(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 May 2016 (12.05.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/073595 A1

(51) International Patent Classification:
C12N 5/0784 (2010.01) *A61K 39/12* (2006.01)
A61K 38/16 (2006.01) *C07K 14/01* (2006.01)

(21) International Application Number:
PCT/US2015/059021

(22) International Filing Date:
4 November 2015 (04.11.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/075,726 5 November 2014 (05.11.2014) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2016/073595 A1

(54) Title: T CELLS AND DENDRITIC CELLS FOR POLYOMAVIRUS THERAPY

(57) Abstract: Disclosed herein are dendritic cells (DCs) expressing one or more polyomavirus antigens. In some examples, these modified DCs are transduced with a viral vector (such as a lenti viral vector) encoding the one or more polyomavirus antigens. The modified DCs are in some examples used to produce polyomavirus antigen-specific T cells. Also disclosed are methods of producing polyomavirus antigen-specific T cells by contacting mononuclear cells with a mixture of peptides from a polyomavirus antigen. The antigen-specific T cells or modified DCs may be administered to a subject to induce or enhance an immune response to polyomavirus in the subject, for example to treat or inhibit polyomavirus infection and/or polyomavirus-associated disease in the subject. Also disclosed herein are methods of making the disclosed modified DCs and polyomavirus antigen-specific T cells.

T CELLS AND DENDRITIC CELLS FOR POLYOMAVIRUS THERAPY

CROSS REFERENCE TO RELATED APPLICATIONS

This claims the benefit of U.S. Provisional Application No. 62/075,726, filed November 5, 2014, which is incorporated herein by reference in its entirety.

FIELD

This disclosure relates to the field of immunology, more specifically to methods and compositions for treating or inhibiting polyomavirus infection, and methods of making the compositions.

BACKGROUND

BK polyomavirus (BKV) persistently infects the urinary tract of nearly all healthy adults. Diseases caused by BKV and other human polyomaviruses are most common in immunocompromised patients and cause significant morbidity and possible mortality in solid organ and hematopoietic stem cell transplant recipients. There is a high seroprevalence of polyomaviruses in the general population, including BKV (90%; multiple serotypes), JC polyomavirus (JCV; 80%), and Merkel cell polyomavirus (MCV; 80%). Reactivation of these viruses in immunosuppressed individuals and transplant recipients can cause severe and sometimes fatal disease, such as polyomavirus-associated nephropathy (PVAN), hemorrhagic cystitis, progressive multifocal leukoencephalopathy (PML), and trichodysplasia spinulosa (TS). MCV is believed to play a causal role in the development of Merkel cell carcinoma (MCC), a rare form of skin cancer. BKV and JCV also potentially play a role in malignancies such as prostate cancer and colon cancer.

There is no specific therapy for polyomavirus infections. The treatment is largely supportive and involves reduction or interruption of immunosuppressive agents. Although pharmacological agents have been used for treatment of BKV disease, they have substantial toxicities and are not very effective. Thus, there is a need for additional safe and effective methods to treat or even prevent polyomavirus infections. Furthermore, given the prevalence of BKV, JCV, and MCV in the population, it would be advantageous to target all polyomaviruses or all BKV serotypes with a single polyomavirus-specific therapy.

SUMMARY

Disclosed herein are polyomavirus antigen-specific T cells and methods of treating or inhibiting polyomavirus infection and/or disease in a subject by administering the antigen-specific T cells to the subject. In some examples, the disclosed antigen-specific T cells induce or enhance an immune response to polyomavirus in a subject. Also disclosed herein are methods of producing polyomavirus antigen-specific T cells and methods of producing dendritic cells (DCs) that can be used in the production of the polyomavirus antigen-specific T cells.

In some embodiments, DCs expressing one or more polyomavirus antigens (in some examples, referred to herein as "modified DCs") are disclosed. In some examples, these modified DCs are transduced with a viral vector (such as a lentiviral vector) encoding the one or more polyomavirus antigens. The modified DCs are in some examples used to produce polyomavirus antigen-specific T cells. In some embodiments, the antigen-specific T cells or modified DCs are administered to a subject to induce or enhance an immune response to polyomavirus in the subject, for example to treat or inhibit polyomavirus infection and/or polyomavirus-associated disease in the subject.

In some embodiments, methods include making modified DCs (such as monocyte-derived DCs (MDDCs) or mature DCs) encoding and/or expressing one or more polyomavirus antigens and methods of making polyomavirus antigen-specific T cells. In some embodiments, the disclosed modified DCs are produced by transducing MDDCs with a viral vector encoding one or more polyomavirus LT antigens (such as one or more of MCV, BKV, and/or JCV LT antigen) or one or more polyomavirus VP1 proteins (such as one or more of MCV, BKV, and/or JCV VP1 protein). In other embodiments, the disclosed polyomavirus antigen-specific T cells (such as LT antigen-specific T cells, small T antigen-specific T cells, VP1 protein-specific T cells, or a combination thereof) are produced by exposing T lymphocytes (such as CD3+ lymphocytes) to the disclosed modified DCs, culturing the cells in the substantial absence of cytokines for a period of time (such as about 3 days), then culturing the cells in the presence of cytokines (such as IL-7 and IL-15) for a period of time (such as about 4 days). In some examples, the methods include a second round of contacting the T lymphocytes with the modified DCs (for example to enrich the population of antigen-specific T cells), culturing the cells in the substantial absence of cytokines for a period of time (such as about 3 days), then culturing the cells in the presence of cytokines (such as IL-2, IL-7 and IL-15) for a period of time (such as about 4 days).

In other embodiments, methods include making polyomavirus antigen-specific T cells. The disclosed methods include contacting monocyte-derived DCs or peripheral blood mononuclear cells (PBMCs) with a mixture of peptides (such as a library of overlapping peptides) from one or more

polyomavirus proteins for a period of time. Cells pulsed with the peptide mixture are co-cultured with PBMCs or lymphocytes for a period of time to induce formation of antigen-specific T cells. In some examples, the mixture of cells are cultured with IL-7 and IL-15 for three days. After three days, IL-2 is also added to the culture and the cells are cultured for an additional 7-14 days. In some embodiments, the peptide mixture is a set of overlapping peptides from all or a portion of a polyomavirus protein (such as LT, ST, or VP1).

In some examples, polyomavirus antigen-specific T cells produced by the methods described herein target multiple polyomaviruses and can be used for treating or inhibiting (or in some cases, even preventing) a broad range of polyomavirus infection and polyomavirus-associated disease. For example, MCV LT and/or ST antigen-specific T cells or BKV LT and/or ST antigen-specific T cells are cross-reactive (that is, recognize LT and/or ST antigen from other polyomaviruses), and can be used to treat or inhibit polyomavirus infection and/or disease generally. Also, BKV VP1 antigen-specific T cells are cross-reactive with multiple BKV subtypes, and can be used to treat or inhibit BKV infection and/or disease generally. BKV VP1 antigen-specific T cells are also cross-reactive with JCV VP1, and to a lesser extent MCV VP1, and can also be used to treat or inhibit polyomavirus infection and/or disease generally.

The foregoing and other features of the disclosure will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic diagram showing an exemplary lentivirus gene transfer vector construct for expression of polyomavirus target antigens.

FIG. 2 is a schematic diagram showing one exemplary method for preparing polyomavirus antigen-specific T cells using modified DCs.

FIGS. 3A and 3B are panels showing pooled data obtained from three donors on the frequency of BKV LT-specific effector T cells expressing TNF-α, IFN-γ, and IL-2, in response to BKV LT, MCV LT, or G250 transduced DC, or DCs pulsed with peptide libraries from BKV LT, JCV LT, WT1 antigen or control DC (gated on $CD3^+$ T cells; FIG. 3A) and representative FACS data from one donor (FIG. 3B).

FIGS. 4A and 4B are panels showing pooled data obtained from three donors on the frequency of MCV LT-specific viable $CD3^+$ effector T cells expressing TNF-α, IFN-γ, and IL-2 in response to BKV LT, MCV LT, or G250 transduced DC, or DCs pulsed with peptide libraries from BKV LT, JCV LT, WT1 antigen or control DC (gated on $CD3^+$ T cells; FIG. 4A) and representative FACS data from one donor (FIG. 4B).

FIGS. 5A and 5B are panels showing pooled data obtained from three donors on the frequency of the effector T cells specific to VP1 of the BKV serotype Ia, expressing TNF-α, IFN-γ, and IL-2 in response to DC transduced with BKV Ia VP1 or BKV IV VP1, MCV VP1, or G250 transduced DC, or DCs pulsed with peptide libraries from BKV VP, JCV VP1, or WT1 antigen or control DC (FIG. 5A) and representative FACS data from one donor (FIG. 5B).

FIGS. 6A and 6B are panels showing pooled data obtained from three donors on the frequency of the effector T cells specific to VP1 of the BKV serotype IV, expressing TNF-α, IFN-γ, and IL-2 cytokines in response to DC transduced with BKV Ia VP1 or BKV IV VP1, MCV VP1, or G250 transduced DC, or DCs pulsed with peptide libraries from BKV VP, JCV VP1, or WT1 antigen or control DC (FIG. 6A) and representative FACS data from one donor (FIG. 6B).

FIGS. 7A and 7B are panels showing pooled data obtained from three donors on the frequency of the MCV VP1-specific effector T cells expressing TNF-α, IFN-γ, and IL-2 cytokines in response to BKV Ia VP1 or BKV IV VP1, MCV VP1, or G250 transduced DC, or DCs pulsed with peptide libraries from BKV VP1, JCV VP1, or WT1 antigen or control DC (FIG. 7A) and representative FACS data from one donor, (FIG. 7B).

FIGS. 8A and 8B are graphs showing cross-reactivity of T cells generated against LT (FIG. 8A) or VP1 (FIG. 8B).

FIG. 9 is a series of panels showing the frequency of BKV- or MCV LT-specific viable $CD3^+$ effector T cells expressing TNF-α, IFN-γ, and IL-2 in response to BKV LT transduced DC before or after sorting based on 4-1BB expression.

FIG. 10 is a schematic diagram showing an exemplary method for generating antigen-specific T cells using stimulation of monocyte-derived DCs with a peptide library (pepmix). Antigen-specific T cells can also be obtained with the method utilizing lymphocytes in place of monocyte-derived DCs.

FIGS. 11A and 11B are a series of panels showing reactivity of T cells generated with a BKV LT peptide library (FIG. 10A) or a BKV VP1 peptide library (FIG. 10B) against polyomavirus antigens.

FIG. 12 is a series of panels showing cross-reactivity of T cells generated with BKV LT or BKV VP1 peptide libraries against BKV or JCV antigens.

FIG. 13 is a schematic diagram of an exemplary GMP protocol for producing polyomavirus antigen-specific T cells by stimulation of PBMCs with a peptide library.

FIG. 14 shows the amino acid sequence of a truncated MCC LT antigen including MCC LT Exon 1 (underlined sequence) and LT Exon 2 (top; SEQ ID NO: 37) and an MCC ST antigen including MCC LT exon 1 (underlined sequence) and ST exon 1 (bottom; SEQ ID NO: 38).

FIG. 15 is a schematic diagram of an exemplary protocol for adoptive immunotherapy of PML in a subject with administering polyomavirus antigen-specific T cells produced from a donor.

FIG. 16 is a schematic diagram of an exemplary protocol for adoptive immunotherapy for Merkel cell carcinoma in a subject by administering autologous polyomavirus antigen-specific T cells.

SEQUENCE LISTING

Any nucleic acid and amino acid sequences listed herein or in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases and amino acids, as defined in 37 C.F.R. § 1.822. In at least some cases, only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand.

SEQ ID NO: 1 is the nucleic acid sequence of an exemplary BKV LT coding sequence that incorporates all known variations that occur in at least two isolates, FIN-2 (genotype IV, GenBank Accession No. AB269822) and CAP-h2 (genotype Ib-1, GenBank Accession No. AY628226) into a single protein and is codon-modified.

SEQ ID NO: 2 is the amino acid sequence of an exemplary BKV LT protein, encoded by SEQ ID NO: 1.

SEQ ID NO: 3 is the nucleic acid sequence of an exemplary codon-modified BKV VP1 genotype Ia coding sequence.

SEQ ID NO: 4 is the amino acid sequence of an exemplary BKV VP1 Ia protein, encoded by SEQ ID NO: 3.

SEQ ID NO: 5 is the nucleic acid sequence of an exemplary codon-modified BKV VP1 genotype IV coding sequence.

SEQ ID NO: 6 is the amino acid sequence of an exemplary BKV VP1 IV protein, encoded by SEQ ID NO: 5.

SEQ ID NO: 7 is the nucleic acid sequence of an exemplary codon-modified MCV LT coding sequence, where the splicing signals for the "57kT" isoform have been silently destroyed.

SEQ ID NO: 8 is the amino acid sequence of an exemplary MCV LT protein, encoded by SEQ ID NO: 7.

SEQ ID NO: 9 is the nucleic acid sequence of an exemplary codon-modified MCV VP1 consensus coding sequence represented by isolate MCC339 and several others.

SEQ ID NO: 10 is the amino acid sequence of an exemplary MCV VP1 protein, encoded by SEQ ID NO: 9.

SEQ ID NO: 11 is the nucleic acid sequence of an exemplary G250 human carbonic anhydrase IX coding sequence.

SEQ ID NO: 12 is the amino acid sequence of an exemplary G250 protein, encoded by SEQ ID NO: 11.

SEQ ID NO: 13 is the nucleic acid sequence of an exemplary JCV LT coding sequence.

SEQ ID NO: 14 is the amino acid sequence of an exemplary JCV LT protein, encoded by SEQ ID NO: 13.

SEQ ID NO: 15 is the nucleic acid sequence of an exemplary JCV VP1 coding sequence.

SEQ ID NO: 16 is the amino acid sequence of an exemplary JCV VP1 protein, encoded by SEQ ID NO: 15.

SEQ ID NO: 17 is the amino acid sequence of an exemplary HIV-1-based lentiviral gene transfer vector (including eGFP coding sequence; nucleotides 2348-3067).

SEQ ID NO: 18 is the amino acid sequence of an Hsc70 domain of polyomavirus LT antigen (HDPKGG).

SEQ ID NO: 19 is the consensus amino acid sequence of a Bub1 domain of polyomavirus LT antigen (WXXWW).

SEQ ID NO: 20 is the consensus amino acid sequence of an Rb domain of polyomavirus LT antigen (LXCXE).

SEQ ID NOs: 21 and 22 are consensus amino acid sequences of zinc binding domains of polyomavirus LT antigen (CXXC and HXXH, respectively).

SEQ ID NO: 23 is the consensus amino acid sequence of a Walker A box domain of polyomavirus LT antigen (G/AXXXXGKT/S).

SEQ ID NO: 24 is the amino acid sequence of an Fbw7 domain of polyomavirus LT antigen (TPPP).

SEQ ID NOs: 25-30 are representative amino acid sequences of Hsc70 domain of polyomavirus LT antigens.

SEQ ID NOs: 31-36 are representative amino acid sequences of Rb domain of polyomavirus LT antigens.

SEQ ID NO: 37 is the amino acid sequence of a truncated MCC LT antigen including MCC LT Exon 1 (amino acids 1-79) and LT Exon 2 (truncated, amino acids 80-216).

SEQ ID NO: 38 is the amino acid sequence of an MCC ST antigen including MCC LT exon 1 (amino acids 1-79) and ST exon 1 (amino acids 80-186).

DETAILED DESCRIPTION

I. Terms and Abbreviations

BKV	BK polyomavirus
CTL	cytotoxic T lymphocyte
DC	dendritic cell
IFN	interferon
IL	interleukin
JCV	JC polyomavirus
LPS	lipopolysaccharide
LT	large T antigen
MCV	Merkel cell polyomavirus
MCC	Merkel cell carcinoma
MDDC	monocyte-derived dendritic cells
PBMC	peripheral blood mononuclear cells
PML	progressive multifocal leukoencephalopathy
PVAN	polyomavirus-associated nephropathy
RCL	replication-competent lentivirus
ST	small T antigen
TNF	tumor necrosis factor
TS	trichodysplasia spinulosa
VP1	viral protein 1

and as otherwise set forth throughout the specification, claims, and abstract.

