could not be performed for some of the T_N donors due to the undetectable levels of Tet⁺ T cells in peripheral blood before antigen stimulation. Taken together, these data indicate that cells derived from the T_{SCM} subset demonstrate superior expansion of antigen-specific cells and may serve as a significant source of other subsets in the T cell memory pool.

6.3.5. *In vitro* expanded T_N and T_{SCM}-derived T cells demonstrate functional activity against specific epitopes as T_{CM}- and T_{EM}-derived T cells

[00183] Given that the epitope specific T_N and T_{SCM} cells maintain a less differentiated phenotype during *in vitro* expansion, the question arises whether this would hamper their functional activity against antigen. Thus, the ability of cells derived from naive and memory T cell subsets to generate cytokines in response to secondary stimulation with antigen was evaluated. CD137 has been described as a marker of antigen-specific CD8⁺ T cells that correlates with functional activity including production of cytokines TNF- α and IFN- γ , as well as cytotoxicity activity (evaluated by CD107a degranulation assay) (Wofl *et al.*, 2007, Blood 110:201-210). Isolated *in vitro* expanded naive- and memory-derived T cells were secondarily stimulated by autologous NLV peptide loaded BLCLs. Following antigen-stimulation, the proportion of CD137 expressing cells within the CD8⁺ T cells increased, as shown for a representative donor in Figure 5A (T_N: 3.36%, T_{SCM}: 37%, T_{CM}, 90.4% and T_{EM} 82%). T cells derived from all subsets (T_N, T_{SCM}, T_{CM} and T_{EM}) were able to produce cytokines. Of note, T cells capable of secreting both TNF- α and IFN- γ were observed in T cells derived from all subsets including T_N and T_{SCM}. To demonstrate cytotoxicity activity of T cells derived from different subsets, the CD107a degranulation assay was performed. Complementing their capacity to secrete cytokines, T_N- and T_{SCM}-derived cells also demonstrated functional cytotoxic activity against peptide loaded autologous targets. The percentage of CD107a-expressing cells within the T_N- and T_{SCM}-derived CD8⁺ T cell population was 20% and 22% respectively (Figure 5B). Taken together, epitope specific T cells can be expanded *in vitro* from T_N and T_{SCM} cells by stimulation with antigen-expressing AAPCs in the presence of IL-7 and IL-15. These epitope specific T_N- and T_{SCM}- derived cells, after expansion *in vitro*, demonstrate a less differentiated memory phenotype than T_{CM}- or T_{EM}-derived epitope specific T cells expanded *in vitro*. However, these antigen-specific T_N- and T_{SCM}-derived cells are functional as evidenced by their ability to release cytokines and degranulate in response to antigen stimulation.

6.3.6. *In vitro* expanded T_{SCM}-derived CMV-specific T cells exhibit an oligoclonal repertoire of public TCRs that is similar to that of T_{CM}- and T_{EM}-derived populations

[00184] Next, the clonal diversity and /or similarity between NLV-Tet⁺ T cells derived from naive as well as memory T cell subsets were examined. To assess clonal differences in the TCR

sequences within NLV-Tet⁺ T cells, T_N⁻, T_{SCM}⁻, T_{CM}⁻ and T_{EM}-derived NLV-specific T cells from two CMV seropositive donors were evaluated. It was first evaluated if there were common TCR sequences within CMV-specific T cells derived from T_N, T_{SCM}, T_{CM} and T_{EM} cells. Accordingly, T_N⁻, T_{SCM}⁻, T_{CM}⁻ and T_{EM}⁻ derived T cells were expanded *in vitro* using artificial antigen presenting cells for 30 days, and the NLV-Tet⁺ T cells and NLV-Tet⁻ T cells were sorted. Then TCR repertoire analysis was performed by next-generation sequencing on the sorted Tet⁺ T cells in comparison to the Tet⁻ T cells. TCR sequencing data were analyzed for similarities in nucleotide sequences by sample overlap. This analysis demonstrates a high overlap between TCR sequences for T_{CM}⁻ and T_{EM}-derived NLV-Tet⁺ T cells post-stimulation (96% overlap in Figure 6C). T_{SCM}-derived cells demonstrated high similarities with T_N⁻ and T_{CM}-derived cells, and less similarities with T_{EM}-derived cells (T_N: 84%; T_{CM}: 91%; and T_{EM}: 57% in Figure 6C). T_N-derived Tet⁺ cells also had a high degree of TCR similarities/overlap with Tet⁺ T_{CM}-derived cells (87%) but very little overlap with T_{EM}-derived cells (14%). No TCR overlap was detected within NLV-Tet⁻ T cells derived from either naive or memory T cells (data not shown). Overall, these data indicate that Tet⁺ T cells recognizing the NLV epitope that are derived from the naive like T_N subset demonstrate a high degree of overlap between TCR sequences expressed by T_{SCM}- and T_{CM}-derived cells. While the TCR sequence detected in T_{SCM} and T_{CM} derived T cells were less frequently detected in the T_{EM}-derived cells, nevertheless, there was a significant number of specific public TCRs common to T cells derived from all memory T cell compartments.

[00185] It was then examined if the same trend of TCR overlap would be present prior to antigen stimulation in peripheral blood. Therefore, T_N⁻, T_{SCM}⁻, T_{CM}⁻ and T_{EM}-derived NLV-Tet⁺ T cells were sorted from another donor prior to stimulation and the TCR sequence was evaluated for clonality and overlap. TCR clonality describes the degree to which one or a few clones dominate the repertoire, with 0 being a flat distribution and 1 being an entirely oligoclonal sample. Prior to antigenic stimulation, T_{EM} NLV-Tet⁺ T cells demonstrated a high degree of clonality, with a clonality index of 0.66 (Figure 6A). In comparison, the T_{CM} NLV-Tet⁺ T cells demonstrated a clonality index of 0.06, suggesting higher TCR clonal diversity, while the circulating T_{SCM} and T_N NLV-Tet⁺ T cells were highly diverse with a clonal index of 0.01 (Figure 6A). In overlap analysis comparing the Tet⁺ T cells in the different subsets, however, NLV-Tet⁺ T cells detected in the T_{EM} fraction were also differentially represented in the T_{CM} repertoire (94% in Figure 6D). On the other hand, T_{SCM} cells, which had a highly diverse TCR repertoire,

demonstrate very little overlap with T_{CM} and T_{EM} TCR sequences (13.2% and 24.6% respectively) (Figure 6D). Strikingly, no TCR overlap between T_N NLV-Tet⁺ T cells and T_{SCM}, T_{CM} or T_{EM} NLV-Tet⁺ T cells was identified in peripheral blood (Figure 6D).

[00186] After 15 days of antigenic stimulation *in vitro*, the expanded NLV-Tet⁺ T cells adopted a restricted clonal diversity, with the clonality index ranging from 0.35 to 0.83 for all subsets (Figure 6B). The overlap analysis data correlated with the previous observation that T_{CM}- and T_{EM}-derived NLV-Tet⁺ T cells bear highly overlapping TCR sequences (97% and 95 % at Day 15 and Day 30 as shown in Figures 6E and 6F). Interestingly, T_{SCM}-derived Tet⁺ T cells developed a restricted TCR repertoire within 15 days post-stimulation, which is highly similar to T_{CM}- and T_{EM}-derived Tet⁺ T cells (T_{SCM}/T_{CM}: 86% and T_{SCM}/T_{EM}: 95% in Figure 6E). Unlike the T_{SCM}-derived Tet⁺ T cells early after re-stimulation, T_N-derived Tet⁺ T cells demonstrated very different TCR sequences compared to T_{CM}- and T_{EM}-derived Tet⁺ T cells with an overlap of 38% and 3.8% respectively (Figure 6E). While a significant fraction of T_N-derived Tet⁺ T cells expressing TCRs shared by T_{CM}-derived Tet⁺ T cells can be detected by day 30, the fraction shared by T_{EM}-derived Tet⁺ T cells remained small throughout 30 days of expansion. Thus, these data suggest that upon antigen stimulation, T_{SCM} constitute the principal and immediate source for replenishing immunodominant NLV-specific T_{CM} and T_{EM} populations in the circulation.

[00187] An additional striking feature of the day 0 T_{CM} and T_{EM} Tet⁺ T cell population and the day 15 T_{SCM}-derived Tet⁺ T cell population is that these Tet⁺ T cells expressed CDR3 sequences CASSPQTGASYGYTF (SEQ ID NO:3) and CASSPKTGAVYGYTF (SEQ ID NO:4) that have previously been reported in public T cell receptors specific for the NLV-peptide (Yang *et al.*, 2015, J Biol Chem 290:29106-29119). While these sequences were also detected in T_N-derived Tet⁺ T cells, their frequency was much lower than in T_{SCM}-, T_{CM}- or T_{EM}-derived Tet⁺ T cells (Table 2). In the dataset of the example herein, the T_N-derived Tet⁺ T cells also contained unique sequences that were not shared with Tet⁺ T cells derived from other subsets. For example, the sequence CASSYVTGTGNYGYTF (SEQ ID NO:5) was only detected in T_N-derived Tet⁺ T cells post-stimulation in high frequencies (89% and 87 % at day 15 and 30 respectively, Table 2). This suggests that T_N-derived Tet⁺ T cells can ultimately potentially expand upon antigen exposure to populate the immunodominant memory T cell pool, and also expand unique clones recognizing the antigen.

[00188] Table 2. Predominant clonotypes represented within naive and memory A2-NL V-specific CD8⁺ T cells in CMV seropositive donors

Source	Amino Acid	TCRBV	TCRBJ	Frequency on different days (%)												
				T _{EM}			T _{CM}			T _{SCM}			T _N			
				0	15	30	0	15	30	0	15	30	0	15	30	
CK-42202 NLV pos	CASSPQTGASYGYTF (SEQ ID NO:3)	TCRBV06-05	TCRBJ01-02	21.29	10.53	8.13	8.06	46.30	62.72	2.56	83.57	67.66	0.00	0.22	0.11	
	CASSYVTGTGNYGYTF (SEQ ID NO:5)	TCRBV06-05	TCRBJ01-02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	88.93	87.14	
	CSVAGTVNEQFF (SEQ ID NO:6)	TCRBV29-01	TCRBJ02-01	0.81	22.99	18.87	0.00	5.79	2.94	0.00	9.72	14.59	0.00	0.00	0.00	0.00
	CAAGGIFGTDQYF (SEQ ID NO:7)	TCRBV27-01	TCRBJ02-03	0.00	4.29	5.79	0.00	6.57	5.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CAWSISDIMNTEAFF (SEQ ID NO:8)	TCRBV30-01	TCRBJ01-01	0.00	1.80	0.83	0.00	12.31	6.95	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CASLEGYTEAFF (SEQ ID NO:9)	TCRBV27-01	TCRBJ01-01	6.20	4.99	3.31	2.42	2.99	1.47	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CAWSVSDPLNTEAFF (SEQ ID NO:10)	TCRBV30-01	TCRBJ01-01	1.66	1.52	2.20	2.42	1.18	0.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CASSPKTGAVYGYTF (SEQ ID NO:4)	TCRBV06-05	TCRBJ01-02			10.79			73.07				79.73			46.41
	CASSHQTSGNITIF (SEQ ID NO:11)	TCRBV19-01	TCRBJ01-03			17.04			0.09				0.00			0.00
	CASLLKTGASYGYTF (SEQ ID NO:12)	TCRBV06-05	TCRBJ01-02			1.17			4.64				0.02			1.10
CASSVLAPTVGSTEAF (SEQ ID NO:13)	TCRBV10-02	TCRBJ01-01			4.37			3.03				0.00			0.00	
CASSYQTGASYGYTF (SEQ ID NO:14)	TCRBV06-05	TCRBJ01-02			4.22			1.81				0.00			0.00	
CASSEIGATNYGYTF (SEQ ID NO:15)	TCRBV06-01	TCRBJ01-02			2.94			0.16				0.00			0.00	

6.3.7. Immunodominance is not caused by the skewed proliferation of T_{SCM} cells in circulation

[00189] The data above show that T_{SCM}-derived T cells demonstrate higher proliferative capacity generating superior expansion of antigen-specific T cells. Next it was tested whether the phenomenon of epitope-specific T cell immunodominance could be a consequence of either over-representation of or preferential proliferation of T_{SCM} cells within T cells responding to immunodominant compared to subdominant epitopes. To address this question, the *in vitro* T cell proliferative capacity of T_{SCM}, T_{CM}, and T_{EM} subsets, respectively, from a CMV seropositive donor co-inheriting HLA-A*0201 and A*2402, upon antigen specific stimulation, was measured. The immunodominant anti-CMV T cell response in donors co-inheriting these alleles has been shown to be directed against the NLV epitope presented by HLA-A*0201 (Lidehall *et al.*, 2005, J Clin Immunol 25:473-481). T cells from the same donor were stimulated with NLV- and QYD-loaded autologous dendritic cells. Within 8 days post-stimulation, NLV-Tet⁺ T cells were enriched to 31.7% and QYD-Tet⁺ T cells to 1.6%, representing the immunodominant and subdominant response respectively (Figure 7A). Strikingly, a similar memory phenotype was found for both A2-NLV- and A24-QYD-Tet⁺ T cells, with no overrepresentation of T_{SCM}-derived T cells within the immunodominant A2-NLV-Tet⁺ T cells in culture (Figure 7B). To further evaluate the relative proliferative potential of immunodominant and subdominant epitope-specific T cells, they were examined for EdU incorporation as proliferation marker and Annexin V expression as apoptosis marker. Similar levels of proliferating cells were found within A2-NLV- and A24-QYD-Tet⁺ T cells, with no preferential proliferation within the immunodominant A2-NLV-Tet⁺ T cells (Figure 7B). However, the A24-QYD-Tet⁺ T cells contained a higher percentage of Annexin V⁺ cells compared to A2-NLV-Tet⁺ T cells (Figure 7B). These data suggest that a higher level of T cell apoptosis within subdominant epitope-specific T cells might promote the preferential enrichment of immunodominant T cells.

6.4. Discussion

[00190] This example described herein characterized HLA-A0201-restricted T_N, T_{SCM}, T_{CM} and T_{EM} cells specific for the immunodominant NLV peptide of CMVpp65 that were isolated by immunoabsorption to tetramers from the blood of healthy seropositive donors, and T cells derived from these subsets after sequential intervals of *in vitro* sensitization. The Tet⁺ and Tet⁻ T

cells from the same T cell subset population were also compared. The results suggest a major role for T_{SCM} cells as a durable reservoir of T cell memory in the recall immune response.