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in Krebs *et al., Lewin's Genes XI*, published by Jones and Bartlett Learning , 2012 (ISBN 1449659853); Kendrew *et al.* (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Publishers, 1994 (ISBN 0632021829); Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by Wiley, John & Sons, Inc., 2011 (ISBN 8126531789); and George P. Rédei, *Encyclopedic Dictionary of Genetics, Genomics, and Proteomics,* 2nd Edition, 2003 (ISBN: 0-471-26821-6).

The following explanations of terms and methods are provided to better describe the present disclosure and to guide those of ordinary skill in the art to practice the present disclosure. The singular forms "a," "an," and "the" refer to one or more than one, unless the context clearly dictates

otherwise. For example, the term "comprising a cell" includes single or plural cells and is considered equivalent to the phrase "comprising at least one cell." As used herein, "comprises" means "includes." Thus, "comprising A or B," means "including A, B, or A and B," without excluding additional elements. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety for all purposes. In case of conflict, the present specification, including explanations of terms, will control.

Although methods and materials similar or equivalent to those described herein can be used to practice or test the disclosed technology, suitable methods and materials are described below. The materials, methods, and examples are illustrative only and not intended to be limiting.

To facilitate review of the various embodiments of this disclosure, the following explanations of specific terms are provided:

Antigen: A molecule that stimulates an immune response. Antigens are usually proteins or polysaccharides or fragments thereof. An epitope is an antigenic determinant. These are particular chemical groups or peptide sequences on a molecule that are antigenic, such that they elicit a specific immune response.

Antigen-presenting cell (APC): Specialized cells that present peptide antigens to T cells via their HLA class I and II molecules. Examples are monocytes, B-lymphocytes and dendritic cells (see below).

Antigen-specific T cell: A CD8+ or CD4+ lymphocyte that recognizes a particular antigen, such as a target antigen. Generally, antigen-specific T cells specifically bind to a particular antigen, but not other antigens. A **target antigen-specific T cell** specifically binds to a particular target antigen, such as such as a polyomavirus antigen, for example LT or VP1. In some examples, antigen-specific T cells are able to react with the same antigen from more than one polyomavirus, such as LT antigen from two or more polyomaviruses (for example, two or more of MCV LT, BKV LT, and JCV LT). In other examples, antigen-specific T cells are able to react with the same antigen from more than one subtype of a polyomavirus (for example, two of more BKV VP1 I, BKV VP1 II, BKV VP1 III, and BKV VP1 IV). In particular examples, antigen-specific T cells that react with the same antigen from more than one polyomavirus or the same antigen from more than one subtype of a polyomavirus are referred to herein as "cross-reactive" T cells.

BK polyomavirus (BKV): A polyomavirus originally isolated from patient B.K. after renal transplantation (Gardner *et al., Lancet* 1:1253-1257, 1971). At least four BKV subtypes are known (subtypes I-IV; *e.g.,* Knowles *et al., J. Med. Virol.* 28:118-123, 1989). BKV is nearly ubiquitous, and up to 90% of healthy individuals are seropositive for BKV. Acute infection is generally asymptomatic and proceeds to latent infection, primarily in the urogenital tract. BKV can

be reactivated in immunocompromised individuals, and can cause significant morbidity, particularly in renal transplant patients.

BKV nucleic acid and amino acid sequences are publicly available. For example, GenBank Accession Nos. V01108, AB211374, AB263920, AB211386, and AB369093 disclose exemplary BKV-I, BKV-II, BKV-III, and BKV-IV nucleic acid sequences, respectively, all of which are incorporated by reference as present in GenBank on October 27, 2014.

Capsid polypeptide: One of three structural proteins that forms the polyomavirus capsid. The polyomavirus capsid is formed from viral protein 1 (VP1), viral protein 2 (VP2), and viral protein 3 (VP3), which form 72 pentameric capsomers. Typically each virion has about 360 copies of VP1 (~5 per capsomer) and 30-60 copies each of VP2 and VP3 (~1 per pentomer).

Cytokine: Proteins made by cells that affect the behavior of other cells, such as lymphocytes. In one embodiment, a cytokine is a chemokine, a molecule that affects cellular trafficking. The term "cytokine" is used as a generic name for a diverse group of soluble proteins and peptides that act as humoral regulators at nanomolar to picomolar concentrations and which, either under normal or pathological conditions, modulate the functional activities of individual cells and tissues. These proteins also mediate interactions between cells directly and regulate processes taking place in the extracellular environment. Examples of cytokines include, but are not limited to, tumor necrosis factor α (TNF-α), interleukin-2 (IL-2), interleukin-7 (IL-7), interleukin-15 (IL-15), and interferon-γ (IFN-γ).

Dendritic cell (DC): Dendritic cells are the principal antigen presenting cells (APCs) involved in primary immune responses. DCs include plasmacytoid dendritic cells and myeloid dendritic cells. Immature DCs originate in the bone marrow and reside in the periphery as immature cells. In the case of injury or infection, the immature DCs capture antigens, which are processed by endosomal or proteosomal pathways for presentation on the cell surface. Antigens processed by the proteosomal pathway bind to major histocompatibility complex (MHC) class I molecules for cell surface presentation to stimulate CD8+ cytotoxic T cells (CTLs). Antigens processed by the endosomal pathway bind to MHC class II molecules for presentation on the cell surface and stimulation of CD4+ helper T cells. DCs also express costimulatory molecules on their cell surface, such as members of the B7 family, TNF family, and intracellular adhesion molecules which participate in activation of T cells.

Immune response: A response of a cell of the immune system, such as a B cell, T cell, macrophage or polymorphonucleocyte, to a stimulus such as an antigen. An immune response can include any cell of the body involved in a host defense response for example, an epithelial cell that

secretes an interferon or a cytokine. An immune response includes, but is not limited to, an innate immune response or inflammation.

Immunocompromised: An immunocompromised subject is a subject who is incapable of developing or unlikely to develop a robust immune response, usually as a result of disease, malnutrition, or immunosuppressive therapy. An immunocompromised immune system is an immune system that is functioning below normal. Immunocompromised subjects are more susceptible to opportunistic infections, for example viral, fungal, protozoan, or bacterial infections, prion diseases, and certain neoplasms.

Subjects who are considered to be immunocompromised include, but are not limited to, subjects with AIDS (or HIV positive), subjects with severe combined immunodeficiency (SCID), diabetics, subjects who have had transplants and who are taking immunosuppressants, and those who are receiving chemotherapy for cancer. Immunocompromised individuals also include subjects with most forms of cancer (other than skin cancer), sickle cell anemia, cystic fibrosis, those who do not have a spleen, subjects with end stage kidney disease (for example, those on dialysis), and those who have been taking corticosteroids or other immune suppressing therapy on a frequent basis within the last year.

Inhibiting or treating a disease: "Inhibiting" a disease refers to inhibiting the full development of a disease, for example, polyomavirus-associated diseases (discussed below). Inhibition of a disease can span the spectrum from partial inhibition to substantially complete inhibition (*e.g.,* including, but not limited to prevention) of the disease. In some examples, the term "inhibiting" refers to reducing or delaying the onset or progression of a disease. "Treatment" refers to a therapeutic intervention that ameliorates a sign or symptom of a disease or pathological condition after it has begun to develop. A subject to be administered a therapeutically effective amount of the disclosed antigen-specific T cells or modified DCs can be identified by standard diagnosing techniques for such a disorder, for example, presence of the disease or disorder or risk factors to develop the disease or disorder.

Isolated: An "isolated" or "purified" biological component (such as a cell, nucleic acid, peptide, protein, protein complex, or virus-like particle) has been substantially separated, produced apart from, or purified away from other components (for example, other biological components in the cell or the organism in which the component naturally occurs). Cells, nucleic acids, peptides and proteins that have been "isolated" or "purified" thus include cells, nucleic acids, and proteins purified by standard purification methods.

The term "isolated" or "purified" does not require absolute purity; rather, it is intended as a relative term. Thus, for example, an isolated biological component is one in which the biological

component is more enriched than the biological component is in its natural environment within a cell, or other production vessel (for example, a cell culture system). Preferably, a preparation is purified such that the biological component represents at least 50%, such as at least 70%, at least 90%, at least 95%, or greater, of the total biological component content of the preparation.

JC polyomavirus (JCV): A polyomavirus originally isolated from a patient (J.C.) with progressive multifocal leukoencephalopathy (Padgett *et al., Lancet* 1:1257-1260, 1971). JCV is genetically similar to BKV and simian virus 40 (SV40). JCV is very common in the general population, with 70-90% of individuals seropositive for JCV. The initial site of infection may be the tonsils or gastrointestinal tract. The primary sites of JC infection are thought to be tubular epithelial cells in the kidney, the lining of the ureters and bladder, and oligodendrocytes and astrocytes in the central nervous system.

JCV nucleic acid and amino acid sequences are publicly available. For example, GenBank Accession Nos. NC_001699, J02226, AB038251, AF015526, and AF281600 disclose exemplary JCV nucleic acid sequences, all of which are incorporated by reference as present in GenBank on October 27, 2014. JCV isolates have been classified into at least eight distinct genotypes, based in part on the amino acid sequences of VP1 proteins of individual isolates (Cubitt *et al.*, *J. Neurovirol.* 7:339-344, 2001).

Large T antigen (LT): Also known as large tumor antigen. LT is a protein that participates in viral replication and is responsible for viral oncogenic activity. LT is highly conserved across the polyomavirus family. MCV includes an additional LT isoform, referred to as 57k or 57kT.

Exemplary BKV LT polypeptide amino acid sequences are publicly available and include GenBank Accession Nos. YP_717940, AB269822, AY628226, and ABC18006, all of which are incorporated herein by reference as present in GenBank on October 27, 2014. Exemplary MCV LT polypeptide amino acid sequences are publicly available and include GenBank Accession Nos. YP_001651046 and AFC36067, both of which are incorporated herein by reference as present in GenBank on October 27, 2014. Exemplary JCV LT polypeptide amino acid sequences are also publicly available and include GenBank Accession Nos. AAC59326, AAC59332, and AAC59338, all of which are incorporated by reference as present in GenBank on October 27, 2014. Exemplary LT nucleic acid and amino acid sequences also include SEQ ID NOs: 1, 2, 7, 8, 13, and 14 disclosed herein. Additional LT amino acid and nucleic acid sequences are publicly available and can be identified by one of ordinary skill in the art.

Lymphocyte: A type of white blood cell involved in the immune defenses of the body. There are two main types of lymphocytes: B cells and T cells.

Merkel cell polyomavirus (MCV): A polyomavirus originally isolated from patients having Merkel cell carcinoma, a rare form of skin cancer (Feng *et al., Science* 319:1096-1100, 2008). MCV is common in the general population with about 50-80% of individuals being seropositive, and with prevalence increasing with age. Merkel cell carcinoma is primarily seen in individuals age 65 years and older and is associated with ultraviolet exposure. There is also a strong association between immunosuppression and Merkel cell carcinoma.

MCV nucleic acid and amino acid sequences are publicly available. For example, GenBank Accession Nos. EU375803, EU375804, JF813003, and JN383841 disclose exemplary MCV nucleic acid sequences, all of which are incorporated by reference as present in GenBank on October 27, 2014.

Monocyte: A large white blood cell in the blood that ingests microbes or other cells and foreign particles and proteins. When a monocyte passes out of the bloodstream and enters tissues, it develops into a macrophage.

Pharmaceutically acceptable carrier: The pharmaceutically acceptable carriers (vehicles) useful in this disclosure are conventional. *Remington: The Science and Practice of Pharmacy*, The University of the Sciences in Philadelphia, Editor, Lippincott, Williams, & Wilkins, Philadelphia, PA, 21st Edition (2005), describes compositions and formulations suitable for pharmaceutical delivery of one or more therapeutic compositions, such as one or more polyomavirus antigen-specific T cells, modified DCs, and/or additional pharmaceutical agents.

In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (for example, powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically-neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

Polyomavirus: A genus of nonenveloped viruses having an icosahedral capsid. The genome of polyomaviruses includes non-structural proteins (large T antigen and small t antigen), a non-coding region including an origin of replication and promoters, and structural proteins (VP1, VP2, and VP3). Polyomaviruses include but are not limited to BK polyomavirus, JC polyomavirus, Merkel cell polyomavirus, and simian virus 40 (SV40). Related human polyomaviruses WU virus

(Gaynor *et al., PLoS Pathog.* 3:e64, 2007), KI virus (Allander *et al., J. Virol.* 81:4130-4136, 2007), and trichodysplasia spinulosa-associated polyomavirus (van der Meijden *et al., PLoS Pathog.* 6:E1001024, 2010) have recently been reported in clinical samples

Polyomavirus infection is generally asymptomatic in healthy subjects. However, polyomavirus infection can occur or be reactivated in immunocompromised individuals and can cause significant morbidity, for example, due to polyomavirus-associated disease. **Polyomavirus-associated nephropathy** (PVAN; also called BK polyomavirus-associated nephropathy or BK virus nephritis) occurs in up to 10% of renal transplant recipients and is believed to be caused by BKV infection or reactivation of latent BKV infection. It causes kidney allograft dysfunction and may lead to loss of the allograft. **Polyomavirus-associated hemorrhagic cystitis** is characterized by inflammation of the bladder leading to dysuria, hematuria, and hemorrhage. It can occur in bone marrow transplant recipients and other individuals who are receiving immunosuppressants or other therapies which decrease immune system function. **Trichodysplasia spinulosa** (TS) is a skin condition characterized by development of papules, spines, and alopecia in the face. It occurs in immunocompromised patients and has recently been found to be associated with the presence of TS-associated polyomavirus (TSV). JCV can reactivate in immunocompromised individuals and can cause **JCV-associated progressive multifocal leukoencephalopathy (PML)**, which is usually fatal. PML occurs in about 10% of patients suffering from HIV-induced AIDS and can also occur in other immunocompromised or immunosuppressed patients, including but not limited to patients treated with rituximab, natalizumab, alemtuzumab, or efalizumab. JCV can also cause urinary tract pathology in some organ transplant recipients. Another polyomavirus-associated disease is Merkel cell carcinoma (discussed above).

Subject: Living multi-cellular vertebrate organisms, a category that includes both human and non-human mammals (such as mice, rats, rabbits, sheep, horses, cows, and non-human primates).

T cell: A white blood cell critical to the immune response. T cells include, but are not limited to, $CD4^+$ T cells and $CD8^+$ T cells. A $CD4^+$ T cell is an immune cell that carries a marker on its surface known as "cluster of differentiation 4" (CD4). These cells, also known as helper T cells, help orchestrate the immune response, including antibody responses as well as killer T cell responses. $CD8^+$ T cells carry the "cluster of differentiation 8" (CD8) marker. In one embodiment, a $CD8^+$ T cell is a cytotoxic T lymphocyte (CTL). In another embodiment, a $CD8^+$ cell is a suppressor T cell.

Therapeutically effective amount: A quantity of a specified agent sufficient to achieve a desired effect in a subject being treated with that agent. In some examples, a therapeutically

effective amount of the polyomavirus antigen-specific T cells and/or modified DCs disclosed herein is an amount sufficient to increase an immune response to one or more polyomaviruses in a subject by at least 10% (such as at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more). In other examples, a therapeutically effective amount is an amount of polyomavirus antigen-specific T cells or modified DCs sufficient to reduce or ameliorate one or more symptoms of a polyomavirus-related disease, such as PVAN, PML, hemorrhagic cystitis, TS, or MCC. In still further example, a therapeutically effective amount is an amount of polyomavirus antigen-specific T cells or modified DCs sufficient to reduce the incidence or severity of a polyomavirus-related disease in a subject infected with one or more polyomaviruses. The therapeutically effective amount (for example an amount increasing resistance to, preventing, ameliorating, and/or treating infection in a subject) will be dependent on, for example, the subject being treated, the manner of administration of the therapeutic composition, and other factors.

Transduced: A virus or vector "transduces" a cell when it transfers nucleic acid into the cell. In some examples a nucleic acid transduced into the cell becomes stably replicated by the cell, either by incorporation of the nucleic acid into the cellular genome, or by episomal replication. As used herein, the term transduction encompasses all techniques by which a nucleic acid molecule is introduced into such a cell, including introduction of plasmid vectors, viral vectors, or naked DNA by electroporation, lipofection, and particle gun acceleration.