[00191] This analysis of antigen-specific T cells in these different T cell subsets has demonstrated that Tet⁺ T_{SCM} in the blood exhibit a CD45RA⁺ CCR7⁺ and CD62L⁺ phenotype similar to T_N cells, but also express CD95. Upon sensitization with antigen, both Tet⁺CD95⁻ T_N and Tet⁺CD95⁺ T_{SCM} cells were regularly detected. Both the Tet⁺ T_N and Tet⁺ T_{SCM} cells expressed significantly higher levels of the co-stimulatory marker CD27 than detected on Tet⁺ T_{CM} and Tet⁺ T_{EM} cells; Tet⁺ T_{SCM} cells demonstrated somewhat higher expression of the activation marker CD127. Conversely, the Tet⁺ T_N and Tet⁺ T_{SCM} cells expressed lower levels of the senescence marker CD57; Tet⁺ T_N cells expressed a significantly lower level of CD28. Strikingly, after *in vitro* sensitization, Tet⁺ T cells derived from all subsets generated a similarly high proportion of cells expressing PD-1, which differed significantly from the low proportion of PD-1⁺ cells in the Tet⁻ T cells derived from these subsets. In addition, the killer-cell lectin like receptor G1 (KLRG1), which is usually considered to be a marker of late differentiation, was expressed to a similar degree in the NLV-peptide responding T_N⁻, T_{SCM}⁻, T_{CM}⁻ and T_{EM}-derived T cells. In contrast, this marker was expressed at a minimal level in the Tet⁻ T_N-derived T cells. Thus, in latently infected healthy seropositive individuals, secondary stimulation with the viral antigen induces expression of both PD-1 and KLRG-1 in all stages of T cell memory. However, the Tet⁺ T_N⁻ and T_{SCM}⁻-derived T cells still differentially express the CD45RA, CCR7 and CD62L markers associated with earlier stages of maturation and the capacity for targeted migration and longer survival.

[00192] At a functional level, Tet⁺ T_N⁻, T_{SCM}⁻, T_{CM}⁻ and T_{EM}-derived T cells all contained cells capable of secreting TNF- α and IFN- γ , and degranulating in response to antigen stimulation, although the proportion of Tet⁺ T_{EM}⁻ and T_{CM}⁻-derived T cells exhibiting these functions were higher than in Tet⁺ T_{SCM}⁻ or T_N-derived T cells. Thus, in contrast to a previous report where CD95⁻ T cells were unable to secrete cytokines after HCMV-specific stimulation (Schmueck-Henneresse *et al.*, 2015, J Immunol 194:5559-5567), the example described herein provides evidence that antigen-responsive T_N-derived T cells do exhibit these effector functions and cannot, thereby, be qualitatively distinguished from antigen-responsive T_{SCM}-derived T cells. A study by Pulko *et al.* (2016, Nat Immunol 17:966-975) has also shown that human memory T cells with a naive phenotype secrete multiple cytokines in response to secondary stimulation with

viral antigens but differ transcriptionally from memory and effector T cells. However, the example described herein did not find that Tet⁺ CD95⁺ T_{SCM} cells are functionally distinguishable from Tet⁺ CD95⁻ T_N cells on the basis of their proliferative response to antigen stimulation. Indeed, their proliferative response also significantly exceeded that of Tet⁺ T_{CM} and Tet⁺ T_{EM} cells.

[00193] A diverse TCR repertoire has been postulated to mediate optimal control of pathogens by increasing the probability that microbial escape mutations would be recognized by one of the TCRαβ pairs represented within the repertoire (Cornberg *et al.*, 2006, J Clin Invest 116:1443-1456; Meyer-Olson *et al.*, 2004, J Exp Med 200:307-319). The TCR sequencing analysis in the example described herein has shown that in the peripheral circulation, the T_N and T_{SCM} cells recognizing the same epitope NLV are more diverse than T_{CM} and T_{EM} cells. This broad TCR repertoire of T_N and T_{SCM} cells recognizing the same pathogen could be hypothesized to provide better control of virus infection. However, upon antigen stimulation, the example herein found that T_{SCM} cells generate T cells that are oligoclonal within 15 days after antigen encounter, and further that these T cells express a repertoire closely related to that of Tet⁺ T_{EM} and Tet⁺ T_{CM} cells that are dominant in the circulation. The fact that T_{SCM}-derived CMV-specific T cells share high overlap of TCR usage with T_{CM} and T_{EM}-derived CMV-specific T cells early post-stimulation, provides evidence that T_{SCM}-derived T cells are particularly effective in reconstituting populations of immunodominant memory T cells in the blood. Moreover, the heterogeneous spectrum of clones generated from T_N cells after antigen stimulation, which differs from that of the other memory T cell subsets, suggests that T_N cells serve as a pool of precursor cells capable of selecting peptide-specific T cells with a broader spectrum of binding characteristics, from which memory T cells of optimal avidity can be selected for expansion if required to control viral variants.

[00194] T cell responses to latent viruses often focus on a small number of the available antigenic epitopes and use a narrow TCR repertoire, a phenomenon termed “immunodominance.” A number of factors have been reported to influence immunodominance, including antigen presentation, peptide-MHC binding affinity and stability or TCR avidity and the frequency of precursors in the naive T cell pool (Khan *et al.*, 2007, J Immunol 178:4455-4465). In the two donors tested in the example herein, an overrepresentation or a preferential proliferation of T_{SCM}-derived T cells responding to immunodominant epitopes was not found,

compared to those responding to subdominant epitopes. Instead, a higher level of T cell apoptosis was observed within subdominant epitope-specific T cells, which might promote the preferential enrichment of immunodominant T cells. Previous studies have also indicated that the characteristics of TCR binding to pMHC complexes may also regulate clonal apoptosis (Tscharke *et al.*, 2015, Nat Rev Immunol 15:705-716). Indeed, TCRs with a subthreshold affinity for pMHC-I preferentially undergo apoptosis early in the response via a process that is regulated by the BCL-2 family members BIM, NOXA and MCL1 (Wensveen *et al.*, 2010, Immunity 32:754-765). Thus, the data of the example herein are in accord with that of others and suggest that apoptosis may also be crucial for shaping adaptive immune responses, thereby enhancing the selection of clones with greater persistence.

[00195] Another feature of the Tet⁺ T cells specific for the NLV HLA-A0201 epitope generated from T_{SCM} cells was their expression of T cell receptor with amino acid sequences identical to sequences previously reported for public T cell receptors specific for the NLV epitope (Yang *et al.*, 2015, J Biol Chem 290:29106-29119). Public T cell receptors are peptide-specific TCRs with highly homologous sequences detected in multiple individuals (Li *et al.*, 2012, Cell Res 22:33-42). Public TCRs have been described in T cells responding to a variety of human viruses (Argaet *et al.*, 1994, J Exp Med 180:2335-2340). NLV peptide-specific T cell repertoires have been shown to exhibit a high prevalence of public TCRs (Wang *et al.*, 2012, Sci Transl Med 4:128ra142; Nguyen *et al.*, 2014, J Immunol 192:5039-5049; Trautmann *et al.*, 2005, J Immunol 175:6123-6132). Indeed, seven public CDR3 α and six CDR3 β motifs account for ~70% of the total NLV-specific TCR response (Wang *et al.*, 2012, Sci Transl Med 4:128ra142). The example herein also identified a common TCR β CDR3 represented within T_N, T_{SCM}, T_{CM} and T_{EM}-derived Tet⁺ T cells post-stimulation that bears the S*_nTG*_nGY (SEQ ID NO:16; *n indicates any amino acid sequence of any length and any amino acid combination) motif which has the highest reported frequency among the other published motifs. Two other studies have also shown the same S*_nTG*_nGY (SEQ ID NO:16; *n indicates any amino acid sequence of any length and any amino acid combination) motif within HLA-A*0201 NLV-specific T cells from both CMV seropositive donors (Hanley *et al.*, 2015, Sci Transl Med 7:285ra263; Neller *et al.*, 2015, Immunol Cell Biol 93:625-633). The crystal structures of two public TCRs in complex with NLV-HLA-A2 have been reported (Yang *et al.*, 2015, J Biol Chem 290:29106-29119). These and other studies (Yang *et al.*, 2015, J Biol Chem 290:29106-29119; Welsh *et al.*, 2010,

Immunol Rev 235:244-266) have provided evidence that different pairings of public CDR3 α and β domains can mediate a multiplicity of high affinity TCRs recognizing the same peptide MHC ligand (Yang *et al.*, 2015, J Biol Chem 290:29106-29119). Current evidence indicates that public TCRs are produced more frequently during random V(D)J gene rearrangements, a random process termed “convergent recombination” (Miles *et al.*, 2011, Immunol Cell Biol 89:375-387). Thereafter, this may be further selected by combinatorial bias. Such public TCRs could potentially propagate more and establish better virus control over other clones in the TCR repertoire.

[00196] In conclusion, first, the example herein demonstrated that antigen-specific T_{SCM} -derived T cells exhibit and maintain a less differentiated phenotype than those derived from other memory T cell populations responding to a single epitope, displaying an expansion profile of tissue homing, co-stimulatory and senescence markers that is intermediate between T_N and T_{CM} -derived cells. In addition, Tet^+ CMVpp65 NLV-specific T cells were regularly detected cells derived from the $CD95^-$ T_N cell compartment. However, antigen-specific T_{SCM} cells differ from Tet^+ T_N cells as well as Tet^+ T_{CM} and Tet^+ T_{EM} cells in that they proliferate to a much higher degree in response to antigen stimulation. Second, antigen-specific Tet^+ T_N and Tet^+ T_{SCM} -derived cells, unlike Tet^- T_N and Tet^- T_{SCM} -derived cells, were found to express PD-1 and KLRG-1 at levels similar to those expressed by Tet^+ T_{CM} - and Tet^+ T_{EM} -derived cells. They also exhibit effector function, indicating the capacity to generate TNF- α , IFN- γ and granzyme B. Third, this example has demonstrated that, in response to antigen stimulation, T_{SCM} cells not only undergo marked proliferation but also rapidly select T cell clones specific for the CMVpp65 NLV epitope presented by HLA-A*0201 that, by TCR sequencing, are public TCRs identical to those expressed by NLV/HLA-A*0201 specific T_{EM} and T_{CM} cells that are immunodominant in the blood of the HLA-A*0201 seropositive donors. Unlike the Tet^+ T_{SCM} cells, Tet^+ T_N cells do not rapidly select clones expressing the immunodominant TCRs detected in T_{EM} and T_{CM} population in the blood. Rather, the Tet^+ T_N cells maintain a distinct repertoire that is markedly more varied than that detected in NLV-specific T_{CM} and T_{EM} cells in the blood, as that detected in T_{SCM} -derived cells upon 15 days of *in vitro* stimulation. Finally, the example herein suggests that while the expansion of T_{SCM} , T_{CM} and T_{EM} cells proliferating in response to the immunodominant epitope NLV presented by HLA-A*0201 is similar to those proliferating in response to the subdominant QYD epitope presented by HLA-A*2402 expressed by the same donor, the

proportions of immunodominant NLV-specific T_{SCM}, T_{CM}, and T_{EM}-derived cells that undergo apoptosis are markedly smaller than that of the subdominant QYD-specific counterparts.

[00197] These findings have implications for the design of T cell-based immunotherapies. This example has provided evidence that the T_{SCM} subset serves as the major durable reservoir for repopulating immunodominant T_{EM} and T_{CM} cells in the circulation upon secondary antigenic challenge. Adoptive transfer of virus-specific T cell lines enriched for T_{SCM} could potentially provide better disease control by providing immunodominant virus-specific T cells capable of rapid and extensive proliferation and enhanced persistence.

7. Incorporation by reference

[00198] All references cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

[00199] Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled.

What is claimed is:

1. A method of generating a population of cells comprising antigen-specific T cells for therapeutic administration to a human patient having or suspected of having a pathogen or cancer, comprising: (a) *ex vivo* sensitizing a population of human blood cells to one or more antigens of the pathogen or cancer over a period of time in culture that is in the range of 9-21 days, wherein at the initiation of said period of time, the population of human blood cells contains at least 50% stem cell-like memory T cells (T_{SCM} cells); and (b) cryopreserving (i) the *ex vivo* sensitized population of human blood cells, or (ii) cells derived therefrom that comprise antigen-specific T cells recognizing the one or more antigens of the pathogen or cancer; thereby producing said population of cells comprising antigen-specific T cells.
2. The method of claim 1, wherein the period of time in culture is in the range of 9-14 days.
3. A method of generating a population of cells comprising antigen-specific T cells for therapeutic administration to a human patient having or suspected of having a pathogen or cancer, comprising transducing a population of human blood cells with a nucleic acid encoding a public TCR recognizing one or more antigens of the pathogen or cancer at a time when the population of human blood cells has been cultured for 3-5 days, wherein the population of human blood cells contains at least 50% T_{SCM} cells; thereby producing said population of cells comprising antigen-specific T cells.
4. A method of generating a population of cells comprising antigen-specific T cells for therapeutic administration to a human patient having or suspected of having a CMV infection, comprising transducing a population of human blood cells with a nucleic acid encoding a public TCR recognizing CMV pp65, wherein the public TCR comprises a β -chain comprising a variable domain, which comprises a CDR3 of SEQ ID NO:3 or SEQ ID NO:4, and wherein the population of human blood cells contains at least 50% T_{SCM} cells; thereby producing said population of cells comprising antigen-specific T cells.