VP1 polypeptide: One of three capsid proteins that make up the outer protein coat of viruses. VP1 is the major capsid protein of polyomaviruses, such as BKV, MCV, and JCV. The VP1 polypeptide interacts with a target cell to provide virion attachment to the target cell.

Exemplary BKV VP1 polypeptide amino acid sequences are publicly available and include GenBank Accession Nos. CAA40239, AEK21505, and BAG84476 all of which are incorporated herein by reference as present in GenBank on October 27, 2014. Exemplary MCV VP1 polypeptide amino acid sequences are publicly available and include GenBank Accession Nos.YP_001651048, ABY65893, and AFC36093, all of which are incorporated herein by reference as present in GenBank on October 27, 2014. Exemplary JCV VP1 polypeptide amino acid sequences are publicly available and include GenBank Accession Nos. AAC59325, AAK97910, and AAG34667, all of which are incorporated herein by reference as present in GenBank on October 27, 2014. Exemplary VP1 polypeptides also include SEQ ID NOs: 3-6, 9, and 10 disclosed herein. Additional VP1 polypeptide amino acid and nucleic acid sequences are publicly available and can be identified by one of ordinary skill in the art.

II. Modified Dendritic Cells and Polyomavirus Antigen-Specific T Cells

Disclosed herein are modified DCs that express one or more polyomavirus antigens, such as a polyomavirus VP1 or LT polypeptide or a fragment thereof. Also disclosed herein are T cells (such as CTLs or helper T cells) that are reactive to the same antigen (such as VP1 or LT) from one or more polyomaviruses. In some examples, the T cells are reactive against an antigen from two or more different polyomaviruses (such as an LT from two or more different polyomaviruses) or against an antigen from two or more different polyomavirus subtypes (such as a VP1 from two or more different BKV subtypes).

The LT antigen of polyomaviruses includes domains that are highly conserved among polyomaviruses (Table 1). Thus, in some embodiments, the disclosed T cells are reactive to LT from multiple (such as two or more) different polyomaviruses (for example, the T cells are cross-reactive with LT from two or more different polyomaviruses). In some examples, the T cells are reactive with LT from two or more of MCV, BKV, and JCV. Exemplary LT amino acid sequences include SEQ ID NO: 2 (BKV LT), SEQ ID NO: 8 (MCV LT), and SEQ ID NO: 14 (JCV LT) disclosed herein. One of ordinary skill in the art can identify additional LT amino acid sequences, including BKV, MCV, and JCV LT amino acid sequences.

Table 1. Large T antigen domains conserved among polyomaviruses

Virus	**J domain and linker**			**Helicase**		**HR**
	Hsc70	**Bub1**	**Rb**	**Zn binding**	**Walker A box**	**Fbw7**
	HDPKGG (SEQ ID NO: 18)	WXXWW (SEQ ID NO: 19)	LXCXE (SEQ ID NO: 20)	CXXC, HXXH (SEQ ID NOs: 21, 22)	G/AXXXXGKT/S (SEQ ID NO: 23)	TPPP (SEQ ID NO: 24)
SV40	+	WEQWW (SEQ ID NO: 25)	LFCSE (SEQ ID NO: 31)	+	+	+
JCV	+	WESWW (SEQ ID NO:26)	LFCHE (SEQ ID NO: 32)	+	+	+

Virus	J domain and linker			Helicase		HR
	Hsc70	**Bub1**	**Rb**	**Zn binding**	**Walker A box**	**Fbw7**
	HDPKGG (SEQ ID NO: 18)	WXXWW (SEQ ID NO: 19)	LXCXE (SEQ ID NO: 20)	CXXC, HXXH (SEQ ID NOs: 21, 22)	G/AXXXXGKT/S (SEQ ID NO: 23)	TPPP (SEQ ID NO: 24)
BKV	+	WESWW (SEQ ID NO: 26)	LFCHE (SEQ ID NO: 32)	+	+	+
WUPyV	+	WDYWW (SEQ ID NO: 27)	LRCNE (SEQ ID NO: 33)	+	+	-
KIPyV	+	SDEWW (SEQ ID NO: 28)	LRCNE (SEQ ID NO: 33)	+	+	-
MCV	+	-	LECDE (SEQ ID NO: 34)	+	+	-
HPyV6	+	WEQWW (SEQ ID NO: 25)	LYCDE (SEQ ID NO: 35)	+	+	-
HPyV7	+	WDQWW (SEQ ID NO: 29)	LYCTE (SEQ ID NO: 36)	+	+	-
TSV	+	WASWW (SEQ ID NO: 30)	LFCHE (SEQ ID NO: 32)	+	+	-

+, sequence present in the indicated protein; -, sequence missing from the indicated protein

VP1 has low to moderate similarity across polyomaviruses. However, VP1 antigens are conserved among subtypes of BKV polyomavirus and among subtypes of JCV polyomavirus. Thus, in some embodiments, the disclosed T cells are reactive to VP1 from multiple (such as two or

more) different subtypes of BKV, for example, the T cells are cross-reactive with VP1 from two or more of BKV subtype I (such as BKV subtype Ia, subtype Ib, or subtype Ic), BKV subtype II, BKV subtype III, and/or BKV subtype IV (such as subtype IVa, subtype IVb, or subtype IVc). In other embodiments, the disclosed T cells are reactive to VP1 from multiple (such as two or more) different subtypes of JCV, for example, the T cells are cross-reactive with VP1 from two or more of JCV subtype 1 (such as JCV subtype 1A), JCV subtype 2 (such as JCV subtype 2A), and/or JCV subtype 3 (such as JCV subtype 3B). In further embodiments, the T cells are cross-reactive with VP1 from two or more different polyomaviruses, such as two or more of BKV VP1, JCV VP1, and MCC VP1.

Exemplary BKV VP1 amino acid sequences include SEQ ID NO: 4 (BKV VP1 Ia) and SEQ ID NO: 6 (BKV VP1 IVc) disclosed herein. Exemplary MCV VP1 amino acid sequences include SEQ ID NO: 10 disclosed herein. Exemplary JCV VP1 amino acid sequences include SEQ ID NO: 16 (JCV VP1 1A) disclosed herein. One of ordinary skill in the art can identify additional VP1 amino acid sequences, including BKV, MCV, and JCV VP1 amino acid sequences.

A. Modified DCs

Disclosed herein are modified DCs that express one or more polyomavirus antigens or a fragment thereof, such as a polyomavirus LT antigen (for example, a BKV, MCV, or JCV LT antigen or portion thereof), a polyomavirus ST antigen (such as a BKV, MCV, or JCV ST antigen, or portion thereof), or a polyomavirus VP1 protein (for example, a BKV, MCV, or JCV VP1 protein), such as the proteins (or fragments thereof) discussed above. In particular embodiments, the DCs are human DCs. In some examples, the DCs been transfected or transduced with a construct including a nucleic acid encoding one or more polyomavirus proteins or fragments thereof. Thus, the DCs disclosed herein, referred to as "modified DCs" are recombinant DCs that include at least one heterologous nucleic acid and/or express at least one heterologous protein or fragment thereof (for example, derived from a species or organism other than the DC itself).

In some embodiments, the modified DCs are DCs (such as human DCs) that have been transduced with a viral vector or construct (such as a lentiviral construct) that includes a nucleic acid encoding at least one polyomavirus antigen or a portion thereof. In some examples, the vector includes a nucleic acid encoding one or more full-length polyomavirus proteins. In some examples, the modified DCs are transduced with a viral construct including a nucleic acid encoding a BKV LT antigen (for example, SEQ ID NO: 1 or a nucleic acid with at least 90%, 95%, 98%, 99%, or more sequence identity with SEQ ID NO: 1), a MCV LT antigen (for example, SEQ ID NO: 7 or a nucleic acid with at least 90%, 95%, 98%, 99%, or more sequence identity with SEQ ID NO: 7), or a JCV LT antigen (for example, SEQ ID NO: 13 or a nucleic acid with at least 90%, 95%, 98%,

99%, or more sequence identity with SEQ ID NO: 13). In other examples, the modified DCs are transduced with a viral construct including a nucleic acid encoding a BKV VP1 antigen (for example, SEQ ID NO: 3 or a nucleic acid with at least 90%, 95%, 98%, 99%, or more sequence identity with SEQ ID NO: 3 or SEQ ID NO: 5 or a nucleic acid with at least 90%, 95%, 98%, 99%, or more sequence identity with SEQ ID NO: 5), a MCV VP1 antigen (for example, SEQ ID NO: 9 or a nucleic acid with at least 90%, 95%, 98%, 99%, or more sequence identity with SEQ ID NO: 9), or a JCV VP1 antigen (for example, SEQ ID NO: 15 or a nucleic acid with at least 90%, 95%, 98%, 99%, or more sequence identity with SEQ ID NO: 15). In some examples the modified DCs are transduced with a viral construct that includes a nucleic acid encoding a truncated MCV LT antigen, such as a nucleic acid encoding a protein with at least 90%, 95%, 98%, 99%, or more sequence identity with SEQ ID NO: 37. In further examples, the modified DCs are transduced with a viral construct that includes a nucleic acid encoding a portion of MCV LT and a portion of MCV ST, such as a nucleic acid encoding a protein with at least 90%, 95%, 98%, 99%, or more sequence identity with SEQ ID NO: 38.

In other examples, the vector includes a nucleic acid encoding one or more portions or fragments (such as one or more epitopes or immunodominant epitopes) of one or more polyomavirus proteins. In some examples, two or more epitopes are included in the vector (for examples, encoding around 9-15 amino acids), for example separated by a linker. The vector may include a nucleic acid encoding one or more polyomavirus epitopes (such as 2, 3, 4, 5, or more epitopes) either directly linked or linked by a nucleic acid encoding a peptide linker. Alternatively, the vector includes a nucleic acid encoding a substantially full-length polyomavirus protein (such as a VP1 or LT polypeptide) and one or more portions or fragments of a polyomavirus protein (for example, linked directly or by a peptide linker). Peptide linker sequences, which are generally between 2 and 25 amino acids in length (and the nucleic acids encoding them), are known in the art and include but are not limited to the glycine(4)-serine spacer described by Chaudhary *et al.* (*Nature* 339:394-397, 1989). Chemical linkers (such as thiol bonds or crosslinking agents) may also be used in some examples, and are known to one of ordinary skill in the art.

The modified DCs disclosed herein include monocyte-derived DCs (MDDCs) transduced with a vector (such as a lentiviral vector) including a nucleic acid encoding one or more polyomavirus antigens and/or fragments thereof. Modified DCs also include mature DCs generated from the transduced MDDCs. Methods of producing the modified DCs of the disclosure are described in Section III and Examples 1 and 2, below.

B. Antigen-Specific T Cells

Also disclosed herein are T cells specific for polyomavirus antigens, such as polyomavirus LT and/or ST antigens or polyomavirus VP1 proteins. In some examples, the T cells are specific for a particular polyomavirus LT antigen, such as MCV LT antigen, BKV LT antigen, or JCV LT antigen, while in other examples, the T cells cross-react with two or more polyomavirus LT antigens, such as two or more of MCV LT antigen, BKV LT antigen, and JCV LT antigen. In further examples, the T cells are specific for a particular polyomavirus ST antigen, such as MCV ST antigen, BKV ST antigen, or JCV ST antigen, while in other examples, the T cells cross-react with two or more polyomavirus ST antigens, such as two or more of MCV ST antigen, BKV ST antigen, and JCV ST antigen. In other examples, the T cells are specific for a particular polyomavirus VP1 protein, such as MCV VP1 protein, BKV VP1 protein, or JCV VP1 protein, while in other examples, the T cells cross-react with two or more polyomavirus VP1 proteins, such as two or more of MCV VP1 protein, BKV VP1 protein, and JCV VP1 protein. In other examples, the T cells cross-react with VP1 proteins from two or more subtypes or serotypes of a polyomavirus. For example, the T cells may cross-react with VP1 proteins from two or more of BKV subtype I (such as Ia, Ib, or Ic), II, III, and/or IV (such as IVa, IVb, or IVc) or may cross-react with VP1 proteins from two or more of JCV subtypes 1, 2, and/or 3.

In some embodiments, the T cells are human T cells (such as human CD3+ lymphocytes) that have been stimulated with APCs (such as MDDCs or mature DCs) expressing or displaying one or more polyomavirus antigen peptides. In other embodiments, the T cells are human T cells (such as human CD3+ lymphocytes) that have been stimulated by contacting the T cells with cells (such as PBMCs or MDDCs) stimulated with a mixture of peptides from one or more polyomavirus antigens (such as a mixture of overlapping peptides from a polyomavirus antigen). In some examples, the T cells are produced by the methods described in Section III and Examples 1-3 and 8 below, for example utilizing the modified DCs and/or peptide libraries disclosed herein.

In some examples, the T cells are administered to a subject (for example, for adoptive immunotherapy) as described below. In some examples, the T cells are autologous T cells, while in other examples, the T cells are allogeneic T cells (for example, fully or partially HLA-matched T cells). In particular examples, the DCs and/or antigen-specific T cells are generated from the same individual, and thus autologous DCs present to autologous T cells. However the DC/T cell pairs may either be obtained from the individual undergoing the treatment (for example, autologous use) or from a donor who may be fully or partially HLA-matched with the recipient (for example, allogeneic or third party use).

III. Methods of Producing Modified Dendritic Cells and Antigen-Specific T Cells

Disclosed herein are methods of producing the modified DCs and antigen-specific T cells described herein (for example, in Section II, above). Target antigen-specific T cells produced by the methods described below (or other methods known to one of skill in the art) can be formulated into a therapeutic composition for administration to a subject, for example with one or more pharmaceutically acceptable carriers. Therapeutic compositions and uses are discussed in Section IV, below.

A. Isolation of Monocytes and Lymphocytes

Monocytes and lymphocytes for use in the disclosed methods can be obtained using any method known in the art. These cell populations can be isolated or purified from peripheral blood drawn from a subject, for example using apheresis (for example leukapheresis) or venous puncture. In some examples, the monocytes and/or lymphocytes are obtained from a subject to be treated with the modified DCs and/or antigen-specific T cells (for example, the cells are autologous), while in other examples, the monocytes and/or lymphocytes are obtained from one or more subjects other than the subject to be treated (for example, the cells are allogeneic). In some examples, the donor is fully or partially HLA-matched with the subject to be treated.

Monocytes can be isolated from blood obtained from the subject using methods known in the art. In one example, monocytes are obtained by elutriation of monocytes. Elutriation systems are commercially available and include ELUTRA® Cell Separation System (TerumoBCT, Lakewood, CO) and JE-50. Elutriation System (Beckman Coulter, Indianapolis, IN). In another example, monocytes are obtained from peripheral blood mononuclear cells (PBMCs) using a kit to deplete non-monocytic cells (for example from Miltenyi Biotec, Auburn, CA) or by positive selection using anti-CD14 magnetic beads as recommended by the manufacturer (Miltenyi Biotec). In another example, PBMCs are prepared by centrifugation over a Ficoll-Paque (Pharmacia, Uppsala, Sweden) density gradient and the monocytes separated from lymphocytes by counterflow centrifugation (for example using an elutriator system) or centrifugation on a continuous Percoll (Pharmacia, Piscataway, NJ) density gradient.

Similarly, lymphocytes can be isolated from blood obtained from the subject using methods known in the art. In one example, lymphocytes are collected by elutriation of the lymphocytes. B cells can also be depleted, for example by cell surface marker selection. In another example, PBMCs are prepared by centrifugation over a Ficoll-Paque density gradient and the lymphocytes separated from monocytes as described above.

The resultant PBMC, monocyte, or lymphocyte product can be cryopreserved prior to use, using standard methods (for example using a combination of Pentastarch and DMSO). An

exemplary cryopreservation method is provided in Example 2, below. To qualify for cryopreservation, the cell culture ideally contains predominately PBMC, monocyte, or lymphocyte cells (for example, at least 50%, 60%, 70%, 80%, 90%, 95%, or more PBMC, monocyte, or lymphocyte cells), for example by flow cytometry.