5. A method of generating a population of cells comprising antigen-specific T cells for therapeutic administration to a human patient having or suspected of having a pathogen or cancer, comprising transducing a population of human blood cells with a nucleic acid encoding a CAR recognizing one or more antigens of the pathogen or cancer at a time when the population of human blood cells has been cultured for 3-5 days, wherein the population of human blood cells contains at least 50% T_{SCM} cells; thereby producing said population of cells comprising antigen-specific T cells.
6. The method of claim 1 or 2, wherein the population of cells comprising antigen-specific T cells is for therapeutic administration to a human patient having or suspected of having a pathogen, wherein the *ex vivo* sensitizing step comprises *ex vivo* sensitizing the population of human blood cells to one or more antigens of the pathogen, and wherein the one or more antigens of the pathogen are one or more antigens of cytomegalovirus (CMV) or one or more antigens of Epstein-Barr virus (EBV).
7. The method of claim 3, wherein the population of cells comprising antigen-specific T cells is for therapeutic administration to a human patient having or suspected of having a pathogen, wherein the public TCR recognizes one or more antigens of the pathogen, and wherein the one or more antigens of the pathogen are one or more antigens of cytomegalovirus (CMV) or one or more antigens of Epstein-Barr virus (EBV).
8. The method of claim 5, wherein the population of cells comprising antigen-specific T cells is for therapeutic administration to a human patient having or suspected of having a pathogen, wherein the CAR recognizes one or more antigens of the pathogen, and wherein the one or more antigens of the pathogen are one or more antigens of cytomegalovirus (CMV) or one or more antigens of Epstein-Barr virus (EBV).
9. The method of claim 1 or 2, wherein the population of cells comprising antigen-specific T cells is for therapeutic administration to a human patient having or suspected of having a cancer, wherein the *ex vivo* sensitizing step comprises *ex vivo* sensitizing the population of human blood cells to one or more antigens of the

- cancer, wherein the cancer is multiple myeloma or plasma cell leukemia, and wherein the one or more antigens of the cancer is Wilms tumor 1 (WT1).
10. The method of claim 3, wherein the population of cells comprising antigen-specific T cells is for therapeutic administration to a human patient having or suspected of having a cancer, wherein the public TCR recognizes one or more antigens of the cancer, wherein the cancer is multiple myeloma or plasma cell leukemia, and wherein the one or more antigens of the cancer is Wilms tumor 1 (WT1).
 11. The method of claim 5, wherein the population of cells comprising antigen-specific T cells is for therapeutic administration to a human patient having or suspected of having a cancer, wherein the CAR recognizes one or more antigens of the cancer, wherein the cancer is multiple myeloma or plasma cell leukemia, and wherein the one or more antigens of the cancer is Wilms tumor 1 (WT1).
 12. The method of any one of claims 1-11, wherein the population of human blood cells contains at least 90% T_{SCM} cells.
 13. The method of any one of claims 1-12, wherein the population of human blood cells contains less than 10% naive T (T_N) cells.
 14. The method of any one of claims 1-13, which further comprises a step of deriving the population of human blood cells from a human cell sample, wherein the deriving step comprises enriching for T_{SCM} cells from the human cell sample, and wherein the enriching step comprises selecting for cells that are CD3⁺ CD62L⁺CD45RO⁻ CD95⁺.
 15. A method of treating a human patient having a pathogen or cancer, comprising: (i) generating a population of cells comprising antigen-specific T cells according to the method of any one of claims 1-14; and (ii) administering the population of cells comprising antigen-specific T cells to the human patient.
 16. A method of treating a human patient having a pathogen or cancer, comprising administering a population of cells comprising antigen-specific T cells to the human patient, wherein the population of cells comprising antigen-specific T cells is the product of a method comprising generating the population of cells comprising antigen-specific T cells according to the method of any one of claims 1-14.

17. The method of claim 15 or 16, wherein the antigen-specific T cells contained in the population of cells comprising antigen-specific T cells are restricted by an HLA allele shared with the diseased cells in the human patient.
18. Use of a population of cells comprising antigen-specific T cells for the manufacture of a medicament for the treatment of a human patient having a pathogen or cancer, wherein the population of cells comprising antigen-specific T cells is the product of a method comprising generating the population of cells comprising antigen-specific T cells according to the method of any one of claims 1-14.
19. The use of claim 18, wherein the antigen-specific T cells contained in the population of cells comprising antigen-specific T cells are restricted by an HLA allele shared with the diseased cells in the human patient.
20. A population of cells comprising antigen-specific T cells suitable for therapeutic administration to a human patient having or suspected of having a pathogen or cancer, wherein the population of cells comprising antigen-specific T cells is the product of a method comprising generating the population of cells comprising antigen-specific T cells according to the method of any one of claims 1-14.
21. A population of cells comprising antigen-specific T cells suitable for therapeutic administration to a human patient having or suspected of having a pathogen or cancer, wherein the population of cells comprising antigen-specific T cells is the product of a method comprising generating the population of cells comprising antigen-specific T cells according to the method of any one of claims 1-14, and wherein the population of cells comprising antigen-specific T cells is cryopreserved.
22. A cell bank comprising a plurality of populations of cells according to claim 20 or 21.

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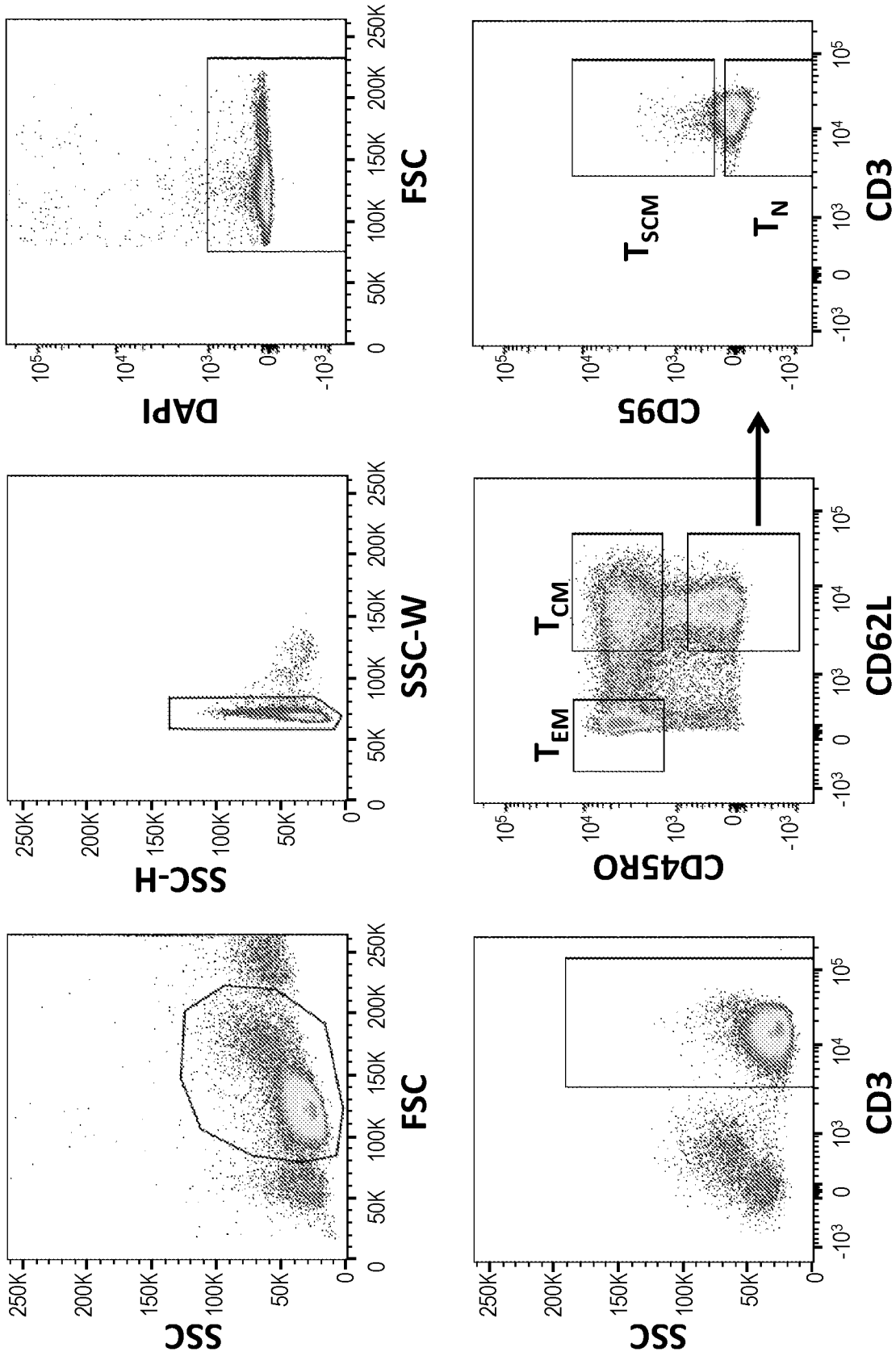
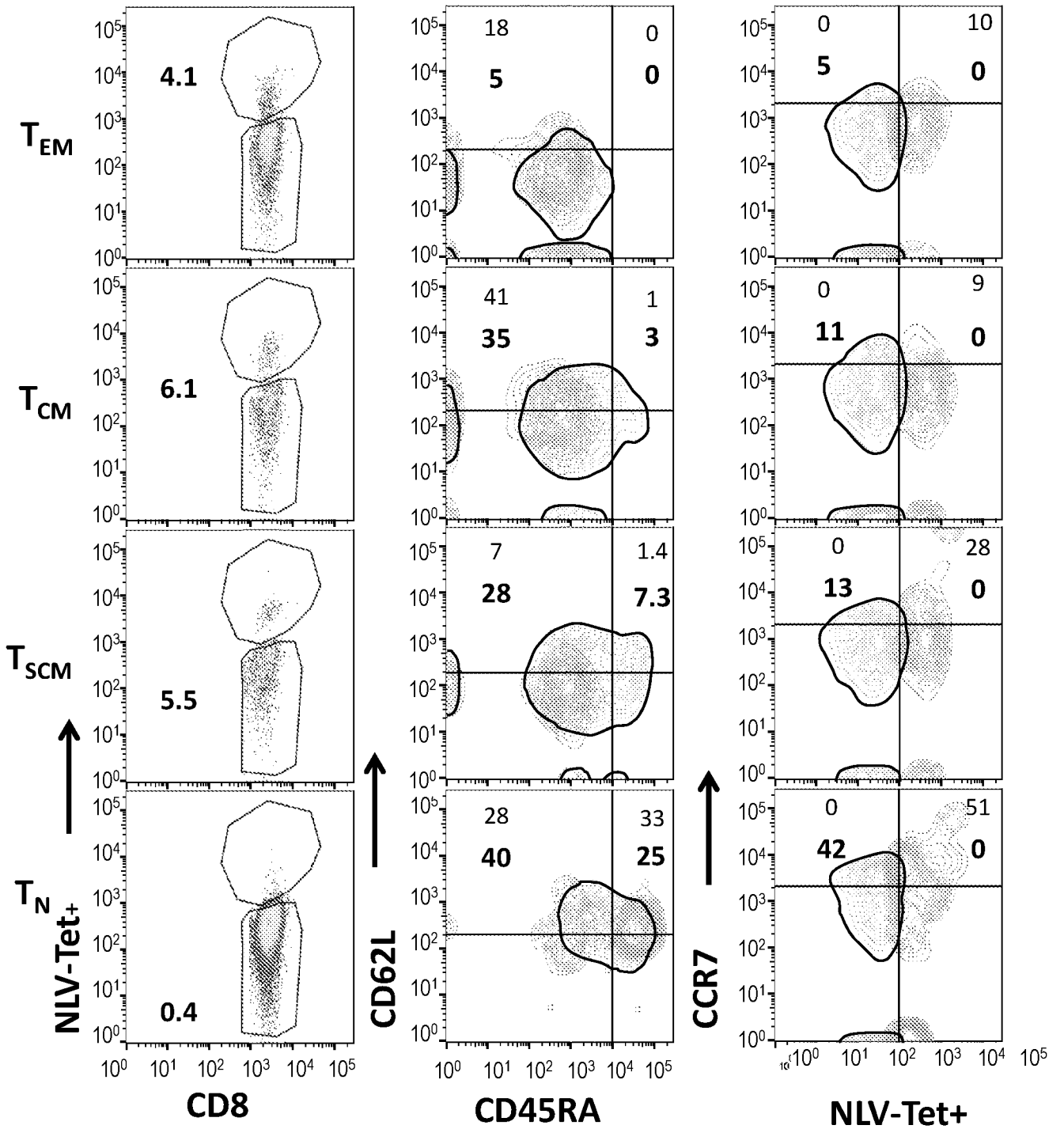


Figure 1

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Day 7



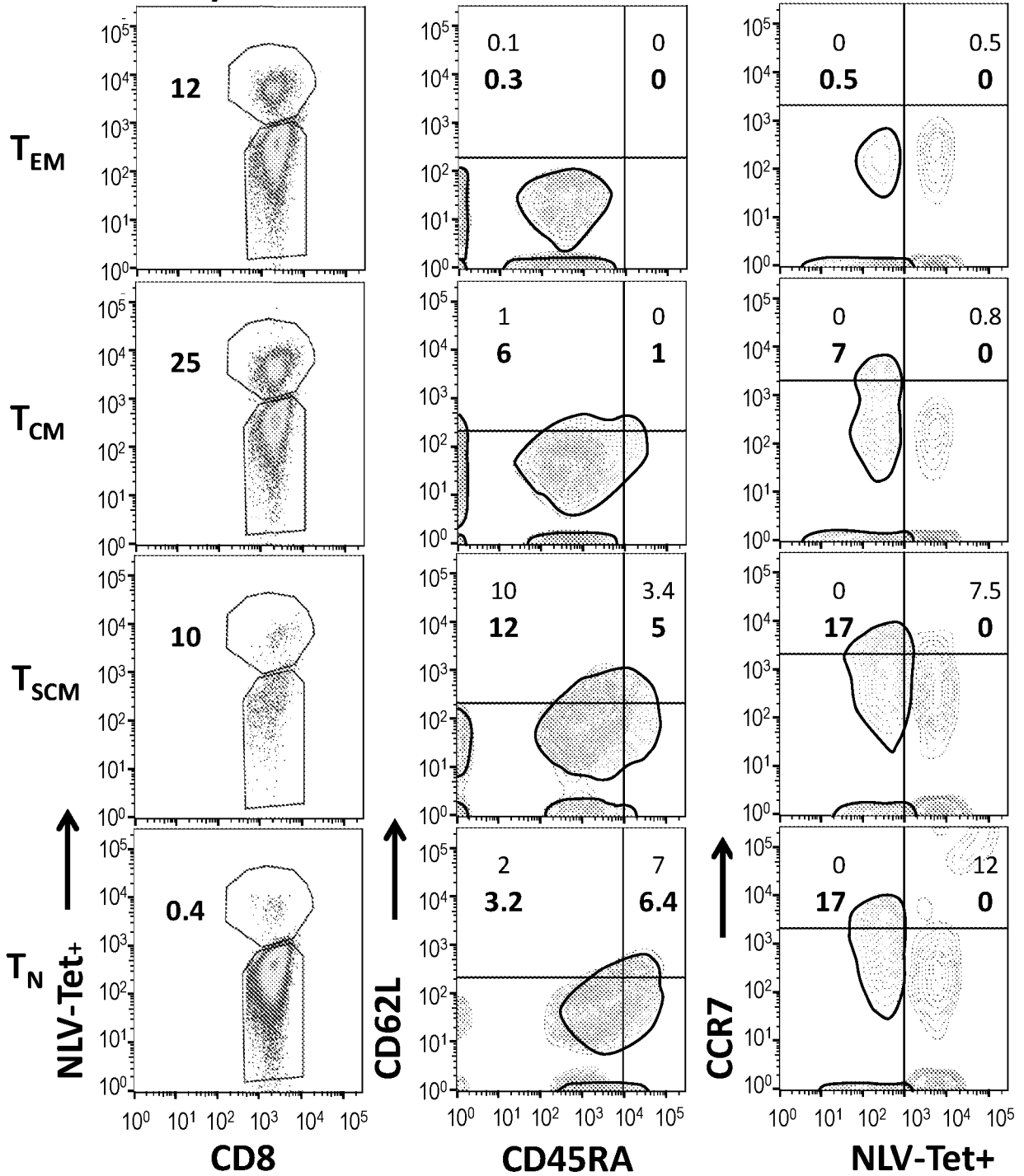
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Bold font: NLV-Tet⁺

Figure 2A

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Day 14

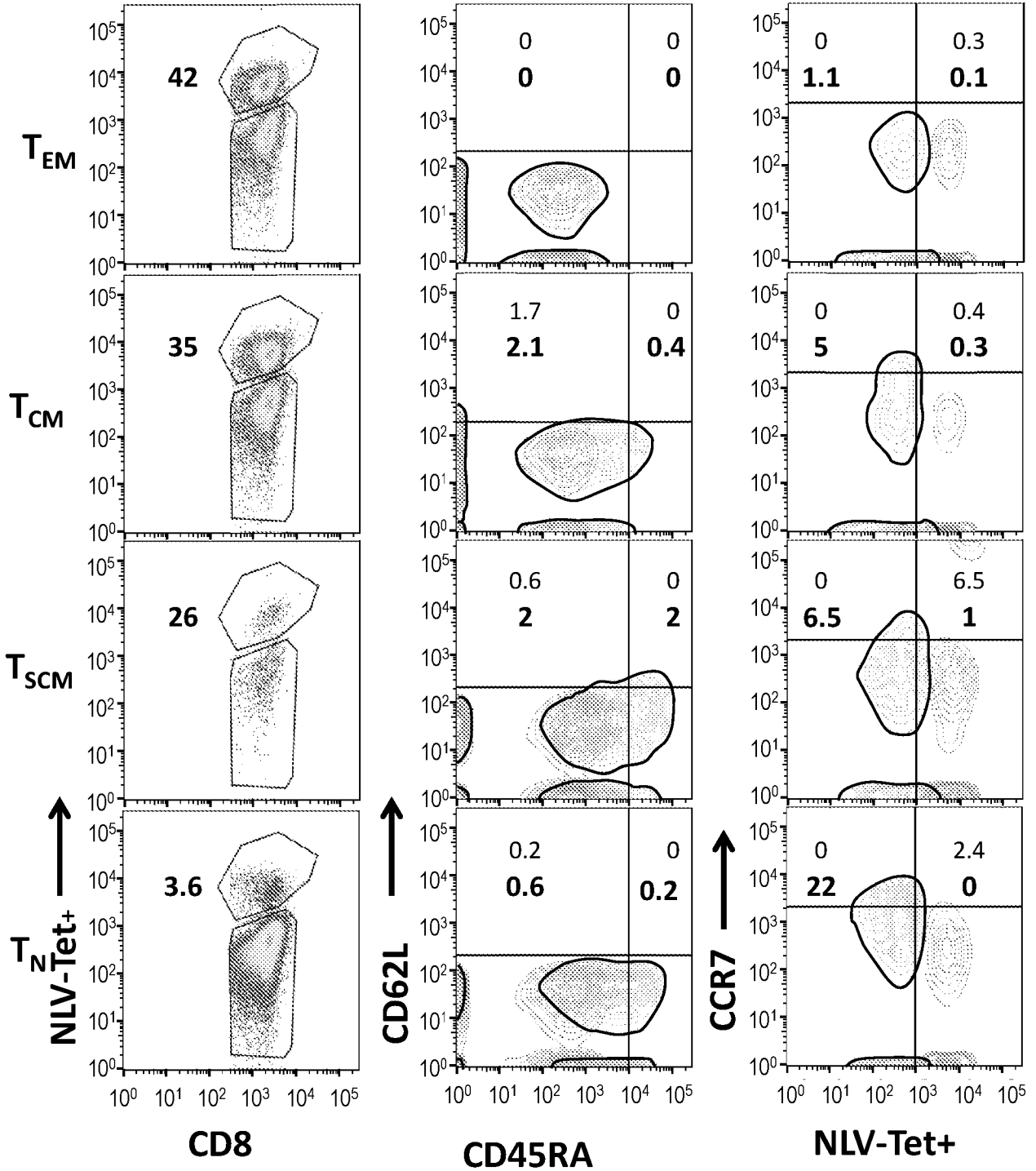


Regular font: NLV-Tet⁺
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Figure 2B

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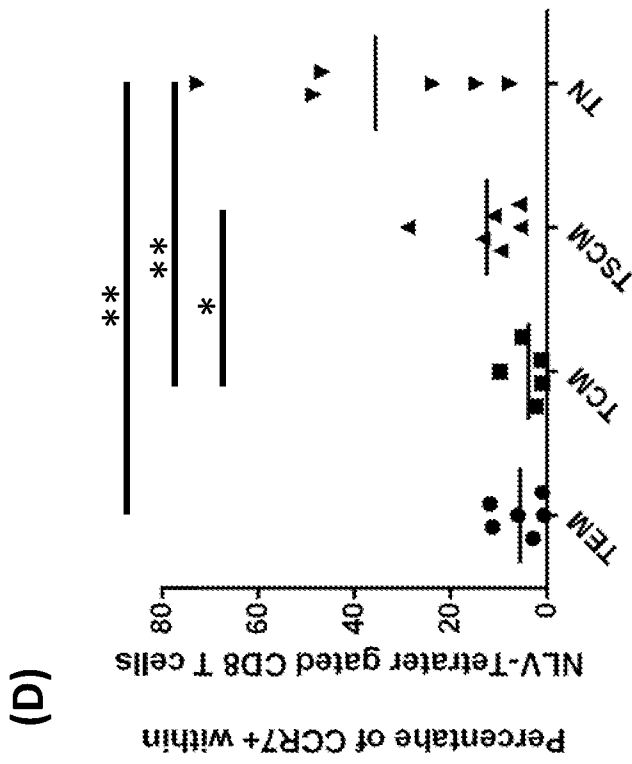
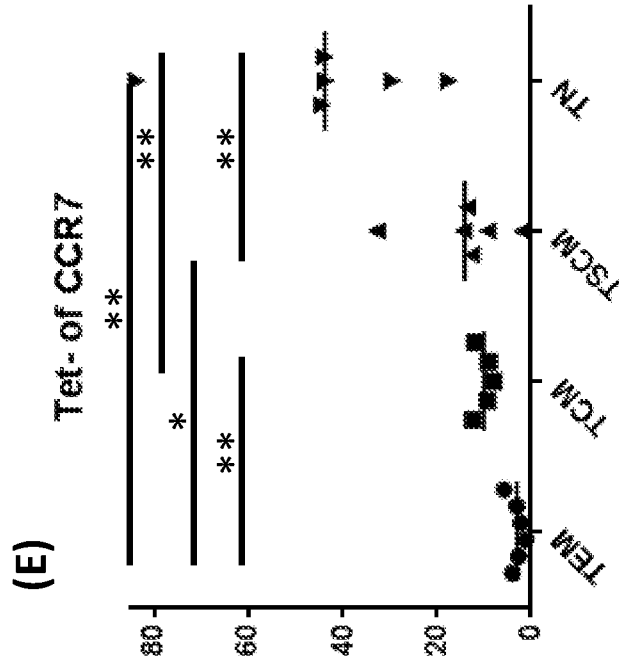
Day 30



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 Bold font: NLV-Tet⁺

Figure 2C

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Figures 2D – 2E

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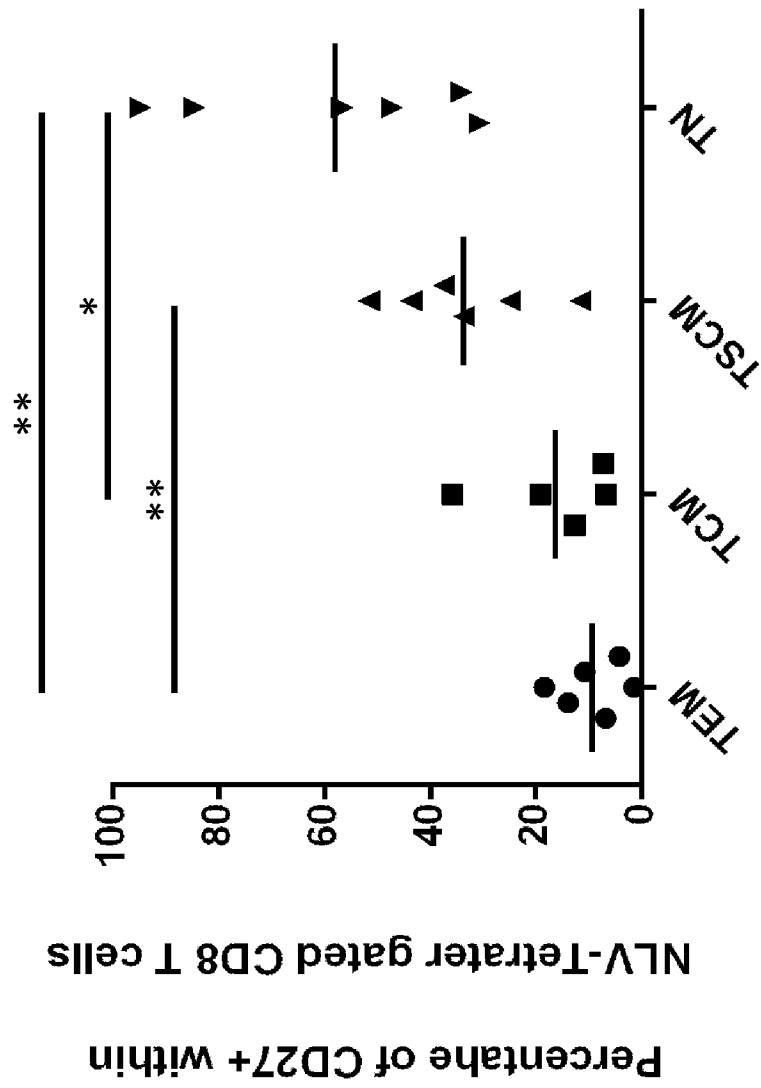
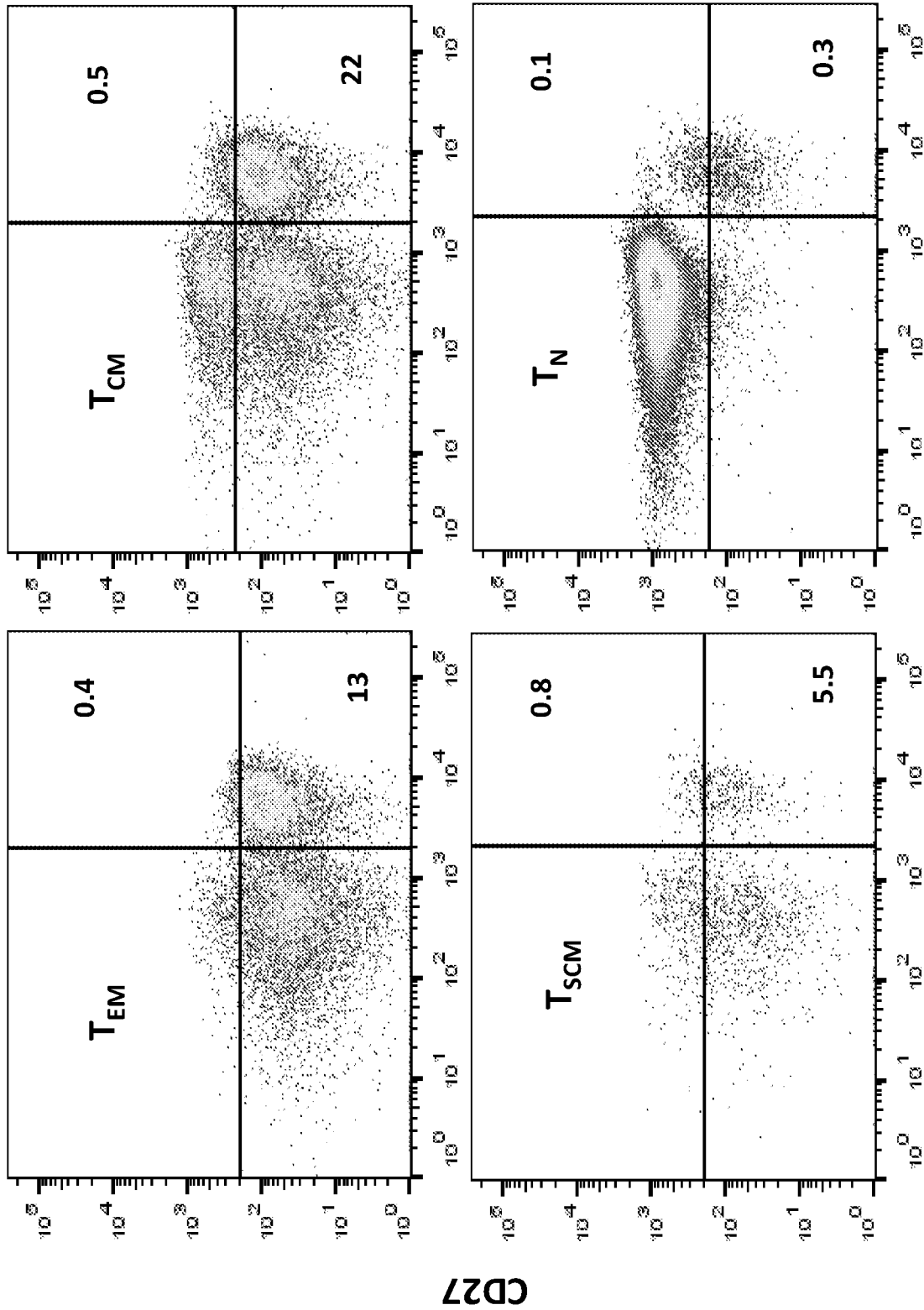