B. Production of Modified Dendritic Cells

In some embodiments, the modified DCs are produced by transduction with one or more viral vectors encoding an antigen (such as polyomavirus, such as a polyomavirus LT protein, ST protein, or VP1 protein, or portion thereof). Without being bound by theory, it is believed that transduction of DCs with one or more viral vectors is advantageous, because it provides for stable expression of the target antigen (as opposed to transient expression from a transfected RNA or DNA). In addition, transduced DCs process and present antigenic targets endogenously, in a physiological manner, similar to that occurring *in vivo* in the case of infection or malignancy. Finally, transduction with viral vectors may also induce maturation of DCs into a highly active subset with optimal stimulatory characteristics (for example, viral vector-delivered antigens can improve antigen presentation by DC through their activation of toll-like receptors on the DC). See, *e.g.*, Chinnasamy *et al., Hum Gene Therapy* 11:1901-1909, 2000; Chinnasamy *et al., Gene Therapy* 12:259-271, 2005, both of which are incorporated by reference herein in their entirety.

Viral vectors suitable for gene delivery to DCs include adenovirus, adeno-associated virus, vaccinia virus, fowlpox, and lentivirus vectors. In particular non-limiting examples disclosed herein, immature DCs are transduced with lentiviral vectors encoding one or more target antigens or a fragment thereof. Without being bound by theory, advantages of using a lentiviral system are believed to include the ability to transduce both dividing cells and non-dividing cells (such as DCs), wide host range due to VSV-G pseudotyping, high titer production, low or no immunogenicity, and random integration.

Disclosed herein are lentiviral vectors or constructs encoding one or more polyomavirus antigens or fragments thereof. An exemplary lentiviral gene transfer construct is shown in FIG. 1. Modified or recombinant DCs containing a disclosed lentiviral vector and expressing one or more polyomavirus antigens or fragments thereof can be produced by transducing immature DCs with lentivirus particles including the vector.

In one embodiment, the methods include preparing immature DCs (such as monocyte-derived DCs (MDDCs)) prior to transduction. In some examples, monocytes are obtained from peripheral blood from one or more subjects, for example as described above.

The monocytes are treated with one or more cytokines to produce MDDCs. In some examples, the monocytes are cultured for a period of time (such as 1-7 days, for example, 3, 4, or 5

days) with addition of granulocyte-macrophage colony-stimulating factor (GM-CSF; about 700-1000 U/ml) and interleukin-4 (IL-4; about 700-1000 U/ml) on days 0 and 2 (for example, 1000 U/ml GM-CSF and 800 U/ml IL-4) to produce MDDCs. Additional cytokines or other compounds can be used to generate MDDCs from monocytes, such as TNF-α, IFN-α, or 15kD granulysin. Culture medium for generation of MDDCs is also commercially available, such as PromoCell Dendritic Cell Generation Medium (PromoCell, Heidelberg, Germany).

MDDCs are transduced with lentiviral vector including a nucleic acid encoding the desired polyomavirus antigen(s) or fragment(s) thereof. Methods of transducing cells with lentiviral vectors are known to one of skill in the art. An exemplary method is described in Example 2, below. In some embodiments, the lentivirus vector including the nucleic acid encoding a polyomavirus target antigen (the vector construct) is packaged in infectious lentivirus particles using a three plasmid system for lentiviral production in a cell line, such as 293T cells. In one example, the cells are transiently transfected with the vector construct, a packaging construct (for example, including gag, pol, and rev genes), and an envelope construct (for example, including VSVG for pseudotyping). The transfected cells are cultured for about 18-72 hours. The virus containing medium, which contains the packaged lentivirus particles is harvested. In some examples, the virus is concentrated (such as by about 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, 100-fold or more) for example, by ultracentrifugation of the culture supernatant. Transduced MDDCs are produced by infecting the MDDCs with the lentiviral particles, for example at a multiplicity of infection (MOI) of about 2 to 50 (such as about 2, 5, 10, 15, 20, 25, 30, 40, or 50). In some examples, the MDDCs are infected at an MOI of about 20-30. In some examples, the transduction is in the presence of one or more additional compounds, such as protamine sulfate (for example, 5-50 μg/ml, such as 5, 10, 20, 30, 40, or 50 μg/ml) or polybrene (for example about 4-40 μg/ml, such as 4, 8, 12, 16, 20, 24, 28, 32, 36, or 40 μg/ml). In additional examples, the transduction is also in the presence of GM-CSF and IL-4 (for example, about 800-1000 U/ml of each).

Mature transduced DCs are produced by treating the transduced MDDCs with one or more maturation agents, such as lipopolysaccharide (LPS) or a cocktail of TNF-α, IL-6, IL-1β, and/or PGE_2. In one example, MDDCs (for example after transduction) are matured with LPS (such as about 30 ng/ml LPS) for about 1-4 days (such as about 24-48 hours). Maturation of the DCs may also be in the presence of GM-CSF and IL-4 (for example, about 800-1000 U/ml of each).

In one embodiment, the presence of mature dendritic cells can be confirmed by antibodies specific for various mature dendritic cell surface markers, such as CD83, CD40, CD86 and HLA-DR. Typically, labeled antibodies specifically directed to the marker are used to identify the cell population, for example using flow cytometry.

In some examples, the transduced MDDCs or mature transduced DCs are used substantially immediately after preparation (for example, within about 6 hours of preparation). In other examples, the transduced MDDCs or mature transduced DCs are stored prior to use (for example by freezing in a suitable medium, such as a medium containing glycerol and/or dimethylsulfoxide). The cells can be frozen at about -80°C or about -180°C (for example, in a liquid nitrogen freezer) until ready for use. In some examples, the cells are frozen at a controlled rate, for example about -1°C/minute until a desired temperature is reached (such as about -70 to -80°C). The cells can then be maintained at -70 to -80°C, or transferred to a liquid nitrogen freezer.

C. Production of Target Antigen-Specific T Cells with Modified Dendritic Cells

Target antigen-specific T cells are generated by stimulating lymphocytes with the mature transduced DCs described above. In some examples, the transduced mature DCs are contacted with lymphocytes (such as T lymphocytes, for example, CD3+ T cells). Methods of isolating lymphocytes or for purifying T lymphocytes (for example from peripheral blood) are known to one of ordinary skill in the art. Exemplary methods for isolating lymphocytes are described above. T lymphocytes can be isolated by positive selection, for example for CD3+ cells, for example using flow cytometry.

The ratio of lymphocytes (such as T lymphocytes) to DCs is about 1:1 to about 200:1 (such as about 2:1, about 3:1, about 4:1, about 5:1, about 6:1, about 7:1, about 8:1, about 9:1, about 10:1, about 20:1, about 50:1, about 100:1, or 200:1). In one example, the ratio of T lymphocytes to DCs is about 5:1 to about 20:1.

In particular examples, the T lymphocytes and DCs are contacted in culture in the substantial absence of cytokines for about 1-3 days. In some examples, culture (or co-culture) in the "substantial absence of cytokines" indicates that cytokines are not added to the culture medium; however, low levels of cytokines may be present in the culture medium (for example, present in components of the medium, such as serum). In addition, cytokines may accumulate in the medium over the course of the culture or co-culture. Thus, "substantial absence of cytokines" does not mean that no cytokines are present in the culture at any time.

After 1-3 days of culture in the substantial absence of cytokines, one or more cytokines (such as one or more of IL-2, IL-7, IL-12, and/or IL-15) are added to the medium and the cells are cultured for about 4-6 days. In some examples, the one or more cytokines are added to the culture medium at a concentration of about 2 ng/ml to about 100 ng/ml (for example, about 2 ng/ml to about 50 ng/ml, about 5 ng/ml to about 20 ng/ml, or about 10 ng/ml to about 20 ng/ml). In other examples, the one or more cytokines are added to the culture medium at a concentration of about 5-100 U/ml (such as about 10 U/ml to about 50 U/ml, about 20 U/ml to about 100 U/ml, or about 10

U/ml to about 20 U/ml). In one example, the T lymphocytes and the mature transduced DCs are co-cultured for about 3 days in the substantial absence of cytokines and then co-cultured in the presence of IL-7 and IL-15 for about 4 days. In one particular example, the cells are co-cultured in the presence of about10 ng/ml IL-7 and about 10 ng/ml IL-15 for about 4 days.

In additional examples, following the co-culture in the presence of one or more cytokines (such as IL-7 and IL-15), the T lymphocytes and DCs are again co-cultured in the substantial absence of cytokines for about 1-3 days, followed by co-culture in the presence of one or more cytokines (for example, one or more of IL-2, IL-7, IL-12, and/or IL-15) for about 4-6 days. In one example, the T lymphocytes and DCs are co-cultured for about 3 days in the substantial absence of cytokines and then co-cultured in the presence of IL-2, IL-7 and IL-15 for about 4 days. In one particular example, the cells are co-cultured in the presence of about 10 U/ml IL-2, about10 ng/ml IL-7, and about 10 ng/ml IL-15 for about 4 days.

In some examples, the target antigen-specific T cells produced by the methods disclosed herein are CTLs (for example, $CD8^+$ T cells); however, one of skill in the art will recognize that other types of target antigen-specific T cells can also be produced, including helper T cells (for example, $CD4^+$ T cells). In particular examples, the methods disclosed herein produce both $CD8^+$ and $CD4^+$ antigen-specific T cells.

An exemplary, non-limiting embodiment of the method is shown in FIG. 2. $CD3^+$ T lymphocytes are primed twice at weekly intervals with mature transduced DCs expressing a target antigen at a ratio of about 5:1. The cells are co-cultured in complete medium in the substantial absence of cytokines for the initial three days of the first priming and then cultured in complete medium with 10 ng/ml IL-7 and 10 ng/ml IL-15 for days 4-7 of the first priming. During the second priming (*e.g.,* with fresh antigen-expressing transduced DCs), the cells are again co-cultured in complete medium in the substantial absence of cytokines for the initial three days, followed by addition of 10 U/ml IL-2, 10 ng/ml IL-7, and 10 ng/ml IL-15 during days 4-7 of the second priming. In one non-limiting example, the complete medium is AIM V® and RPMI 1640 media 50:50 mix (Life Technologies, Grand Island, NY) supplemented with 5% heat-inactivated normal AB serum (Gemini Bio-Product, Woodland, CA) and 2 mM L-glutamine, 100 U/mL Penicillin, and 100 µg/mL Streptomycin (Invitrogen, Carlsbad, CA).

In some examples, the T cells are enriched for T cells specific for the target antigen, for example, the population of T cells that are specific for the target antigen is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more of the total population of T cells obtained using the disclosed methods. The specificity of the T cells produced by the methods described herein can be assessed by methods known to one of skill in the art.

Exemplary methods are described in Example 3, below. In one example, an assay to demonstrate that the expanded population of T cells is specific for the target antigen includes utilizing labeled CD3 and a labeled cytokine (such as TNF-α, IL-2, or IFN-γ) to determine the percent of target antigen-specific T cells that are present, wherein target antigen-specific T cells are both $CD3^+$ and cytokine-positive. In some examples, the method further includes using labeled CD4 and labeled CD8 to determine the percent of target antigen-specific T cells that are present within a particular CD population, wherein target antigen-specific T cells are those that are cytokine-positive and $CD8^+$ or $CD4^+$. Flow cytometry can be used to conduct such assays. In another example, the cytotoxicity of the antigen-specific T cells can be determined. Methods for determining cytotoxicity are known in the art, for example a ^{51}Cr-release assay (for example see Walker *et al. Nature* 328:345-8, 1987; Qin *et al. Acta Pharmacol. Sin.* 23(6):534-8, 2002; all herein incorporated by reference).

In some embodiments, the antigen-specific T cells produced by the methods described herein are enriched by selecting antigen-specific cells prior to formulation in a therapeutic product and use (for example, by selection for 4-1BB positive cells). However, it is believed that enrichment or purification is not routinely necessary. Prior experience with antigen-specific T cell expansion has demonstrated that in the absence of antigenic stimulation the main repertoire of T cells become anergic and die. Thus, in some examples, substantially only T cells stimulated and expanded by the selected antigen-loaded DC survive and function after culture (for example after 1-14 days, such as about 3 days, 5 days, 7 days, 10 days, or 14 days in culture).

In some examples, the antigen-specific T cells are used (for example, administered to a subject) substantially immediately after preparation (for example, within about 6 hours of preparation). In other examples, the antigen-specific T cells are stored prior to use (for example by freezing in a suitable medium, such as a medium containing glycerol and/or dimethylsulfoxide). The cells can be frozen at about -80°C or about -180°C (for example, in a liquid nitrogen freezer) until ready for use. In some examples, the cells are frozen at a controlled rate, for example about -1°C/minute until a desired temperature is reached (such as about -70 to -80°C). The cells can then be maintained at -70 to -80°C, or transferred to a liquid nitrogen freezer.

D. Production of Target Antigen-Specific T Cells with Peptide Mixes

Target antigen-specific T cells are generated by stimulating MDDCs or PBMCs with a mixture of peptides derived from one or more polyomavirus antigens. In some examples, the peptides (also referred to as a "pepmix" or peptide libraries) are contacted with PBMCs. In other examples, the peptides are contacted with monocyte-derived DCs. Methods of isolating or preparing PBMCs, lymphocytes, and/or monocyte-derived DCs are known as one of ordinary skill

in the art. Exemplary methods of isolating PBMCs include leukapheresis or gradient centrifugation. Exemplary methods for preparing monocyte-derived DCs and isolating lymphocytes (such as T lymphocytes) are described above.

The peptide mixtures used in the methods described herein are libraries of overlapping peptides that span all or a portion of a polyomavirus protein sequence. The pepmix is believed to include one or more immunodominant epitopes. The peptides are at least 10 amino acids long (for example, 10-30 amino acids, 12-18 amino acids, 15-25 amino acids long). In some examples, the pepmix includes overlapping 15 amino acid peptides (15mers). The peptides overlap with one another by 10-15 amino acids (for example, overlap by 10, 11, 12, 13, 14, or 15 amino acids). In one specific example, the peptides in the pepmix overlap by 11 amino acids. An exemplary method for preparing a pepmix is shown in FIG. 10. For example, a set of overlapping peptides from a polyomavirus protein or portion thereof is synthesized and pooled in a pepmix. Alternatively, overlapping peptides are synthesized from two or more polyomavirus proteins or portions thereof and are pooled in a single pepmix.

In some embodiments, the pepmix includes overlapping peptides from one polyomavirus antigen (such as an LT, ST, or VP1 protein). In other embodiments, the pepmix includes overlapping peptides from two or more polyomavirus antigens. For example, the pepmix may include overlapping peptides from the same protein from two different polyomaviruses (such as MCV LT and BKV LT or BKV VP1 and JCV VP1), from different proteins (such as LT and ST or LT and VP1), or a combination thereof (for example, MCV LT and BKV VP1). A pepmix can be generated by synthesizing a set of overlapping peptides based on the sequence of a polyomavirus protein. For example, a pepmix can be synthesized from any one of SEQ ID NOs: 2, 4, 6, 8, 10, 14, 16, 37, or 38. Pepmixes for polyomavirus proteins are also commercially available, for example from Miltenyi Biotec (San Diego, CA) or JPT Peptide Technologies (Berlin, Germany).

In some embodiments, the methods include contacting MDDCs with a pepmix. The stimulated MDDCs are then mixed with PBMCs or lymphocytes. The ratio of lymphocytes (such as T lymphocytes) to DCs is about 1:1 to about 200:1 (such as about 2:1, about 3:1, about 4:1, about 5:1, about 6:1, about 7:1, about 8:1, about 9:1, about 10:1, about 20:1, about 50:1, about 100:1, or 200:1). In one example, the ratio of T lymphocytes to DCs is about 5:1 to about 20:1. The mixture of DCs and lymphocytes (or PBMCs) is cultured with medium including IL-7 and IL-15 for three days. After three days, the cells are cultured with medium including IL-7, IL-15, and IL-2 for another 7-14 days. In some examples, the cells are pulsed with the same or a different pepmix on day 8 to 14 and cultured in the same cytokine combination as described above (IL-7/IL-

15 medium for 3 days and then add IL-2. An exemplary non-limiting embodiment of one method of preparing polyomavirus antigen-specific T cells is shown in FIG. 10.