Figure 3A

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Tetramer
Figure 3B

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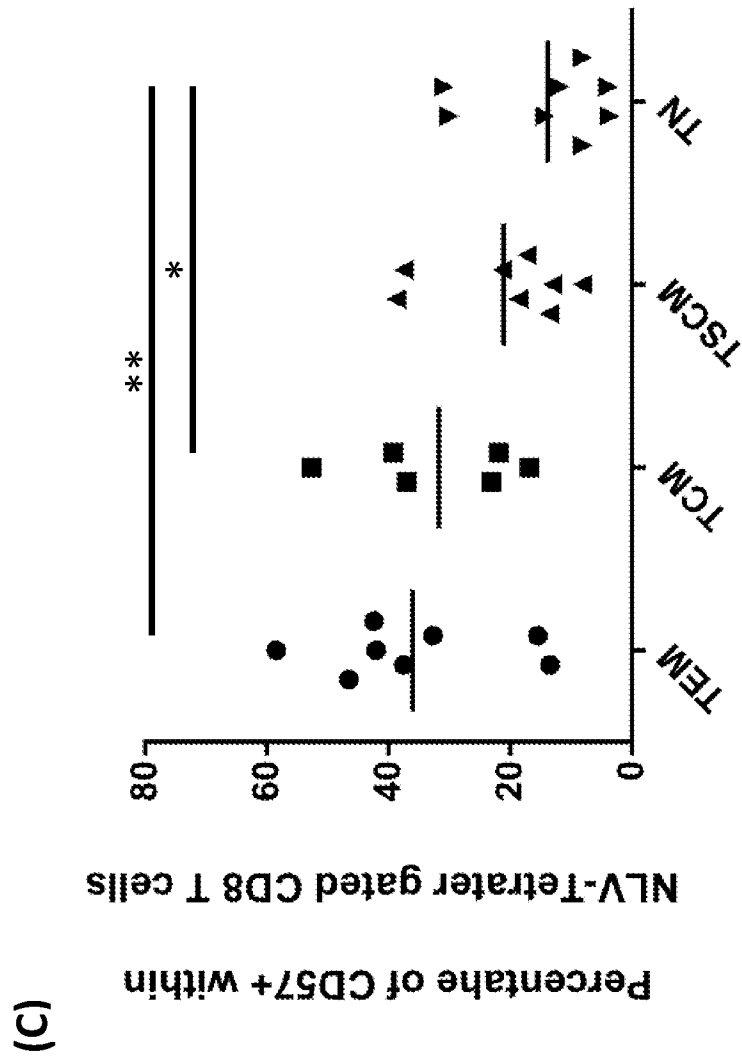
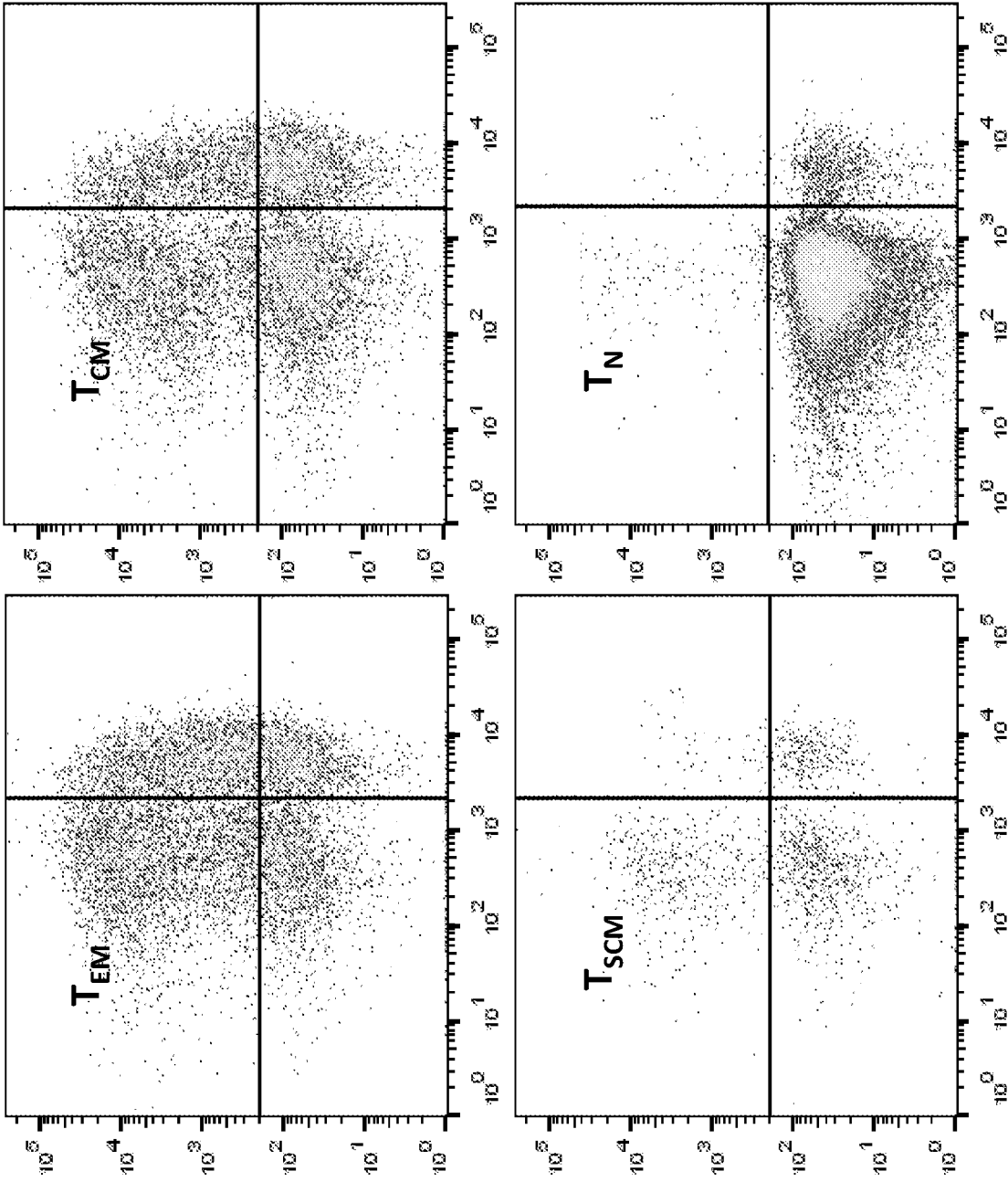


Figure 3C

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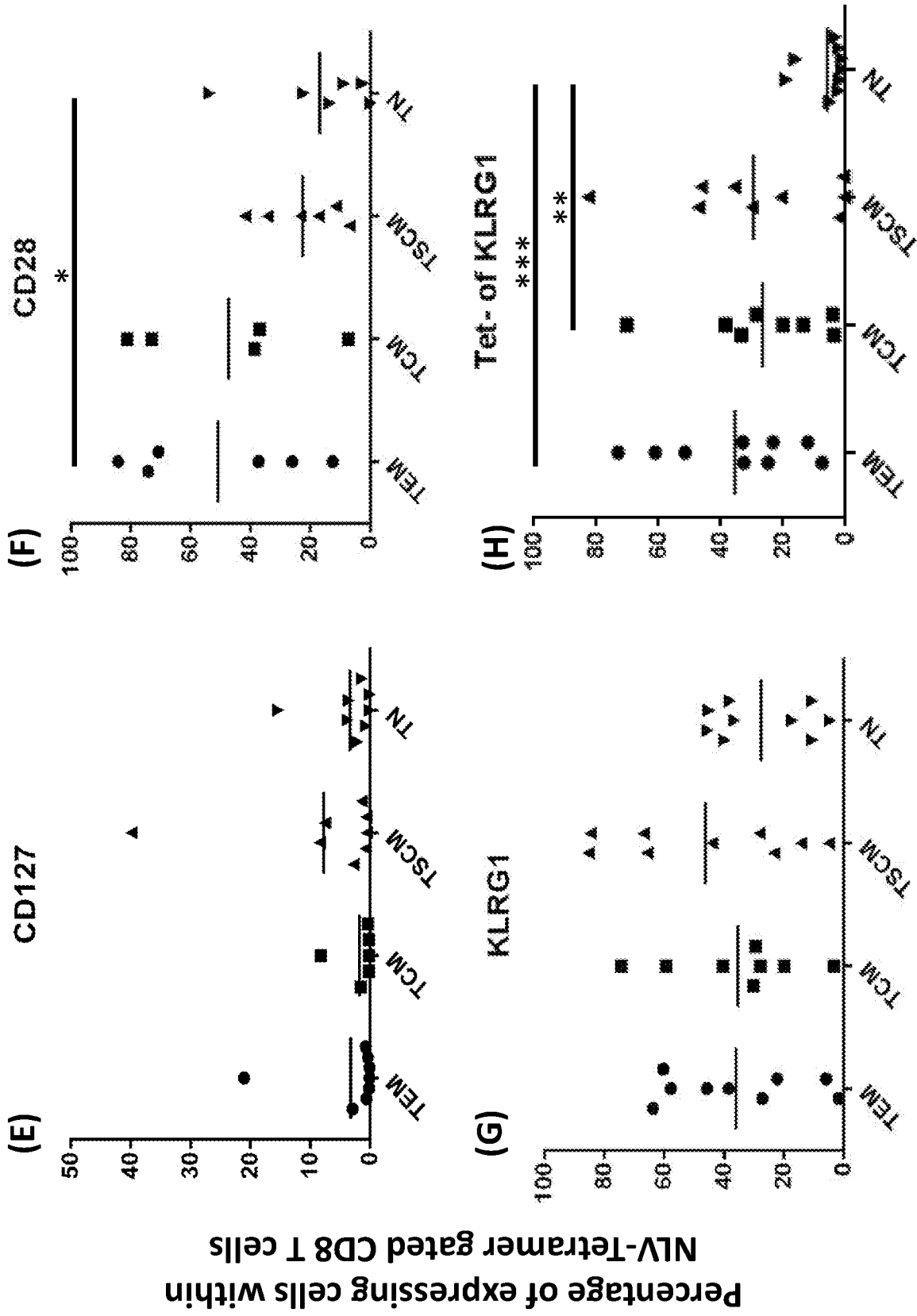


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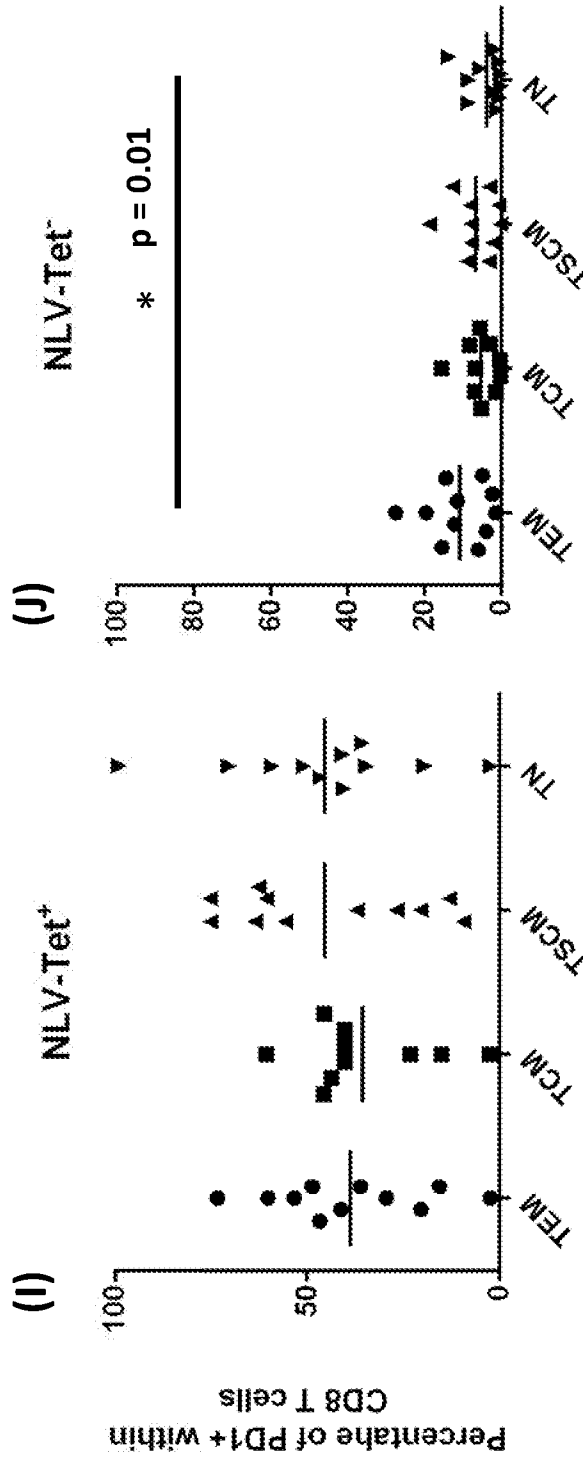
Tetramer
Figure 3D

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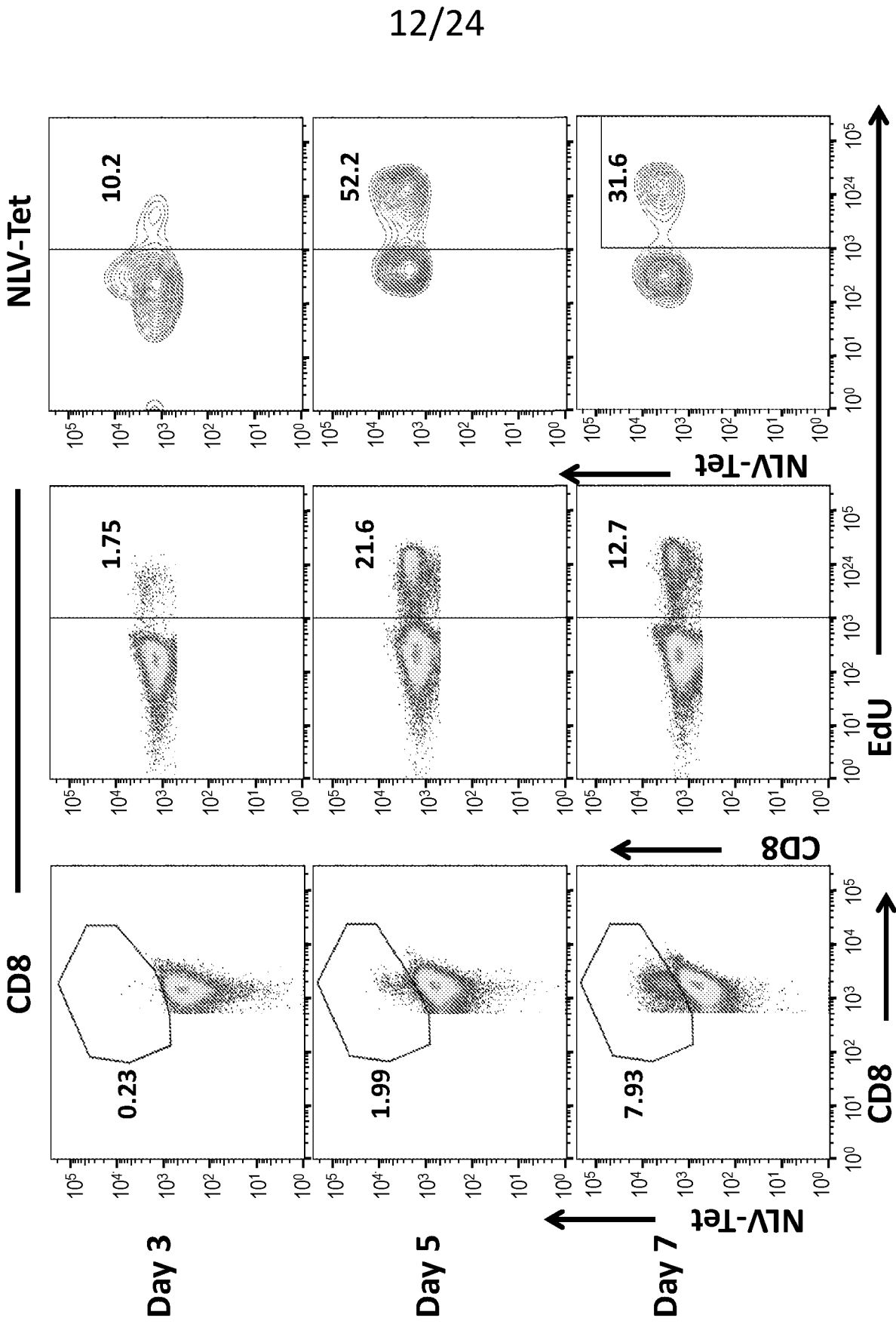


Figures 3E - 3H

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Figures 3I – 3J



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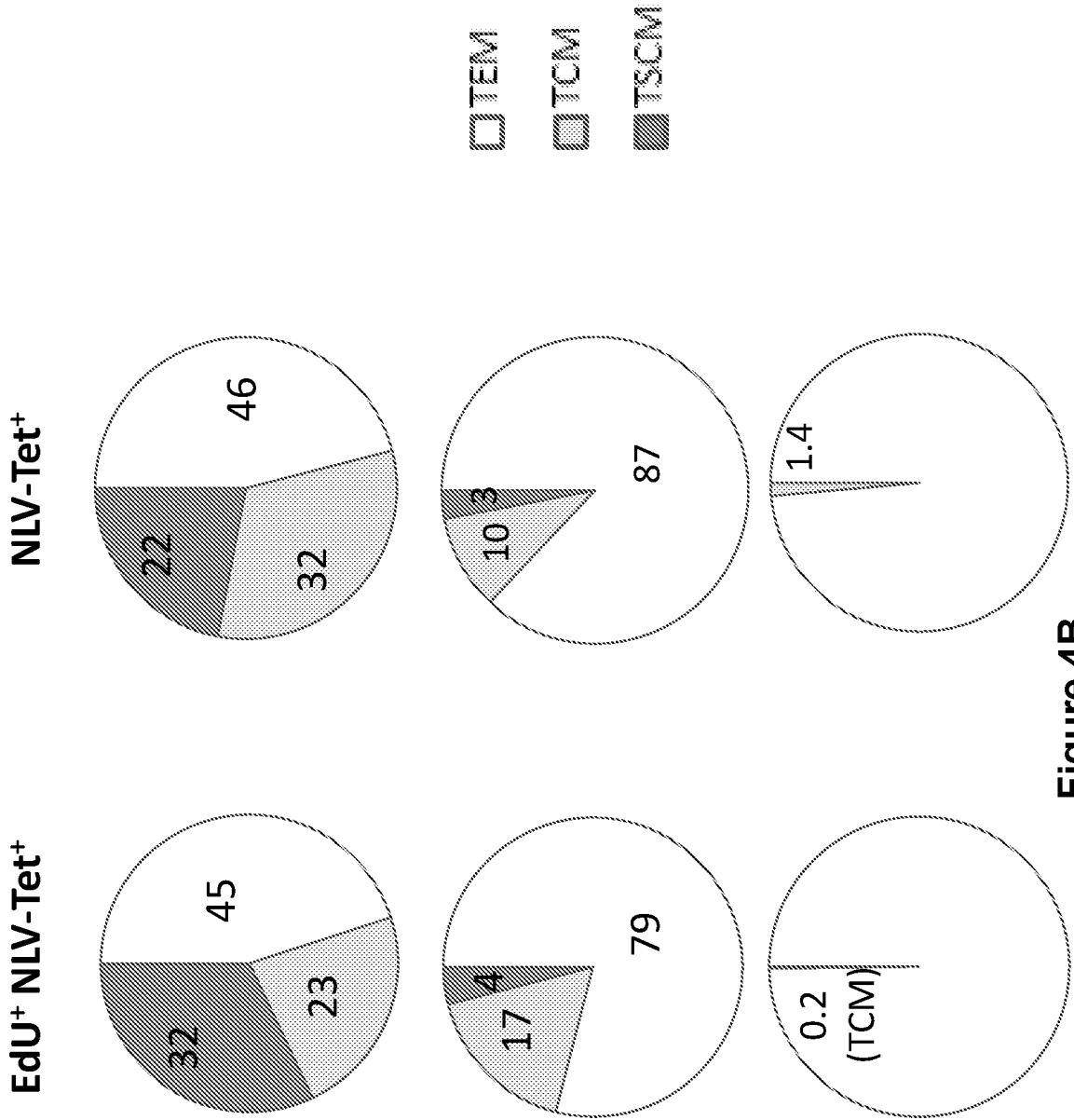


Figure 4B

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Antigen specific cells after 14 days expansion

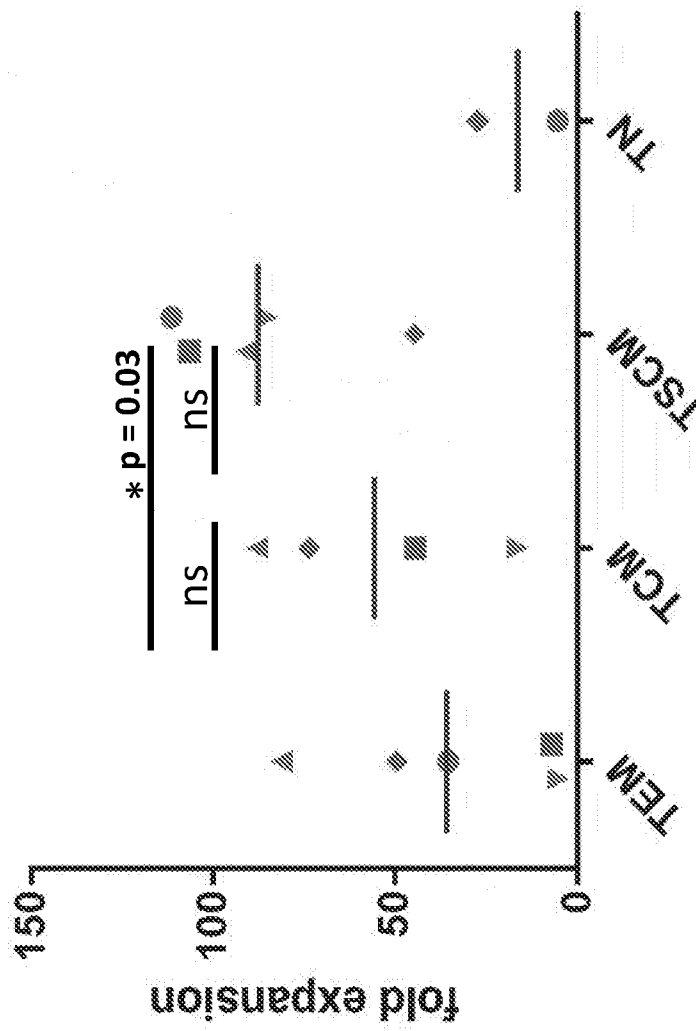


Figure 4C

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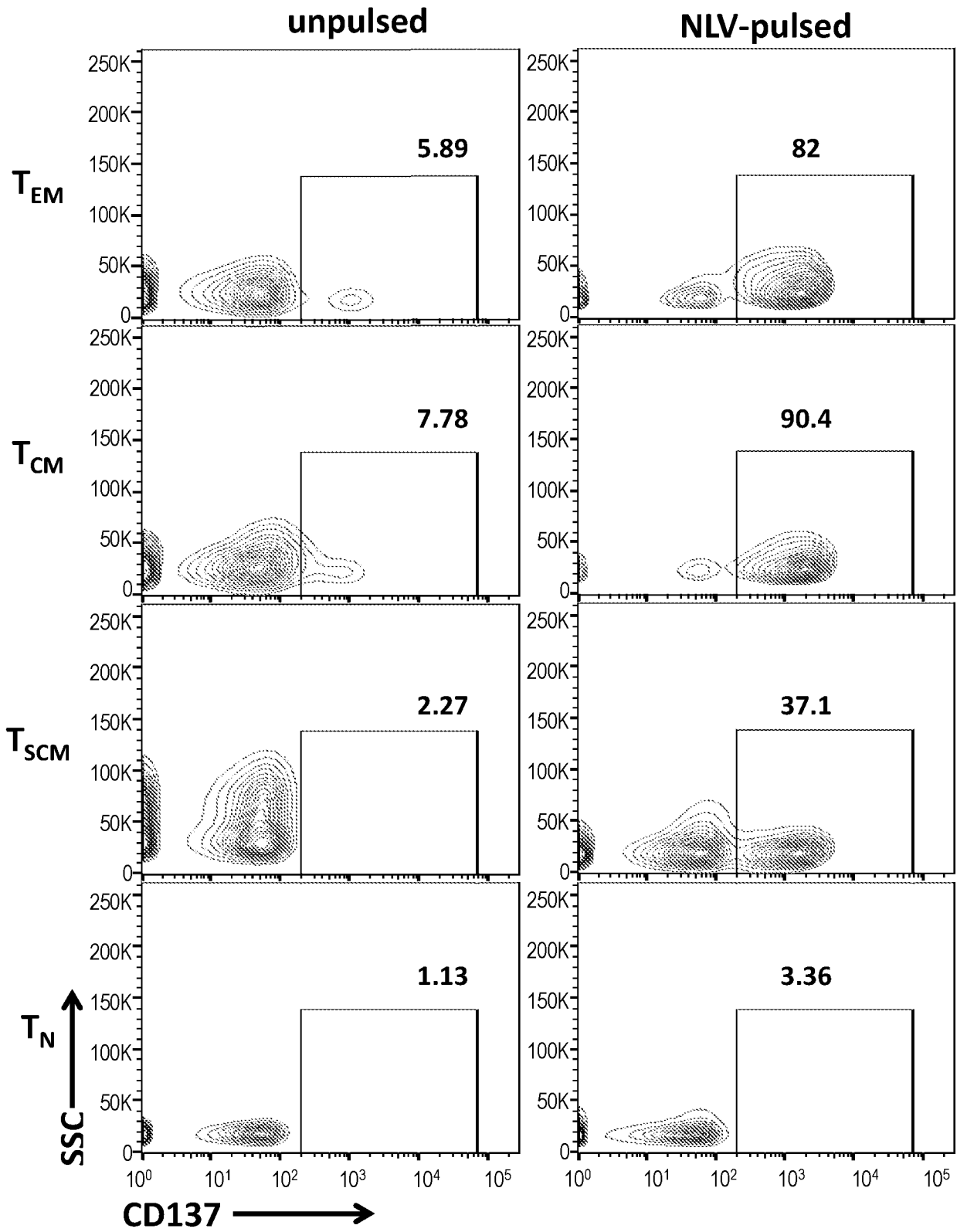