One of ordinary skill in the art can identify appropriate culture medium, for example AIM V® and RPMI 1640 media 50:50 mix (Life Technologies, Grand Island, NY) supplemented with 5% heat-inactivated normal AB serum (Gemini Bio-Product, Woodland, CA) and 2 mM L-glutamine, 100 U/mL Penicillin, and 100 µg/mL Streptomycin (Invitrogen, Carlsbad, CA). In some examples, IL-7, IL-15, and/or IL-2 are included in the culture medium at a concentration of about 2 ng/ml to about 100 ng/ml (for example, about 2 ng/ml to about 50 ng/ml, about 5 ng/ml to about 20 ng/ml, or about 10 ng/ml to about 20 ng/ml) or at a concentration of about 5-100 U/ml (such as about 10 U/ml to about 50 U/ml, about 20 U/ml to about 100 U/ml, or about 10 U/ml to about 20 U/ml). In one particular example, the cells are co-cultured in the presence of about 10 ng/ml IL-7 and about 10 ng/ml IL-15, and about 10 U/ml IL-2 (when present).

In other embodiments, the methods include contacting mononuclear cells (*e.g.*, PBMCs) with one or more pepmixes (for example, to produce "stimulator" cells). The stimulator cells are then contacted with naïve mononuclear cells (*e.g.*, PBMCs that have not been contacted with a pepmix) and cultured for a period of time to produce polyomavirus antigen-specific T cells. In some examples, the stimulator cells and naïve cells ("responder" cells) are mixed at a ratio of 1:1; however, one of ordinary skill in the art can determine other appropriate ratios for use in the methods. In some examples, the stimulator cells are irradiated (for example, with about 25 Gy) prior to mixing with the responder cells. Without being bound by theory, it is believed that this maintains the cells in an antigen-presenting state, rather than dividing and diluting the population of APCs.

The mixture of stimulator and responder cells is cultured with medium including IL-7 and IL-15 for three days. After three days, the cells are cultured with medium including IL-7, IL-15, and IL-2 for another 7-14 days. In some examples, the cells are pulsed with the same or a different pepmix on day 8. If a second round of stimulation is performed, the same cytokine cocktail is used in the same sequence (IL-7/IL-15 medium for 3 days and then add IL-2). An exemplary non-limiting embodiment of one method of preparing polyomavirus antigen-specific T cells is shown in FIG. 13.

One of ordinary skill in the art can identify appropriate culture medium, for example AIM V® and RPMI 1640 media 50:50 mix (Life Technologies, Grand Island, NY) supplemented with 5% heat-inactivated normal AB serum (Gemini Bio-Product, Woodland, CA) and 2 mM L-glutamine, 100 U/mL Penicillin, and 100 µg/mL Streptomycin (Invitrogen, Carlsbad, CA). In some examples, IL-7, IL-15, and/or IL-2 are included in the culture medium at a concentration of about 2

ng/ml to about 100 ng/ml (for example, about 2 ng/ml to about 50 ng/ml, about 5 ng/ml to about 20 ng/ml, or about 10 ng/ml to about 20 ng/ml) or at a concentration of about 5-100 U/ml (such as about 10 U/ml to about 50 U/ml, about 20 U/ml to about 100 U/ml, or about 10 U/ml to about 20 U/ml). In one particular example, the cells are co-cultured in the presence of about 10 ng/ml IL-7 and about 10 ng/ml IL-15, and about 10 U/ml IL-2 (when present).

In some examples, the target antigen-specific T cells produced by either of the method embodiments discussed in this section are CTLs (for example, $CD8^+$ T cells); however, one of skill in the art will recognize that other types of target antigen-specific T cells can also be produced, including helper T cells (for example, $CD4^+$ T cells). In particular examples, the methods disclosed herein produce both $CD8^+$ and $CD4^+$ antigen-specific T cells.

In some examples, the T cells are enriched for T cells specific for the target antigen, for example, the population of T cells that are specific for the target antigen is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more of the total population of T cells obtained using the disclosed methods. In other examples, the T cells are enriched for activated T cells, for example, the population of T cells that are activated is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more of the total population of T cells obtained using the disclosed methods. In one example, activated T cells can be selected for using a marker of activated T cells such as 4-1BB (CD137), for example as described in Example 5.

In some embodiments, the antigen-specific T cells are enriched by selecting antigen-specific cells and/or activated cells prior to formulation in a therapeutic product and use (described in Section IV, below). However, it is believed that enrichment or purification is not routinely necessary. Prior experience with antigen-specific T cell expansion has demonstrated that in the absence of antigenic stimulation the main repertoire of T cells become anergic and die. Thus, in some examples, substantially only T cells stimulated and expanded by the selected antigen-loaded DC survive and function after culture (for example after 1-14 days, such as about 3 days, 5 days, 7 days, 10 days, or 14 days in culture). The specificity of the T cells produced by the methods described herein can be assessed by the same methods as described in Section C, above.

In some examples, the antigen-specific T cells are used (for example, administered to a subject) substantially immediately after preparation (for example, within about 6 hours of preparation). In other examples, the antigen-specific T cells are stored prior to use (for example by freezing in a suitable medium, such as a medium containing glycerol and/or dimethylsulfoxide). The cells can be frozen at about -80°C or about -180°C (for example, in a liquid nitrogen freezer) until ready for use. In some examples, the cells are frozen at a controlled rate, for example about -

1°C/minute until a desired temperature is reached (such as about -70 to -80°C). The cells can then be maintained at -70 to -80°C, or transferred to a liquid nitrogen freezer.

IV. Methods of Treating or Inhibiting Polyomavirus Infection

Disclosed herein are methods of treating or inhibiting polyomavirus infection and/or polyomavirus-associated disease (such as PVAN, PML, hemorrhagic cystitis, TS, or MCC). The disclosed methods also include eliciting or enhancing an immune response against one or more polyomaviruses in the subject. In some embodiments the methods include administering to a subject (such as a subject having or at risk of polyomavirus infection or polyomavirus-associated disease) one or more of the disclosed polyomavirus antigen-specific T cells and/or modified DCs.

In some embodiments, the methods further include selecting a subject in need of enhanced immunity to one or more polyomaviruses (such as BKV, MCV, JCV, and/or TSV). In some examples, a subject in need of enhanced immunity to a polyomavirus is a subject with a polyomavirus infection and/or polyomavirus-associated disease (such as PVAN, PML, hemorrhagic cystitis, TS, or MCC). In other examples, a subject in need of enhanced immunity to polyomavirus is a subject at risk of polyomavirus infection or at risk of polyomavirus-associated disorders, such as PVAN, polyomavirus-associated hemorrhagic cystitis, PML, TS, or MCC. Subjects in need of enhanced immunity to polyomavirus include subjects who are immunocompromised, for example subjects who are infected with human immunodeficiency virus (HIV), subjects with SCID, diabetics, subjects who are receiving chemotherapy for cancer, subjects who are receiving immunosuppressive therapy (such as corticosteroids, a calcineurin inhibitor, such as tacrolimus, cyclosporine, or pimecrolimus, or other therapies that decrease immune system function, such as rituximab, natalizumab, efalizumab, or alemtuzumab), and/or elderly subjects (for example, human subjects 65 years of age or older). In some examples, subjects who are receiving immunosuppressive therapy include individuals who have received or are a candidate for an organ transplant (such as a renal transplant or other solid organ transplant or a bone marrow transplant). In a particular example, a subject in need of enhanced immunity to one or more polyomaviruses is a renal transplant recipient or a bone marrow (hematopoietic stem cell) transplant recipient. In other examples, subjects in need of enhanced immunity to polyomaviruses include those who are candidates for organ transplantation or those who are candidates for immunosuppressive therapy. In further examples, a subject in need of enhanced immunity to polyomaviruses may include a subject who has or is at risk for cancer (for example, prostate cancer, colon cancer, or bladder carcinoma).

A. Administering Polyomavirus Antigen-Specific T Cells

Disclosed herein are methods of eliciting or increasing an immune response or treating or inhibiting polyomavirus infection in a subject having or at risk of polyomavirus infection by administering to the subject a therapeutically effective amount of one or more target antigen-specific T cells, for example, polyomavirus antigen-specific T cells prepared by the methods disclosed herein. In some examples, a therapeutically effective amount of the polyomavirus antigen-specific T cells is an amount sufficient to increase an immune response to one or more polyomaviruses (such as BKV, JCV, or MCV) in a subject by at least 10% (such as at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more). In other examples, a therapeutically effective amount is an amount sufficient to reduce or ameliorate one or more symptoms of a polyomavirus-related disease, such as PVAN, PML, polyomavirus-associated hemorrhagic cystitis, TS, or MCC. In still further examples, a therapeutically effective amount is an amount sufficient to reduce the incidence or severity of a polyomavirus-related disease in a subject infected with one or more polyomaviruses. The amount of cells effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* assays can be employed to identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each subject's circumstances. Effective doses can be extrapolated from dose-response curves derived from *in vitro* or animal model test systems or in clinical trials with polyomavirus-infected individuals.

In some examples, the polyomavirus antigen-specific T cells disclosed herein are administered to a subject at a dose of about 10^5 $CD3^+$ cells/kg to about 10^9 $CD3^+$ cells/kg, such as from about 10^6 cells/kg to about 10^8 cells/kg, such as from about 5 x 10^6 cells/kg to about 75 x 10^6 cells/kg, such as about 25 x 10^6 cells/kg, or about 50 x 10^6 cells/kg. In other examples, subject is administered about 10^5 cells, about 10^6 cells, about 10^7 cells, about 10^8 cells, about 10^9 cells, about 10^{10} cells, about 10^{11} cells, or more. In particular non-limiting examples, the subject is administered at least about 10^5 $CD3^+$ cells/kg, for example, about 10^6-10^7 $CD3^+$ cells/kg. The polyomavirus antigen-specific T cells can be administered by any means known to one of skill in the art, either locally or systemically, such as by intravenous injection, intramuscular injection, subcutaneous injection, intraperitoneal injection, oral administration, nasal administration, or intradermal administration. In some specific embodiments, administration is by intravenous injection. In other specific embodiments, the antigen-specific T cells are administered by

instillation into the peritoneal cavity, pleural cavity, or by injection into the cerebrospinal fluid (for example, by lumbar puncture).

Polyomavirus antigen-specific T cells, such as those produced by the methods described herein, can be administered in a single dose or in multiple doses (such as 2, 3, 4, 5, or more doses), as determined by a clinician. In some examples, the cells are administered at intervals of approximately 1 day, 3 days, 1 week, 2 weeks, monthly, twice yearly, or yearly, depending on the response desired and the response obtained (such as induction of an immune response to one or more polyomaviruses and/or reduction or even elimination of one or more symptoms associated with one or more polyomavirus-related diseases). Either single or multiple doses will be administered, as determined by the research protocol and clinical status of the recipient.

In some examples, once the desired response is obtained (for example, establishment of a persisting repertoire of antigen-specific T cells or reduction of symptoms), no further antigen-specific T cells are administered. This response may be achieved with a single administration of antigen-specific T cells, in at least some examples. However, if there is evidence of non-persistence of the infused antigen-specific T cells and/or persisting symptoms associated with polyomavirus infection (including viremia and/or one or more symptoms of a polyomavirus-related disease) or if the subject has persisting immune deficiency, one or more additional administrations of a therapeutically effective amount of antigen-specific T cells can be administered. Immunization protocols (such as amount of T cells, number of doses and timing of administration) can be determined experimentally, for example by using animal models (such as mice or non-human primates), and/or by clinical testing in humans.

The polyomavirus antigen-specific T cells disclosed herein can be administered with a pharmaceutically acceptable carrier, such as buffered saline. Standard procedures and buffers can be used. One of ordinary skill in the art can select one or more pharmaceutically acceptable carriers suitable for use with the cells disclosed herein. *Remington: The Science and Practice of Pharmacy*, The University of the Sciences in Philadelphia, Editor, Lippincott, Williams, & Wilkins, Philadelphia, PA, 21st Edition (2005), describes compositions and formulations suitable for pharmaceutical delivery of the cells herein disclosed. In general, the nature of the carrier will depend on the mode of administration being employed. For instance, parenteral formulations usually include injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, serum, plasma, serum substitutes, pharmacologically approved tissue culture medium supplemented with autologous serum or blood group AB serum from a blood bank, combinations thereof, or the like,

as a vehicle. The carrier and composition can be sterile, and the formulation suited to the mode of administration.

In some examples, other therapeutic agents are administered with the antigen-specific T cells (*e.g.*, administered before, during, or after administration of the antigen-specific T cells), depending on the desired effect. Exemplary therapeutic agents include, but are not limited to, antiviral agents (for example, cidofovir, brincidofovir, or cytosine arabinoside (AraC)), antibiotics (for example, fluoroquinolines, such as ciprofloxacin or levofloxacin), immune stimulants (such as interferon-alpha), cytokines (such as IL-2), leflunomide, mefloquine, intravenous immunoglobulin (IVIG), or one or more peptides of the same antigen used to stimulate T cells *in vitro*. In a particular example, compositions containing the disclosed antigen-specific T cells also include one or more therapeutic agents.

In some embodiments, one or more of the modified DCs disclosed herein are administered before, during, or after administration of the polyomavirus antigen-specific T cells. In some examples, the polyomavirus antigen-specific T cells and modified DCs are administered in a prime-boost regimen. For example, the polyomavirus antigen-specific T cells are administered to a subject as a "prime" and the modified DCs are subsequently administered to the subject as one or more "boosts." In some examples, a subject is administered a priming dose of one or more of the disclosed polyomavirus antigen-specific T cells (for example, produced by the methods disclosed herein) followed by at least one boost dose of one or more (such as 1, 2, 3, or 4) of the modified polyomavirus antigen expressing DCs disclosed herein. The boost can be administered about 7, 10, 14, 30, 60, 90, or more days after administration of the prime. Additional boosts can be administered at subsequent time points, if determined to be necessary or beneficial. In one non-limiting example, a prime-boost regimen includes administration of antigen-specific T cells on day 0 and antigen-expressing DCs on day 10. Immunization protocols (such as amount of T cells and modified DCs, number of doses (prime and boost) and timing of administration) can be determined experimentally, for example by using animal models (such as mice or non-human primates) and/or by clinical testing in humans.

B. Administering Modified Dendritic Cells

Also disclosed herein are methods of eliciting or increasing an immune response to one or more polyomaviruses or treating or inhibiting polyomavirus infection in a subject having or at risk of polyomavirus infection by administering to the subject a therapeutically effective amount of one or more modified DCs containing a nucleic acid encoding one or more polyomavirus antigens or a fragment thereof, for example, modified DCs disclosed herein. In some examples, a therapeutically effective amount of the modified DCs is an amount sufficient to increase an immune response to

one or more polyomaviruses in a subject by at least 10% (such as at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more). In other examples, a therapeutically effective amount is an amount sufficient to reduce or ameliorate one or more symptoms of a polyomavirus-related disease, such as PVAN, PML, hemorrhagic cystitis, TS, or MCC. In still further example, a therapeutically effective amount is an amount sufficient to reduce the incidence or severity of a polyomavirus-related disease in a subject infected with one or more polyomaviruses. The amount of modified DCs effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* assays can be employed to identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each subject's circumstances. Effective doses can be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

In some examples, the modified DCs disclosed herein are administered to a subject at a dose of about 10^5 cells/kg to about 10^9 cells/kg, such as from about 10^6 cells/kg to about 10^8 cells/kg, such as from about 5 x 10^6 cells/kg to about 75 x 10^6 cells/kg, such as about 25 x 10^6 cells/kg, or about 50 x 10^6 cells/kg. In other examples, subject is administered about 10^5 cells, about 10^6 cells, about 10^7 cells, about 10^8 cells, about 10^9 cells, about 10^{10} cells, about 10^{11} cells, or more. In particular non-limiting examples, the subject is administered at least about 10^5 cells/kg, for example, about 10^6-10^7 cells/kg. The modified DCs can be administered by any means known to one of skill in the art, either locally or systemically, such as by intravenous injection, intramuscular injection, subcutaneous injection, intraperitoneal injection, oral administration, nasal administration, or intradermal administration. In some specific embodiments, administration is by intravenous injection. In other specific embodiments, the DCs are administered by instillation into the peritoneal cavity, pleural cavity, or by injection into the cerebrospinal fluid (for example, by lumbar puncture).