Figure 5A-1

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NLV-pulsed

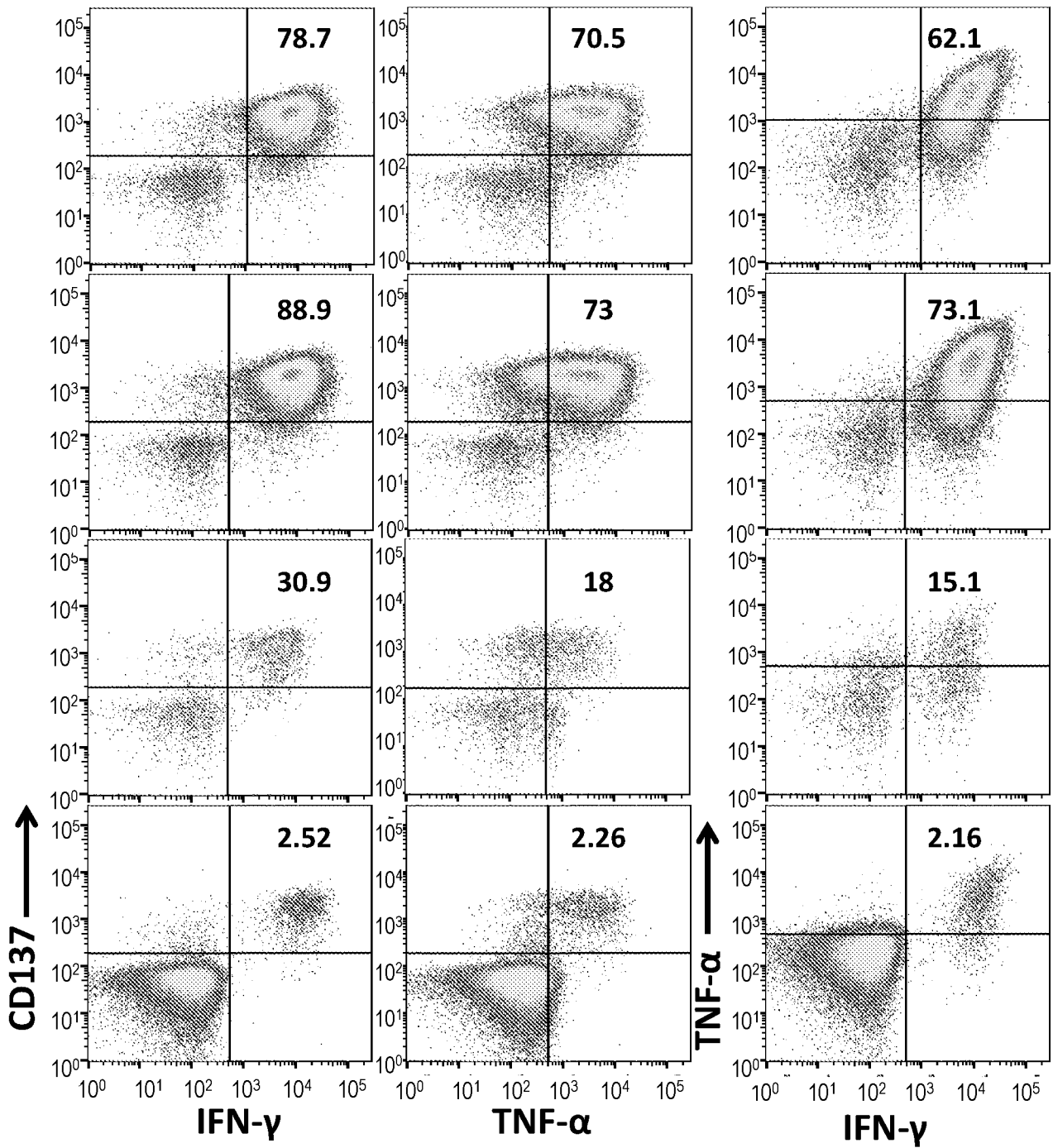


Figure 5A-2

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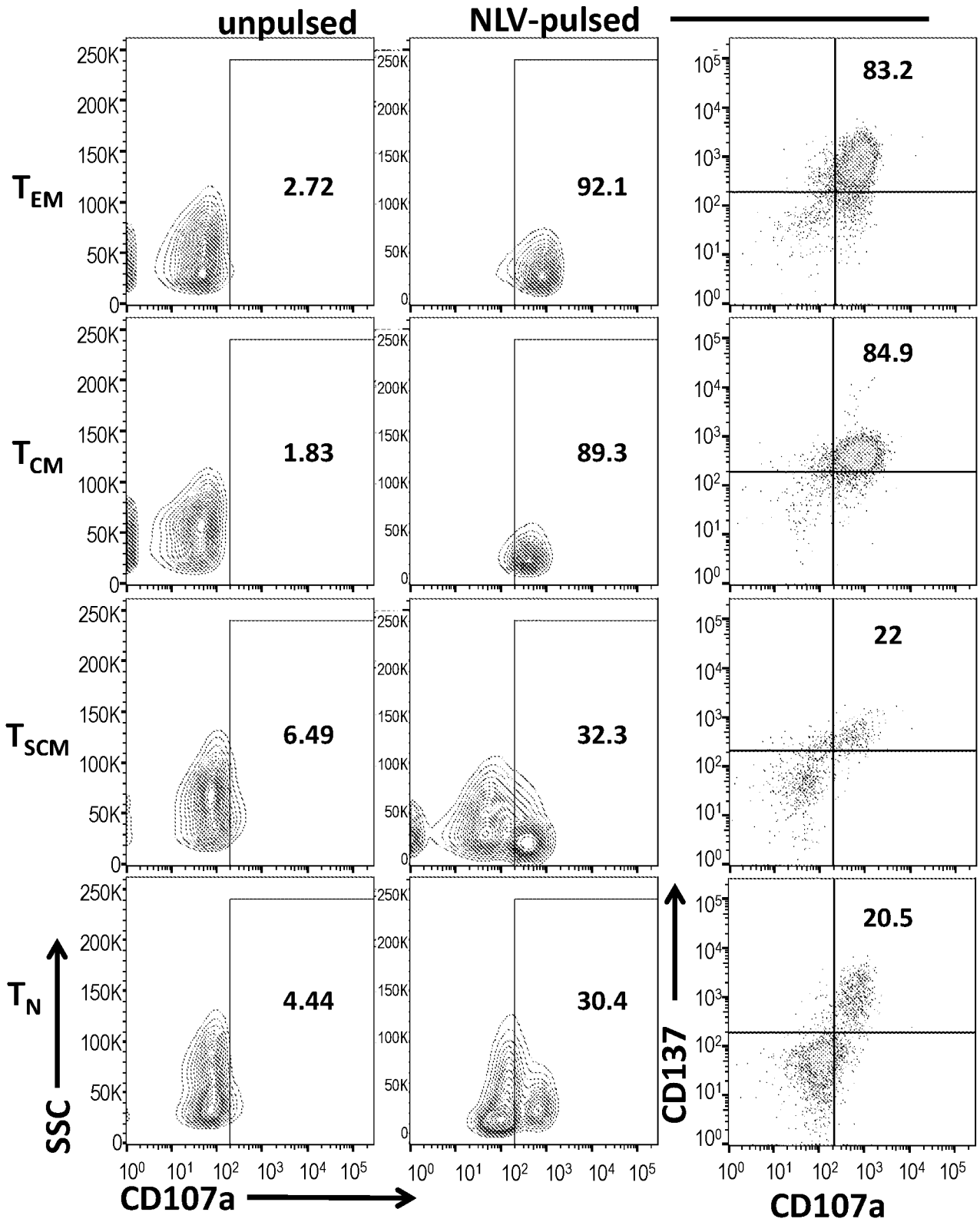


Figure 5B

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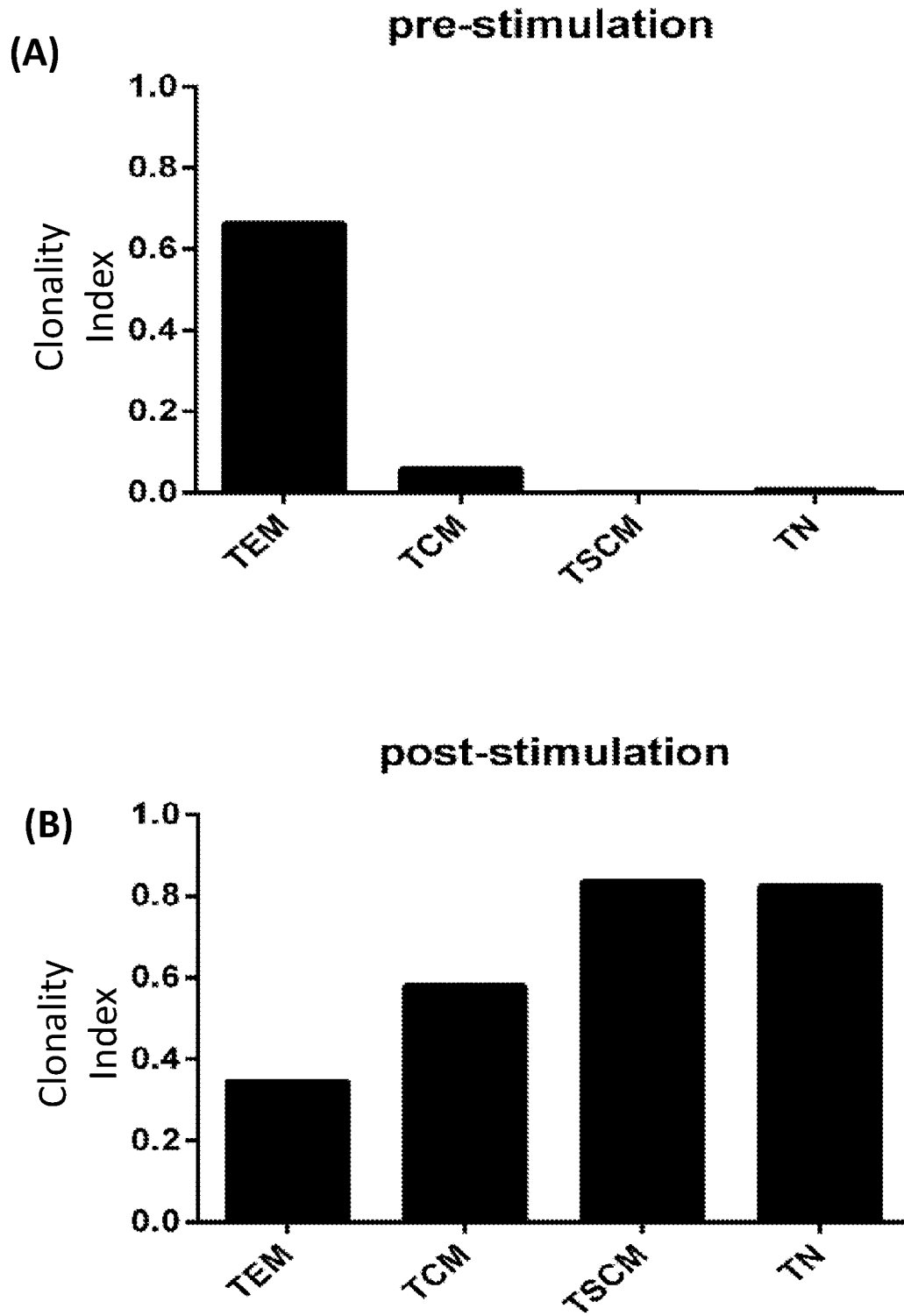
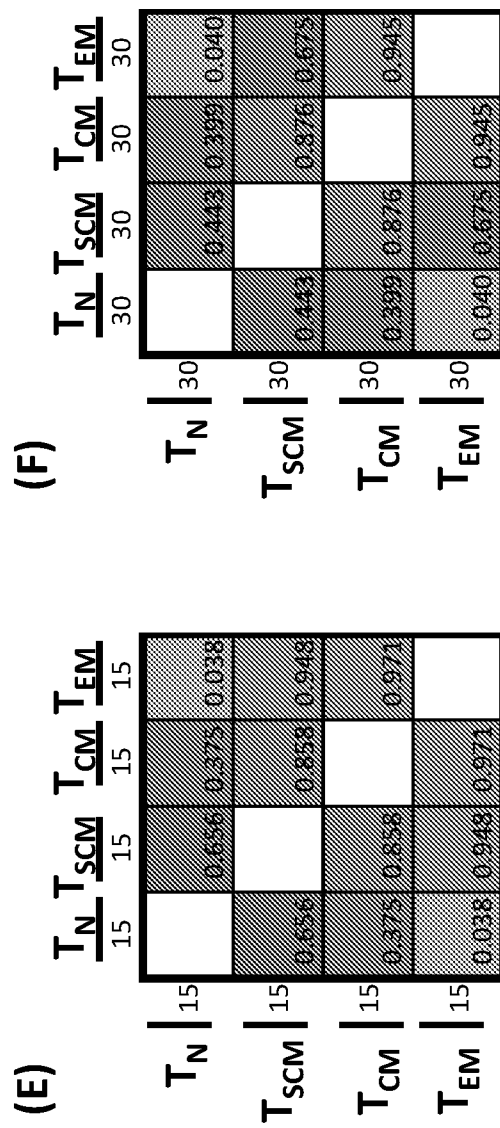
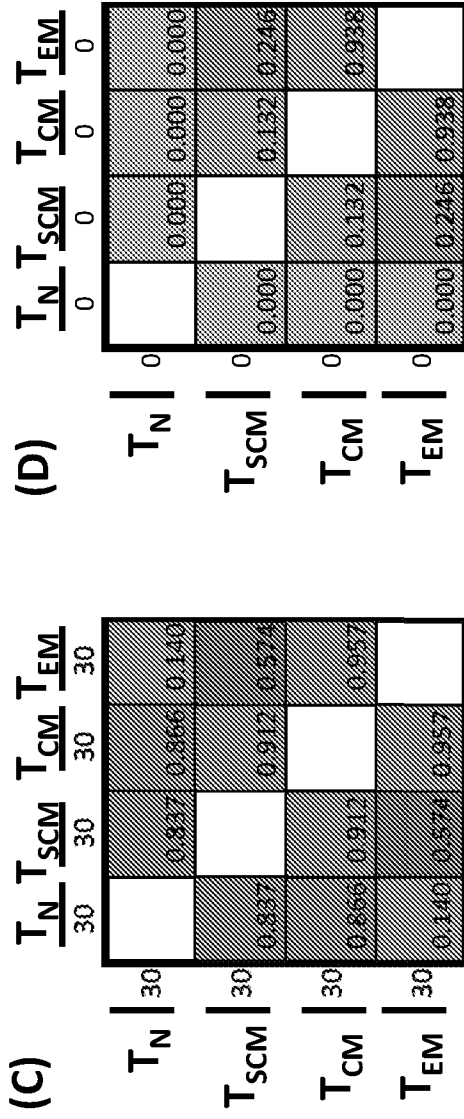


Figure 6A -6B



Figures 6C – 6F

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		T_N			T_{SCM}			T_{CM}			T_{EM}		
		0	15	30	0	15	30	0	15	30	0	15	30
T_N	0		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	15	0.000		0.955	0.003	0.656	0.289	0.008	0.375	0.257	0.207	0.038	0.009
	30	0.000	0.955		0.004	0.759	0.443	0.013	0.451	0.399	0.223	0.063	0.040
T_{SCM}	0	0.000	0.003	0.004		0.862	0.748	0.132	0.535	0.724	0.246	0.152	0.026
	15	0.000	0.656	0.759	0.862		0.990	0.876	0.858	0.957	0.897	0.948	0.892
	30	0.000	0.289	0.443	0.748	0.990		0.748	0.733	0.876	0.702	0.815	0.675
T_{CM}	0	0.000	0.008	0.013	0.132	0.876	0.748		0.661	0.753	0.938	0.403	0.110
	15	0.000	0.375	0.451	0.535	0.858	0.733	0.661		0.968	0.965	0.971	0.969
	30	0.000	0.257	0.399	0.724	0.957	0.876	0.753	0.968		0.797	0.940	0.945
T_{EM}	0	0.000	0.207	0.223	0.246	0.897	0.702	0.938	0.965	0.797		0.961	0.955
	15	0.000	0.038	0.063	0.152	0.948	0.815	0.403	0.971	0.940	0.961		0.934
	30	0.000	0.009	0.040	0.026	0.892	0.675	0.110	0.969	0.945	0.955	0.934	

Figure 6G

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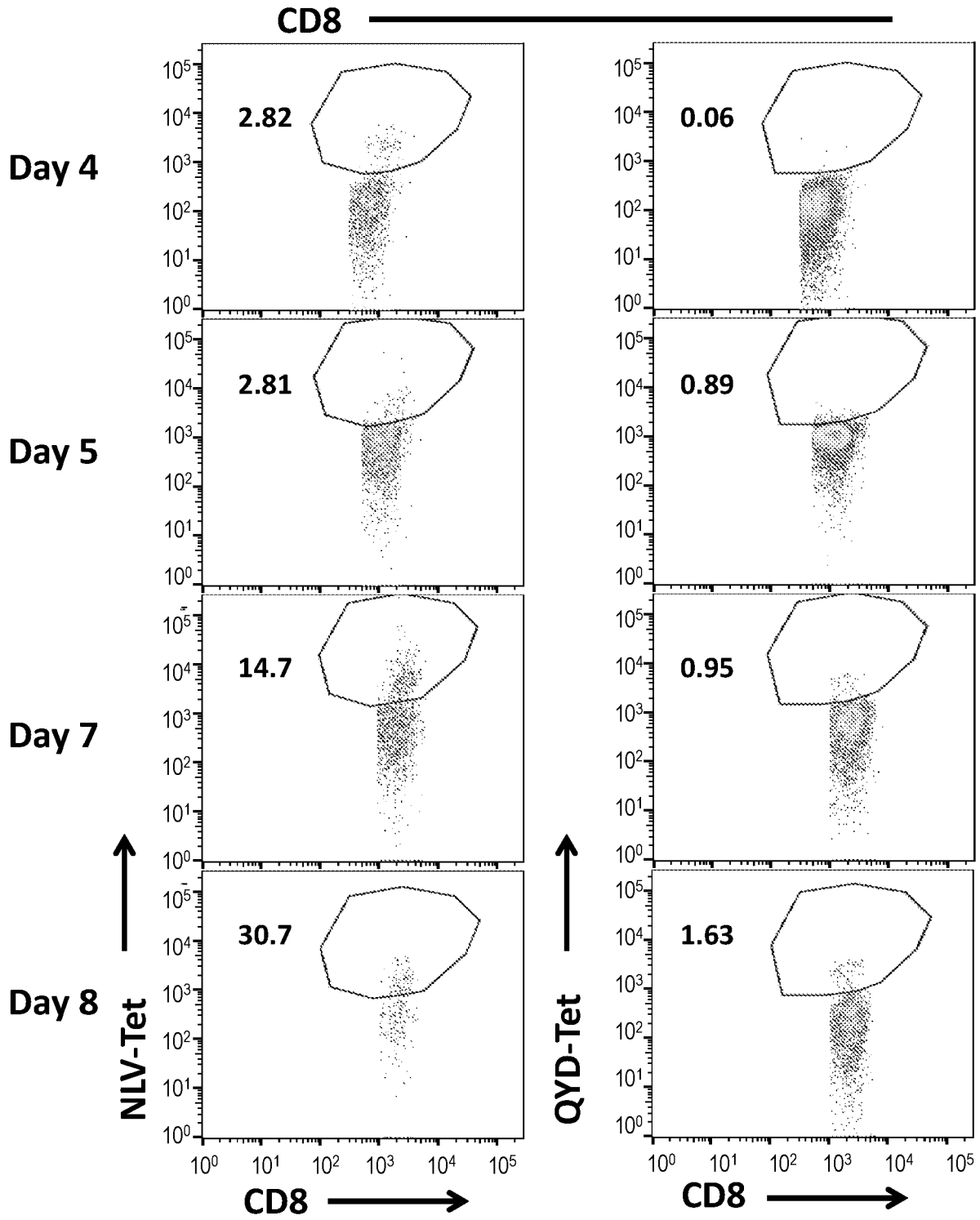


Figure 7A-1

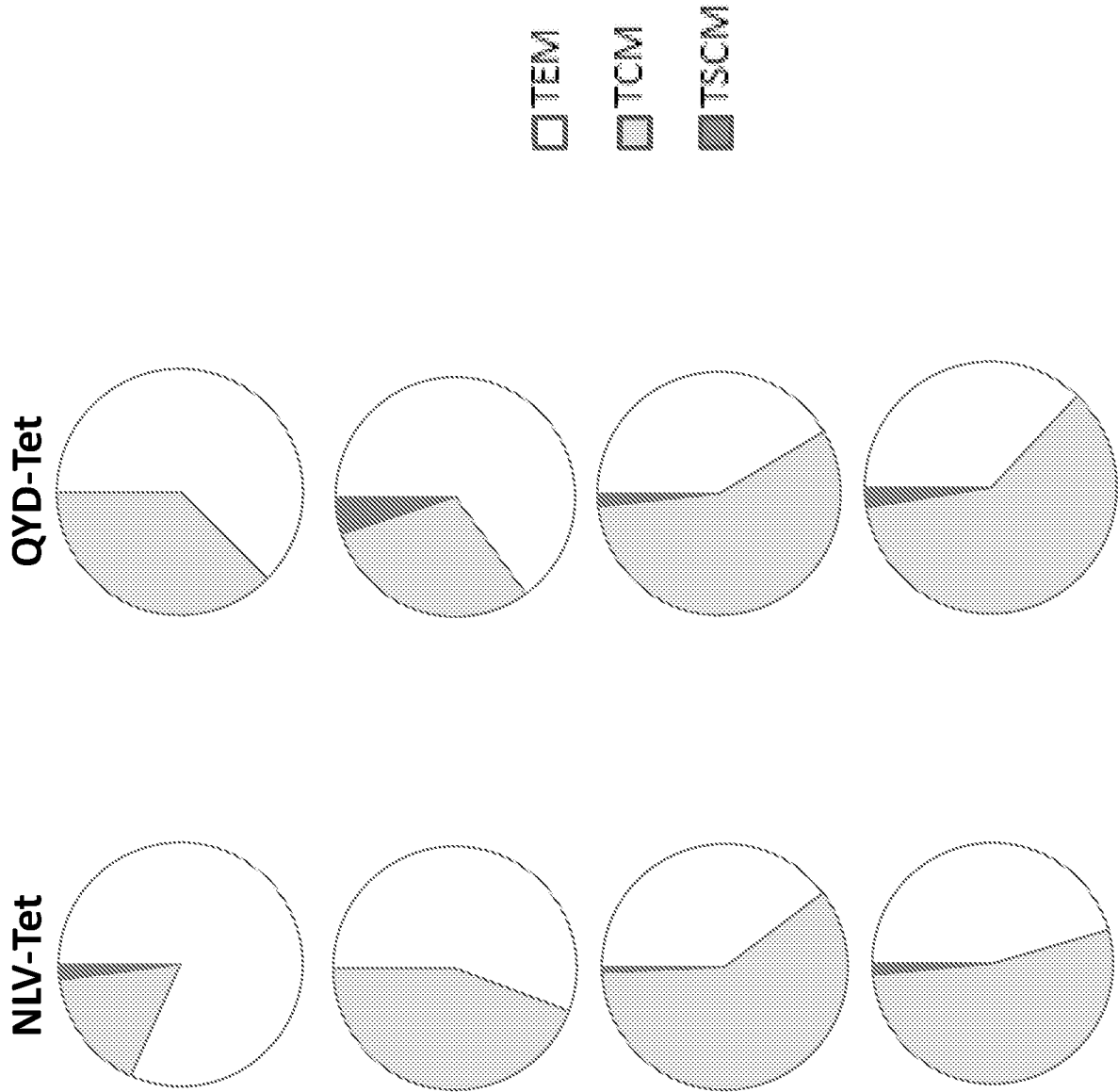


Figure 7A-2

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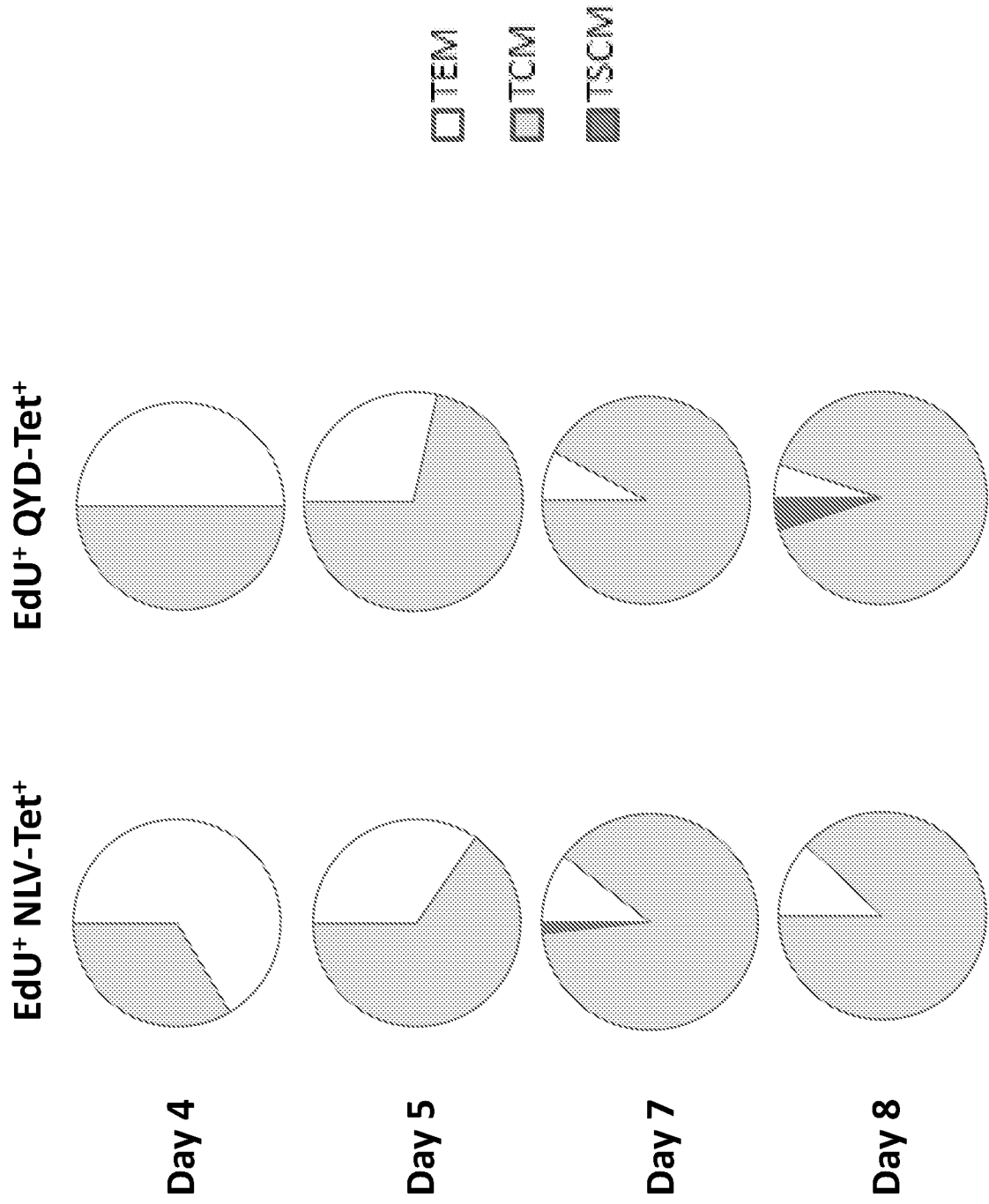


Figure 7B-1

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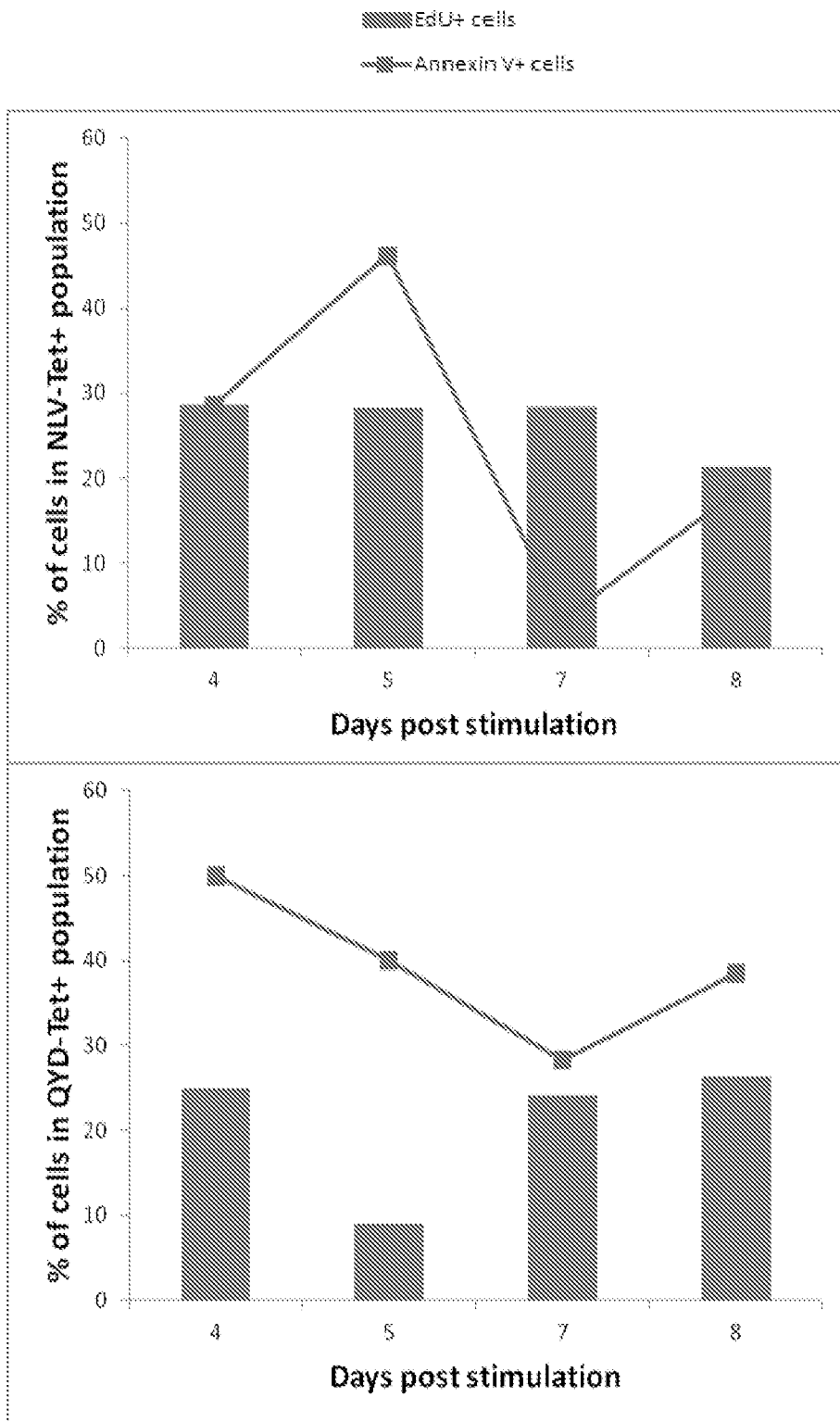


Figure 7B-2

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