The modified DCs, such as those produced by the methods described herein, can be administered in a single dose or multiple doses as determined by a clinician. In some examples, the cells are administered at intervals of approximately 1 day, 3 days, 1 week, 2 weeks, monthly, twice yearly, or yearly, depending on the response desired and the response obtained (such as induction of an immune response to one or more polyomaviruses and/or reduction or even elimination of one or more symptoms associated with one or more polyomavirus-related diseases). In particular, non-limiting examples, the DCs are administered at intervals of about 3-4 weeks at least twice (such as about 2-8 administrations). In some examples, once the desired response is obtained, no further

DCs are administered. However, if the recipient displays one or more symptoms associated with polyomavirus infection (including viremia and/or one or more symptoms of a polyomavirus-related disease), a therapeutically effective amount of modified DCs can be administered at that time. Immunization protocols (such as amount of DCs, number of doses and timing of administration) can be determined experimentally, for example by using animal models (such as mice or non-human primates), followed by clinical testing in humans.

The modified DCs disclosed herein can be administered with a pharmaceutically acceptable carrier, such as buffered saline. One of ordinary skill in the art can select one or more pharmaceutically acceptable carriers suitable for use with the cells disclosed herein. *Remington: The Science and Practice of Pharmacy*, The University of the Sciences in Philadelphia, Editor, Lippincott, Williams, & Wilkins, Philadelphia, PA, 21st Edition (2005), describes compositions and formulations suitable for pharmaceutical delivery of the cells herein disclosed. In general, the nature of the carrier will depend on the mode of administration being employed. For instance, parenteral formulations usually include injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, serum, plasma, serum substitutes, pharmacologically approved tissue culture medium supplemented with autologous serum or blood group AB serum from a blood bank, combinations thereof, or the like, as a vehicle. The carrier and composition can be sterile, and the formulation suited to the mode of administration.

In some examples, other therapeutic agents are administered with the modified DCs. Other therapeutic agents can be administered before, during, or after administration of the modified DCs, depending on the desired effect. Exemplary therapeutic agents include, but are not limited to, anti-viral agents, immune stimulants such as interferon-alpha, cytokines, or one or more peptides of the same antigen expressed by the modified DCs. In a particular example, compositions containing the disclosed modified DCs also include one or more therapeutic agents.

The present disclosure is illustrated by the following non-limiting Examples.

Example 1

Viral Antigens and Lentiviral Vectors

This example describes the polyomavirus antigens and construction of lentiviral vectors for their expression in dendritic cells.

Gene sequences and plasmids: The full length coding sequences of BKV LT, BKV VP1 Ia, BKV VP1 IV, MCV LT, and MCV VP1 viral antigens and G250 (human carbonic anhydrase

IX) were synthesized by GeneArt DNA synthesis technology (Life Technologies, Grand Island, NY) flanked by two restriction sites, AscI and SalI, at the 5′ end and 3′ end, respectively. Details of each coding sequence are provided in Table 2. Where indicated, the sequences were optimized (referred to as "codon-modified") for expression in mammalian cells using GeneOptimizer® (GeneArt, Life Technologies, Grand Island, NY) to select the proper codons to be used during translation of amino acid sequence to codon sequence. The coding sequences were cloned into the human immunodeficiency virus (HIV)-1-based lentiviral gene transfer vector pRRLsin18.PPT.MSCV.eGFP.WPRE, replacing the eGFP sequences using standard molecular biology techniques (FIG. 1). The final constructs were verified by DNA sequencing then alignment against original DNA sequence using multiple-alignment algorithm AlignX in Vector NTI application software (Invitrogen). The sequences of the antigens (nucleic acid and protein) and the gene transfer vector are provided in the Sequence Listing submitted herewith.

Table 2. Antigen coding sequences

Antigen	Description	SEQ ID NO:
BKV LT	The open reading frame sequence was incorporated with all known variations that occur in at least two isolates, FIN-2 (genotype IV, accession AB269822) and CAP-h2 (genotype Ib-1, accession AY628226) into a single protein and was codon-modified	1
BKV VP1 Ia	Codon-modified BKV VP1 coding sequence based on genotype Ia isolate BK-D	3
BKV VP1 IV	Codon-modified BKV VP1 based on genotype IVc2 isolate A-66H	5
MCV LT	Splicing signals for the "57kT" isoform of the MCV LT antigen have been silently destroyed	7
MCV VP1	Codon-modified MCV VP1 consensus sequences represented by isolate MCC339 and several others	9
G250	Full length coding sequence of the human Carbonic anhydrase IX gene	11

The human immunodeficiency virus (HIV)-1-based gene transfer vectors used in this study were pRRLsin18.PPT.MSCV.BKV-LT.Wpre (Lenti-BKV LT), pRRLsin18.PPT.MSCV.BKV Ia-Vp1.Wpre (Lenti-BKV VP1 Ia), pRRLsin18.PPT.MSCV.BKV IV-Vp1.Wpre (Lenti-BKV VP1 IVc2), pRRLsin18.PPT.MSCV.MCV LT.Wpre (Lenti-MCV LT), pRRLsin18.PPT.MSCV.MCV Vp1.Wpre (Lenti-MCV VP1), and pRRLsin18.PPT.PGK.G250.Wpre (Lenti-G250). The vesicular stomatitis virus G protein (VSV-G) envelope encoding construct pMD.G, and the packaging construct pCMV ΔR8.91 were kindly provided by Prof. D. Trono, Department of Genetics and Microbiology, CMU, Geneva, Switzerland.

Lentiviral vector production: The lentiviral vector particles were produced in 293T cells (American Type Culture Collection, Manassas, VA) by three-plasmid transfection using a calcium phosphate transfection kit (Invitrogen) as previously described (Chinnasamy *et al. Blood.* 96:1309-1316, 2000), except that Opti-MEM-1 containing 5% HS was used as culture medium during transfection. The viral supernatants were concentrated 50-fold by ultracentrifugation at 50,000*g* for 1.5 hours at 4°C. Viral pellets were resuspended in complete medium and stored frozen at -80°C until use. The titer of the viral preparations and the presence of replication-competent lentivirus

(RCL) were determined as described previously (Escarpe *et al., Mol. Ther.* 8:332-341, 2003). No RCL was detectable in any of the vector preparations used in this study.

Example 2

Lentiviral Transduced Dendritic Cells

This example describes generation of DCs transduced with lentiviral vectors encoding viral antigens.

Cells: Peripheral blood mononuclear cells (PBMC) from healthy donors were collected by apheresis (Amicus Separator; Fenwal, Lake Zurich, IL, USA) on an institutional review board-approved protocol in the Department of Transfusion Medicine, National Institutes of Health (Bethesda, MD, USA). Human PBMCs were obtained from healthy donor leukopacs and leukocytes enriched by centrifugation over Ficoll. Monocytes were obtained from healthy donors by leukapheresis and elutriation (Transfusion Medicine Department, Clinical Center, National Institutes of Health, Bethesda, MD). Monocytes and lymphocytes were isolated from the PBMC concentrates on the day of collection by elutriation (Elutra, Terumo BCT, Lakewood, CO), according to the manufacturer's recommendations. Cells were enriched by centrifugation over Ficoll and cryopreserved using a controlled rate freezer (Kryosave; Integra, Planer plc, Sunbury-on-Thames, UK) in media containing 5% dimethylsulfoxide (Edwards Lifesciences, Irvine, CA), 6% pentastarch (Pharmaceutical Development Section, Pharmacy Department, Clinical Center, National Institutes of Health) and 4% human serum albumin (Baxter Health Care Corporation, Los Angeles, CA).

Before use, cells were thawed, washed, and resuspended in complete medium, (CM) supplemented with 5% human AB serum (HS) and rested overnight. The cells were thawed and washed in 50:50 mix of AIM V and RPMI 1640 media supplemented with 5% heat-inactivated normal AB serum (Gemini Bio-Product, Woodland, CA) and 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin (Invitrogen, Carlsbad, CA) CM and 30U/ml DNase I (Sigma-Aldrich, St. Louis, MO).

Preparation of monocyte-derived dendritic cells (MDDC): Elutriated monocytes were cultured at 1×10^6 cell/ml in 12-well plates in complete media Recombinant human rIL-4 (800 U/ml) and GM-CSF (1000 U/ml, PeproTech, Rocky Hill, NJ) were added at Days 0 and 2 and cultured at 37°C in 5% CO_2 and 95% humidity. Four days after culture, cells were phenotypically characterized and used in subsequent experiments as described below. On Day 4, MDDCs were transduced with lentiviral vector expressing the indicated antigen and matured with lipopolysaccharide (LPS; 30 ng/ml) for 24-48 hours before use as APCs.

Lentiviral transduction of DCs: Immature DC cultures (Day 4) were transduced with lentiviral vectors at a multiplicity of infection (MOI) of approximately 30, in the presence of protamine sulfate (10 μg/ml, Sigma-Aldrich), in 24-well plate containing 1 ml/well of DC culture medium supplemented with rhGM-CSF (1000U/ml) and rhIL-4(800U/ml). After 48 hours transduction, cells were washed and cultured in fresh DC culture medium containing GM-CSF (1000U/ml) and IL-4 (800U/ml) in the presence of 30 ng/ml endotoxin-free LPS (Sigma-Aldrich) for 24-48 hours. In some conditions, untransduced were DCs matured with LPS and pulsed with indicated peptides. The viability of DC cultures was monitored periodically before and after transduction using trypan blue (Sigma-Aldrich) staining.

Example 3

Polyomavirus Antigen-Specific Effector T Cells

This example describes a method for generating polyomavirus antigen-specific effector T cells using modified DCs.

To generate and expand effector T cells with specificity against the selected BKV and MCV viral antigens *ex vivo*, purified autologous $CD3^+$ T cells were primed twice at weekly intervals with Lenti-BKV-LT, Lenti-BKV Ia-VP1, Lenti-BKV IVc2-VP1, Lenti-MCV LT, or Lenti-MCV VP1 transduced autologous DCs at a responder:stimulator ratio of 5:1. Cells were cultured in a 96-well-plate at a density of $1x10^6$ cells/mL CM in a total volume of 200 μl per well. Cultures received IL-7 (10 ng/ml) and IL-15 (10 ng/ml) during first priming and IL-2 (10 U/ml) in addition to IL-7 and IL-15 during second priming step. No cytokines were present during the initial 3 days of both priming steps (FIG. 2). Resulting effector T cell cultures were tested for reactivity against target cells expressing the relevant or irrelevant target antigen. Target cells included autologous DC lentivirally transduced to express relevant BKV or MCV viral antigens or an irrelevant antigen G250 in a 6 hour co-culture assay. Where indicated, the effector cells were also tested for reactivity against autologous DC targets pulsed with a peptide library (pepmix) containing 15mer peptides overlapping by 11 amino acids spanning the entire protein sequence of the BKV or JCV LT or VP1 antigens or the irrelevant control antigen Wilms tumor-1 (WT1) ((Miltenyi Biotec, Auburn, CA; JPT Peptide Technologies GmbH, Berlin, Germany). Autologous DCs transduced with a lentiviral construct expressing G250, pulsed with WT1 pepmix, or with no manipulation served as specificity control targets.

Briefly, effector cells were co-cultured for 6 hours with indicated target cells at 5:1 ratio. The protein transport inhibitors Brefeldin A (GolgiPlug) or Monensin (GolgiStop) (BD Biosciences, San Jose, CA) that prevent the secretion of cytokines thus trapping them in

intracellular compartments were added to the cultures 1 hour after initiation of the co-culture. At the end of the co-culture, cells were subjected to intracellular cytokine staining and flow cytometry to determine the expression of TNF-α, IFN-γ, IL-2, and IL-17A.

To determine the intracellular cytokines in various subsets of antigen-experienced $CD3^+$ T cells aliquots of cells were stained with the following fluorochrome labeled mouse mAbs: anti-CD3 brilliant violet 605 (BioLegend, San Diego, CA), anti-CD4 V500 (BD Biosciences, San Jose, CA), and anti-CD8 Cy7-allophycocyanin, anti-CD45RO allophycocyanin (BD Biosciences), and anti-CD27 Cy5-Phycoerythrin. Subsequently, cells were fixed and permeabilized with Cytofix/Cytoperm (BD PharMingen, San Jose, CA) solution according to the manufacturer's instructions and stained with the following mouse anti-human cytokine antibodies: anti-IL2 fluorescein isothiocyanate, anti-TNFα Cy7-Phycoerythrin, and anti-IFNγ Alexa Fluor® 700 (all from eBioscience, Inc., San Diego, CA). Flow cytometry data were acquired using the BD Fortessa™ flow cytometer (BD Biosciences) and Flowjo™ software.

Effector T cells generated against BKV LT specifically released polyfunctional cytokines in response to their cognate antigen and cross-reacted with cells expressing MCV LT (FIGS. 3A and 3B). Similarly, effector T cells generated against MCV LT specifically released polyfunctional cytokines in response to their cognate antigen and cross-reacted with cells expressing BKV LT (FIGS. 4A and 4B). T cells generated against BKV LT, JCV LT, or MCV LT were cross-reactive with LT from each polyomavirus (FIG. 8A). The cells were generated using lenti-transduced DCs.

Effector T cells generated against MCV VP1 specifically recognized their cognate antigen, but were inefficient in recognizing BKV VP1 (FIGS. 5A and 5B). Similarly, effector T cells generated against BKV Ia VP1 or BKV IV VP1 specifically recognized their cognate antigen, but only inefficiently recognized MCV VP1 (FIGS. 6A-6B and 7A-7B). However, T cells generated against VP1 from BKV serotype Ia and IV were cross-reactive (FIGS. 6A-6B and 7A-7B). T cells generated against BKV VP1 or JC VP1 were highly cross-reactive against both BKV VP1 and JCV VP1, but less reactive against MCV VP1 and T cells generated against MCV VP1 were only weakly cross-reactive against BKV VP1 or JCV VP1 (FIG. 8B). The cells were generated using lenti-transduced DCs.

Similarly, the BKV LT-specific T cells were highly cross reactive against the peptide libraries derived from the LT of JC virus (FIGS. 3A-3B). The effector T cells against BKV VP1 antigen of both serotypes Ia and IV efficiently recognized the JCV VP1 pepmix pulsed autologous DCs and secreted polyfunctional cytokines (FIGS. 5A, 5B, 6A, and 6B), although they poorly recognized the MCV VP1 expressing autologous DCs as shown earlier.

Example 4

Generating JCV Antigen-Specific Effector T Cells

This example describes particular methods for the generation of JCV antigen-specific effector T cells. However, one skilled in the art will appreciate that methods that deviate from these specific methods can also be used to successfully generate JCV antigen-specific effector T cells.

Lentivirus vectors encoding JCV LT antigen (SEQ ID NO: 13) or JCV VP1 (SEQ ID NO: 15) are constructed as described in Example 1. Immature DCs are prepared as described in Example 2 and cultures (Day 4) are transduced with JCV LT antigen or VP1 lentiviral vectors at a multiplicity of infection (MOI) of approximately 30, in the presence of protamine sulfate in DC culture medium supplemented with hGM-CSF and hIL-4. After 48 hours transduction, cells are washed and cultured in fresh DC culture medium containing hGM-CSF and hIL-4 in the presence of 30 ng/ml endotoxin-free LPS for 24-48 hours.

$CD3^+$ T cells are primed twice at weekly intervals with Lenti-JCV-LT or Lenti-JCV-VP1 transduced autologous DCs at a responder: stimulator ratio of 5:1. Cells are cultured in a 96-well-plate at a density of $1x10^6$ cells/mL CM in a total volume of 200 µl per well. Cultures are treated with IL-7 and IL-15 during first priming and IL-2 in addition to IL-7 and IL-15 during second priming step. No cytokines are present during the initial 3 days of both priming steps. Resulting effector T cell cultures are tested for reactivity against target cells expressing the relevant or irrelevant antigen, or related antigens (such as LT or VP1 from a MCV or BKV), by intracellular cytokine staining and/or flow cytometry as described in Example 3.

Example 5

Enriching Polyomavirus Antigen-Specific T Cells

This example describes methods of enriching a population of polyomavirus antigen-specific T cells.

BKV LT-specific T cells were produced using the methods described in Example 3. Activated T cells were enriched by sorting based on 4-1BB (CD137) expression. Briefly, in vitro generated LT-specific T cells were stimulated with LT antigen for 24 hours, labeled with anti-CD137 antibody and FACS-sorted. Resulting effector T cell cultures were tested for reactivity against target cells expressing BKV LT, MCV LT, or control by intracellular cytokine staining and/or flow cytometry as described in Example 3. As shown in FIG. 9, enriching based on 4-1BB expression dramatically increased the percentage of reactive cells in the population.

This method for enriching polyomavirus antigen-specific T cells can be used regardless of how the T cells are produced. For example, an enrichment step can be used following production of T cells using lentivirally transduced DCs expressing polyomavirus antigen or stimulation with one or more peptides from a polyomavirus protein (such as a peptide library from one or more polyomavirus antigens).

Example 6

Polyomavirus Antigen-Specific Effector T Cells

This example describes an alternative method for generating polyomavirus antigen-specific effector T cells.

Polyomavirus antigen-specific T cells were generated a peptide library (pepmix) containing 15mer peptides overlapping by 11 amino acids spanning the entire protein sequence of the BKV LT or VP1 antigens or the irrelevant control antigen Wilms tumor-1 (WT1) ((Miltenyi Biotec, Auburn, CA; JPT Peptide Technologies GmbH, Berlin, Germany) as shown in FIG. 10. T cells were generated as shown in figure 10, but using just PBMC rather than DCs as antigen presenting cells, are shown in figure 12. Cells shown in figure 11 were generated with lentiviral transduced DCs. Figure 11 shows cross-reactivity and polyfunctionality of PyV-reactive cells generated this way (see Example 4)..

T cells generated with the BKV LT pepmix were reactive with BKV LT, JCV LT, and MCV LT (FIG. 11A and 12). T cells generated with the BKV VP1 pepmix were reactive with BKV VP1, JCV VP1, and MCV VP1 (FIG. 12). This method generates very active products, for example, as shown in Table 3.

Table 3. Reactivity of BKV stimulated T cells

Donor ID	Positive (SEB) %IGN-γ/%TNF-α	BKV Peptide Stimulated % IFN-γ	BKV Peptide Stimulated % TNF-α
W092115050061	39.9/38.5	22.8	30.5
W092115050122	41.0/61.1	38.8	49.9
W092115050196	12.3/10.7	15.2	12.4

Example 7

Determining Cytotoxicity of Antigen-Specific Effector T Cells

This example describes particular methods for determining the cytotoxicity of polyomavirus antigen-specific effector T cells. However, one skilled in the art will appreciate that methods that deviate from these specific methods can also be used to successfully cytotoxicity of antigen-specific effector T cells.

Polyomavirus antigen-specific T cells (such as MCV LT antigen, BKV LT antigen, JCV LT antigen, MCV VP1, BKV VP1, or JCV VP1 specific T cells) are produced using the methods in Examples 1-6. The antigen-specific T cells are tested for cytotoxicity using ^{51}Cr-relaease assay against cells expressing the target protein (such as cell lines stably infected with BKV, MCV, or JCV, or cells transduced to express the target antigen). See, *e.g.,* Walker *et al. Nature* 328:345-8, 1987; Qin *et al. Acta Pharmacol. Sin.* 23(6):534-8, 2002.

Example 8

Methods for Producing Polyomavirus Antigen-Specific T Cells from a Subject

This example provides exemplary methods for producing polyomavirus antigen-specific T cells from a subject, for example, for use in adoptive immunotherapy. However, one skilled in the art will appreciate that methods that deviate from these specific methods can also be used to successfully prepare polyomavirus antigen-specific T cells.

Peripheral blood mononuclear cells (PBMC) are obtained from a subject. The subject may be a donor (such as a partially HLA-matched donor for a subject in need of treatment) or may be from the subject to be treated with the resulting polyomavirus antigen-specific T cells. The mononuclear cells may be obtained by leukapheresis. As illustrated in FIG. 13, the PBMC are divided into two aliquots (for example, aliquots of about 2 x 10^8 cells each). One aliquot is reserved. One aliquot is pulsed with polyomavirus peptides, such as a peptide library including overlapping peptides from one or more of BKV LT, BKV VP1, MCV LT and/or ST at about 0.5 μg/ml (8 μl/peptide). The pulsed cells are irradiated (for example at 25 Gy).

The pulsed and irradiated cells are then mixed with the reserved aliquot of cells at a 1:1 ratio (*e.g.,* 1.5 x10^6 cells/ml) in GMP-grade culture media (for example X-vivo or AIM-V)and 5-10% human serum including 10-50ng/ml of IL-7 and IL-15 (day 0). On day 3, IL-2 is added to the media (for example, by replacing half of the medium with medium containing 30 IU/ml IL-2). The cells are maintained in culture at about 1.5 x 10^6 cells/ml until day 12, when they are maintained at 2 x 10^6 cells/ml. On day 10-14, the cells are harvested, and optionally cryopreserved.

Example 9

Methods of Treating or Inhibiting Polyomavirus Infection

This example provides exemplary methods for treating or inhibiting polyomavirus infection or polyomavirus-associated disease in a subject. However, one skilled in the art will appreciate that methods that deviate from these specific methods can also be used to successfully treat or inhibit polyomavirus infection and/or disease in a subject.

In particular examples, the method includes selecting a subject having, thought to have, or at risk of having polyomavirus infection or polyomavirus-associated disease. Subjects having or thought to have polyomavirus infection or polyomavirus-associated disease include those with $>10^7$ polyomavirus copies per 10 mL urine or histopathological identification of viral alterations in a renal biopsy. Subjects at risk of polyomavirus infection or disease include those who have had or are candidates for organ transplantation (such as renal transplant or bone marrow transplant), and immunocompromised individuals.

Subjects selected for treatment are administered a therapeutically effective amount of a disclosed composition. In some examples, polyomavirus antigen-specific T cells disclosed herein are administered to the subject at doses of about 10^5-10^7 cells/kg. The mode of administration can be any used in the art, including but not limited to intravenous, intraperitoneal, or intrathecal administration. The amount of agent administered to the subject can be determined by a clinician, and may depend on the particular subject treated. Specific exemplary amounts are provided herein (but the disclosure is not limited to such doses).

The inhibition of polyomavirus infection and/or disease in a subject is monitored at time points following administration of the composition. In some examples, a decrease in viremia or a decrease in signs and symptoms of polyomavirus-associated disease in the subject indicates that the antigen-specific T cells are therapeutically effective.

Example 10

Methods of Treating or Inhibiting PML

This example provides exemplary methods for treating or inhibiting PML in a subject. However, one skilled in the art will appreciate that methods that deviate from these specific methods can also be used to successfully treat or inhibit PML.

A schematic of an exemplary protocol for treating a patient with PML is shown in FIG. 15. A donor subject who is at least a partial HLA match for a subject with PML is selected. Cells are collected from the donor by leukapheresis, and polyomavirus antigen-specific T cells are produced using the methods described in Example 3 or Example 8. BK LT and VP1 pepmixes are used to

treat both JC and BK infection. The polyomavirus antigen-specific T cells are administered to the subject with PML by infusion. Safe dose will be determined in a clinical study. Up to 5x10e6 CD3+ T cell/kg will be given in the initial studies, additional doses will be permitted based on clinical responses and safety profile in individual patients. The status of the subject with PML is monitored periodically throughout the protocol, for example by lumbar puncture and measurement of viral load, cellularity, protein and glucose levels (standard tests for viral encephalitis, MRI (*e.g.*, number or size of lesions), and/or neurological evaluation (*e.g.*, cortical signs/symptoms, behavioral or neuropsychological changes, visual deficits, seizures, or hemiparesis). A decrease in one or more signs or symptoms of PML, such as decrease in size or number of lesions, decrease in virus in CSF, and/or improvement in one or more neurological symptoms indicate inhibition of PML in the subject.

Example 11

Methods of Treating or Inhibiting Merkel Cell Carcinoma

This example provides exemplary methods for treating or inhibiting Merkel cell carcinoma (MCC) in a subject. However, one skilled in the art will appreciate that methods that deviate from these specific methods can also be used to successfully treat or inhibit MCC.

A schematic of an exemplary protocol for treating a patient with MCC is shown in FIG. 16. Cells are collected from a partially HLA-matched donor or the subject with MCC by leukapheresis, and polyomavirus antigen-specific T cells are produced using the methods described in Example 3 or Example 8. The polyomavirus antigen-specific cells are produced using DCs transduced with a lentiviral vector encoding a polyomavirus LT (such as MCV LT, BKV LT, or JCV LT) or with a peptide mix generated from MCV LT and/or ST (such as generated from SEQ ID NOs: 37 or 38). Preconditioning will be defined in the clinical research protocol and might include cyclophosphamide and fludarabine chemotherapy. The polyomavirus antigen-specific T cells are administered to the subject with PML by infusion at high doses, up to all cells available. The initial trial will most likely include cell dose escalation to determine safe dose. The initial planned dose of MCC-specific T cells will be 1x10e7 CD3+ T cells/kg. Additional doses of MCC cells will be permitted.. Interleukin-2 at doses of 10000 to 600000 IU/kg every 8 hours will be administered subcutaneously or intravenously to the patient for up to 7days following infusionof the polyomavirus antigen-specific T cells. The status of the subject with MCC is monitored periodically throughout the protocol, for example by clinical examination or using serial CT scanning to evaluate objective responses using standard RECIST criteria._____. A decrease in size

of primary tumor or metaststases as defined by standard RECIST criteria will indicate inhibition of MCC in the subject.

In view of the many possible embodiments to which the principles of the disclosure may be applied, it should be recognized that the illustrated embodiments are only examples and should not be taken as limiting the scope of the invention. Rather, the scope of the invention is defined by the following claims. We therefore claim as our invention all that comes within the scope and spirit of these claims.

We claim:

1. A method of producing polyomavirus antigen-specific T cells, comprising:

contacting a first population of mononuclear cells with a mixture of peptides from a polyomavirus antigen to produce a population of stimulated cells;

contacting the population of stimulated cells with a second population of mononuclear cells to produce a mixture of cells; and

culturing the mixture of cells in the presence of IL-7 and IL-15.

2. A method of producing polyomavirus antigen-specific T cells, comprising:

contacting a population of monocyte-derived dendritic cells with a mixture of peptides from a polyomavirus antigen to produce a population of stimulated cells;

contacting the population of stimulated cells with a population of mononuclear cells to produce a mixture of cells; and

culturing the mixture of cells in the presence of IL-7 and IL-15.

3. The method of claim 1 or claim 2, wherein the mixture of peptides from the polyomavirus antigen comprises overlapping 15 amino acid peptides from the antigen.

4. The method of any one of claims 1 to 3, wherein the polyomavirus antigen comprises a Merkel cell polyomavirus large T antigen, a Merkel cell polyomavirus small T antigen, a BK polyomavirus large T antigen, a Merkel cell polyomavirus VP1 polypeptide, a BK polyomavirus serotype I VP1 polypeptide or a BK polyomavirus serotype IV VP1 polypeptide.

5. The method of any one of claims 1 to 4, wherein culturing the cells in the presence of IL-7 and IL-15 comprises culturing the cells for about 10-14 days.

6. The method of any one of claims 1 to 5, further comprising culturing the mixture of cells in the presence of IL-2 after three days of culture.

7. A method of producing polyomavirus antigen-specific T cells, comprising:

contacting a plurality of T cells with dendritic cells expressing a heterologous polyomavirus antigen in the substantial absence of cytokines followed by the presence of IL-7 and IL-15, thereby producing polyomavirus antigen-specific T cells.

8. The method of claim 7, wherein the dendritic cells expressing a heterologous polyomavirus antigen comprise dendritic cells transduced with a viral vector comprising a nucleic acid encoding the polyomavirus antigen.

9. The method of claim 7 or claim 8, wherein the polyomavirus antigen comprises a large T antigen, a small T antigen, or a VP1 polypeptide.

10. The method of claim 9, wherein the polyomavirus antigen comprises a Merkel cell polyomavirus large T antigen, a Merkel cell polyomavirus small T antigen, a BK polyomavirus large T antigen, a Merkel cell polyomavirus VP1 polypeptide, a BK polyomavirus serotype I VP1 polypeptide or a BK polyomavirus serotype IV VP1 polypeptide.

11. The method of any one of claims 8 to 10, wherein the viral vector is a lentiviral vector.

12. The method of any one of claims 7 to 11, wherein the contacting further comprises contacting the plurality of T cells with the dendritic cells in the substantial absence of cytokines followed by the presence of IL-2, IL-7 and IL-15.

13. The method of any one of claims 7 to 12, wherein the T cells are contacted with the dendritic cells in the substantial absence of cytokines for about three days, followed by the presence of IL-7 and IL-15 or the presence of IL-2, IL-7, and IL-15 for about four days.

14. The method of any one of claims 7 to 13, wherein the T cells are human T cells.

15. A population of polyomavirus antigen-specific T cells produced by the method of any one of claims 1 to 14.

16. The method of any one of claims 1 to 15, further comprising administering the polyomavirus antigen-specific T cells to a subject.

17. The method of claim 16, wherein the polyomavirus antigen-specific T cells are allogeneic T cells or autologous T cells.

18. A population of dendritic cells transduced with a viral vector comprising a nucleic acid encoding a polyomavirus antigen.

19. The population of dendritic cells of claim 18, wherein the viral vector encodes a polyomavirus large T antigen or a VP1 polypeptide.

20. The population of dendritic cells of claim 19, wherein the polyomavirus antigen comprises a Merkel cell polyomavirus large T antigen, a Merkel cell polyomavirus small T antigen, a BK polyomavirus large T antigen, a Merkel cell polyomavirus VP1 polypeptide, a BK polyomavirus serotype I VP1 polypeptide or a BK polyomavirus serotype IV VP1 polypeptide.

21. The population of dendritic cells of any one of claims 18 to 20, wherein the viral vector is a lentiviral vector.

22. The population of dendritic cells of any one of claims 18 to 21, wherein the dendritic cells comprise monocyte-derived dendritic cells and/or mature dendritic cells.

23. The population of dendritic cells of any one of claims 18 to 22, wherein the dendritic cells are human dendritic cells.

24. A method of inducing an immune response to a polyomavirus in a subject, comprising:

transducing dendritic cells with a viral vector comprising a nucleic acid encoding a polyomavirus antigen to produce dendritic cells stably expressing the polyomavirus antigen;

contacting the dendritic cells stably expressing the polyomavirus antigen with a plurality of T cells in the substantial absence of cytokines followed by the presence of IL-7 and IL-15 to produce a population of polyomavirus antigen-specific T cells; and

administering to the subject the population of polyomavirus antigen-specific T cells.

25. A method of inducing an immune response to a polyomavirus in a subject, comprising:

contacting a first population of mononuclear cells with a mixture of peptides from a polyomavirus antigen to produce a population of stimulated cells;

contacting the population of stimulated cells with a second population of mononuclear cells to produce a mixture of cells;

culturing the mixture of cells in the presence of IL-7 and IL-15 for 10-14 days to produce a population of polyomavirus antigen-specific T cells; and

administering to the subject the population of polyomavirus antigen-specific T cells.

26. The method of claim 24 or claim 25, wherein the polyomavirus antigen comprises a large T antigen, a small T antigen, or a VP1 polypeptide.

27. The method of claim 26, wherein the polyomavirus antigen comprises a Merkel cell polyomavirus large T antigen, a Merkel cell polyomavirus small T antigen, a BK polyomavirus large T antigen, a Merkel cell polyomavirus VP1 polypeptide, a BK polyomavirus serotype I VP1 polypeptide or a BK polyomavirus serotype IV VP1 polypeptide.

28. The method of any one of claims 26 to 27, wherein the polyomavirus antigen-specific T cells cross-react with two or more of BKV large T antigen, MCV large T antigen, or JCV large T antigen.

29. The method of any one of claims 24 to 28, wherein the polyomavirus antigen-specific T cells cross-react with two or more BKV serotype VP1 proteins.

30. The method of any one of claims 24 to 29, wherein the subject is infected with BKV, JCV, or MCV.

31. The method of any one of claims 24 to 30, wherein the subject has a polyomavirus-associated disease.

32. The method of claim 31, wherein the polyomavirus-associated disease is progressive multifocal leukoencephalopathy, Merkel cell carcinoma, polyomavirus-associated nephropathy, hemorrhagic cystitis, or trichodysplasia spinulosa (TS).

33. A method of inducing an immune response to a polyomavirus in a subject, comprising administering to the subject a population of polyomavirus large T antigen-specific T cells or BKV VP1-specific T cells produced by contacting dendritic cells transduced with a viral vector comprising a nucleic acid encoding the polyomavirus large T antigen or BKV VP1 polypeptide with a plurality of T cells in the substantial absence of cytokines followed by the presence of IL-7

and IL-15, to produce a population of polyomavirus large T antigen-specific T cells or BKV VP1-specific T cells.

34. A method of inducing an immune response to a polyomavirus in a subject, comprising administering to the subject a population of polyomavirus large T antigen-specific T cells or BKV VP1-specific T cells produced by:

contacting a first population of mononuclear cells with a mixture of peptides from a polyomavirus antigen to produce a population of stimulated cells;

contacting the population of stimulated cells with a second population of mononuclear cells to produce a mixture of cells; and

culturing the mixture of cells in the presence of IL-7 and IL-15 for 10-14 days, to produce a population of polyomavirus large T antigen-specific T cells or BKV VP1-specific T cells.

FIG. 1

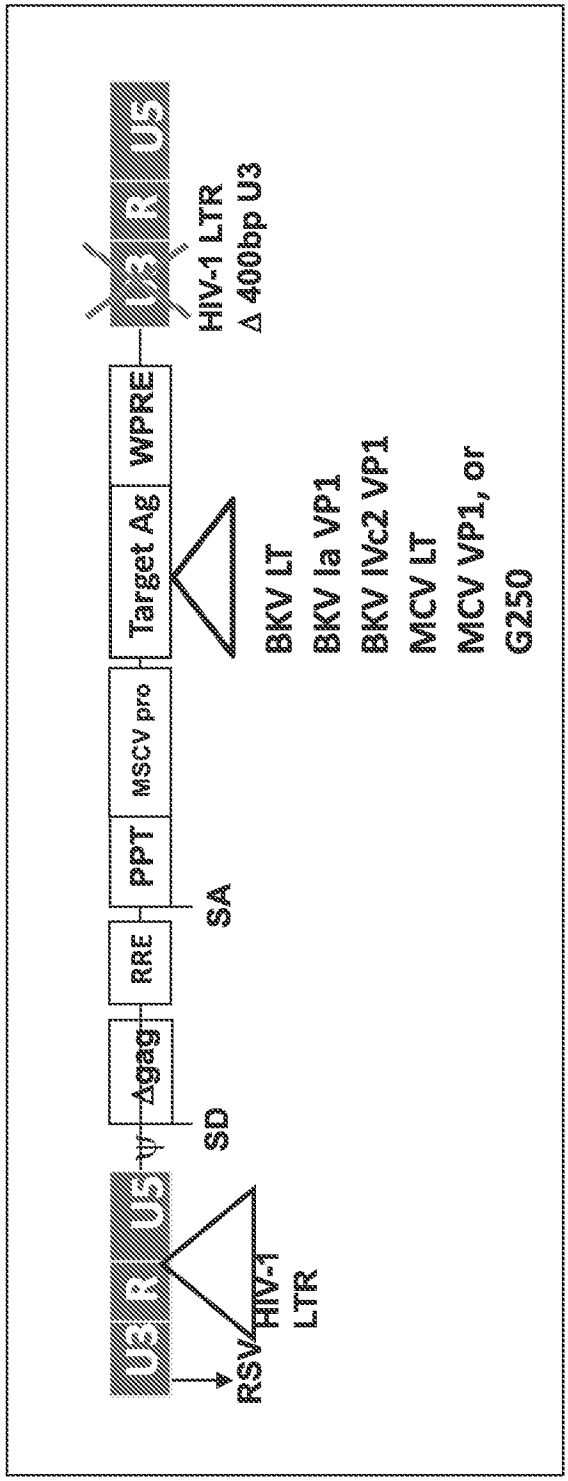

U3
R
U5
RSV
HIV-1
LTR
ψ
Δgag
SD
RRE
SA
PPT
MSCV pro
Target Ag
WPRE
U3
R
U5
HIV-1 LTR
Δ 400bp U3
BKV LT
BKV Ia VP1
BKV IVc2 VP1
MCV LT
MCV VP1, or
G250

FIG. 2

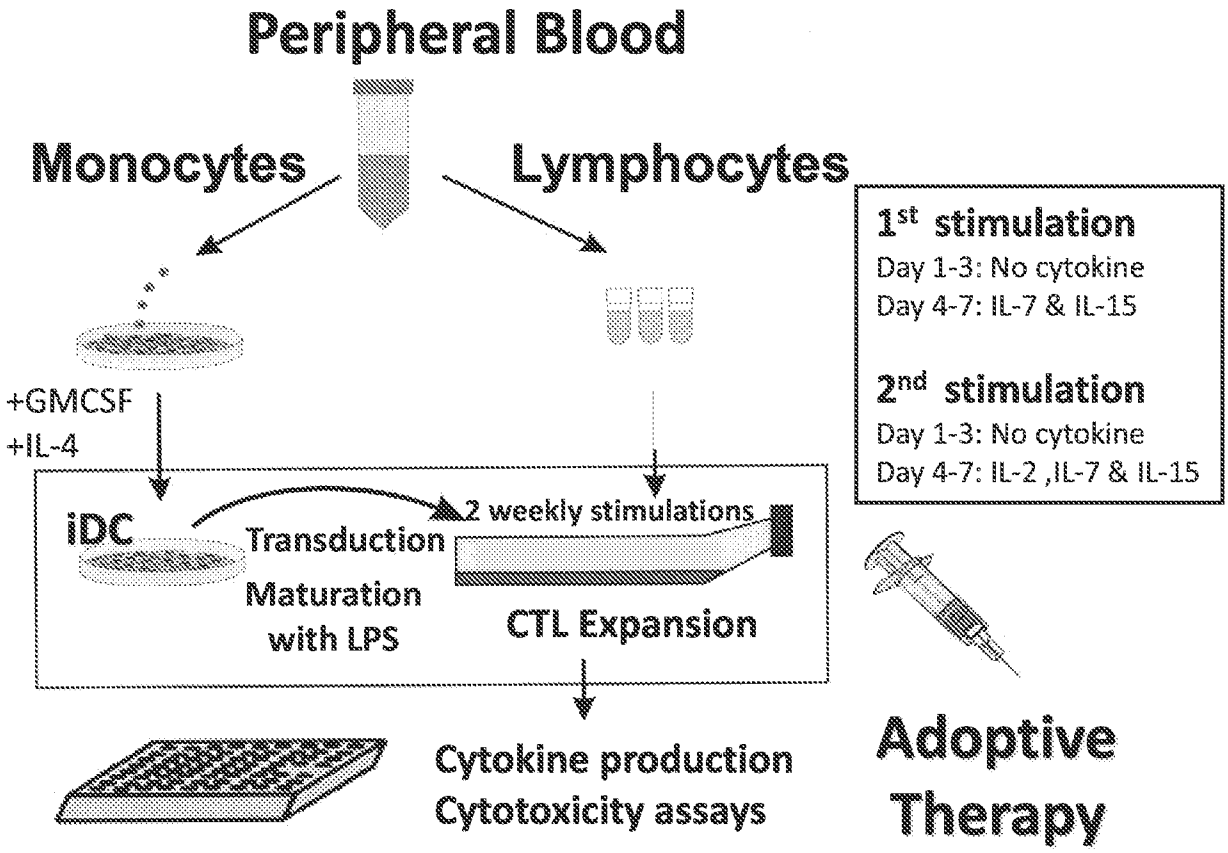
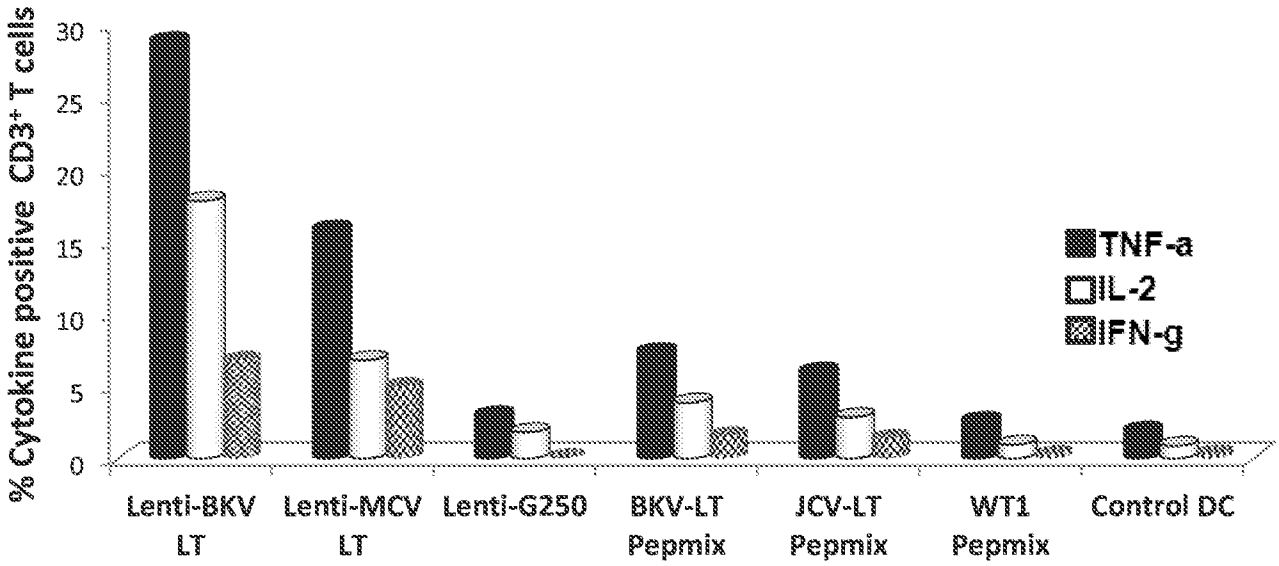

Peripheral Blood
Monocytes
Lymphocytes
1st stimulation
Day 1-3: No cytokine
Day 4-7: IL-7 & IL-15
2nd stimulation
Day 1-3: No cytokine
Day 4-7: IL-2 ,IL-7 & IL-15
+GMCSF
+IL-4
iDC
Transduction
Maturation
with LPS
2 weekly stimulations
CTL Expansion
Cytokine production
Cytotoxicity assays
Adoptive
Therapy

FIG. 3A


% Cytokine positive CD3+ T cells
30
25
20
15
10
5
0
TNF-a
IL-2
IFN-g
Lenti-BKV LT
Lenti-MCV LT
Lenti-G250
BKV-LT Pepmix
JCV-LT Pepmix
WT1 Pepmix
Control DC

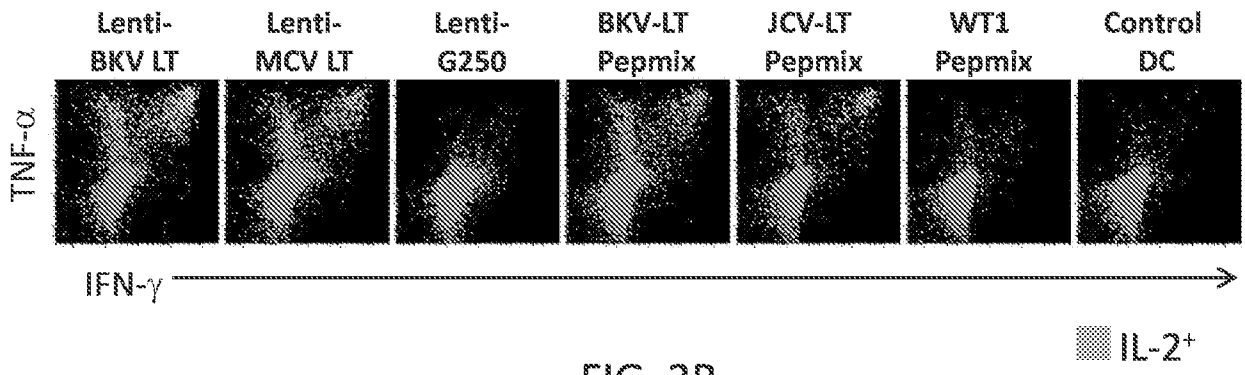
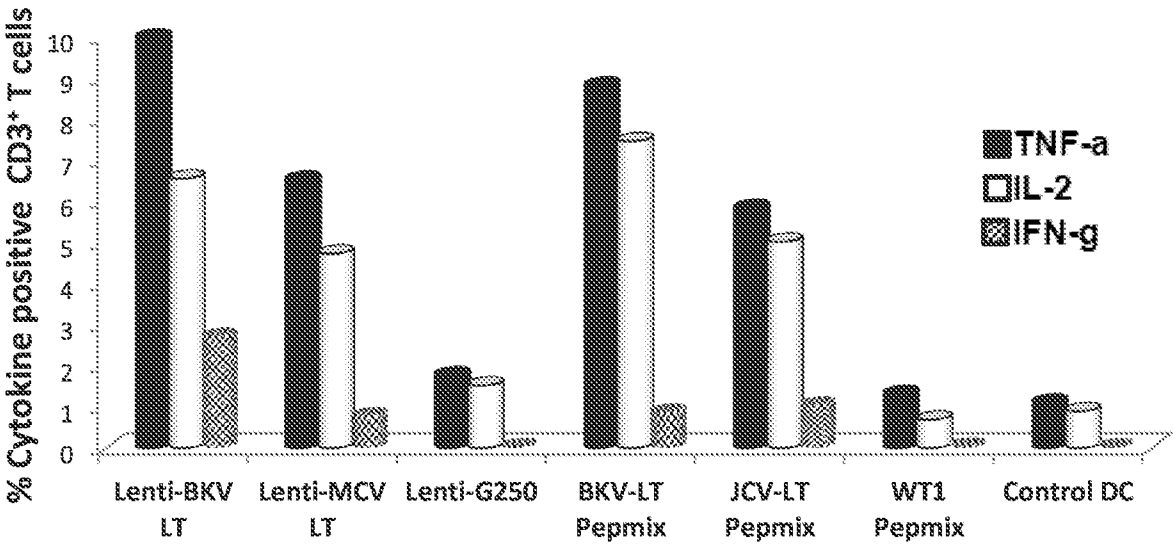

Lenti-BKV LT
Lenti-MCV LT
Lenti-G250
BKV-LT Pepmix
JCV-LT Pepmix
WT1 Pepmix
Control DC
TNF-α
IFN-γ
IL-2+

FIG. 3B

FIG. 4A


% Cytokine positive CD3+ T cells
10
9
8
7
6
5
4
3
2
1
0
TNF-a
IL-2
IFN-g
Lenti-BKV LT
Lenti-MCV LT
Lenti-G250
BKV-LT Pepmix
JCV-LT Pepmix
WT1 Pepmix
Control DC

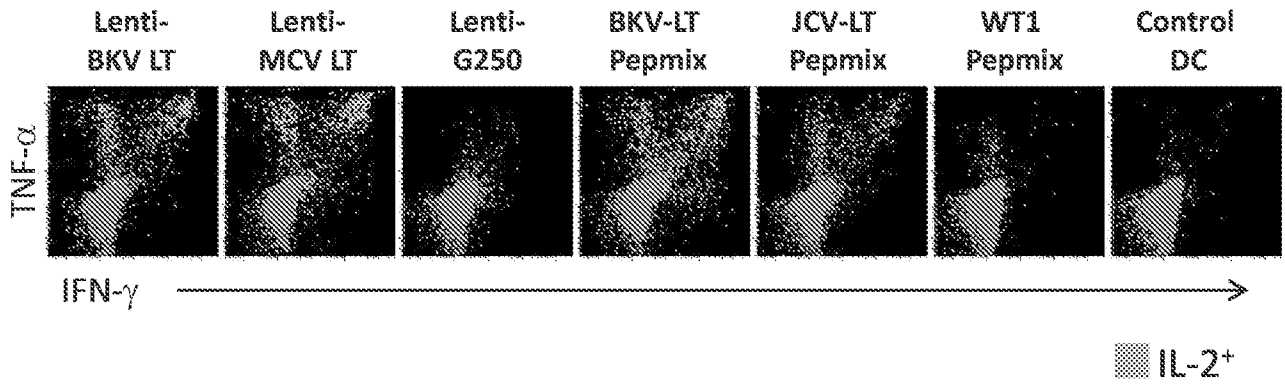
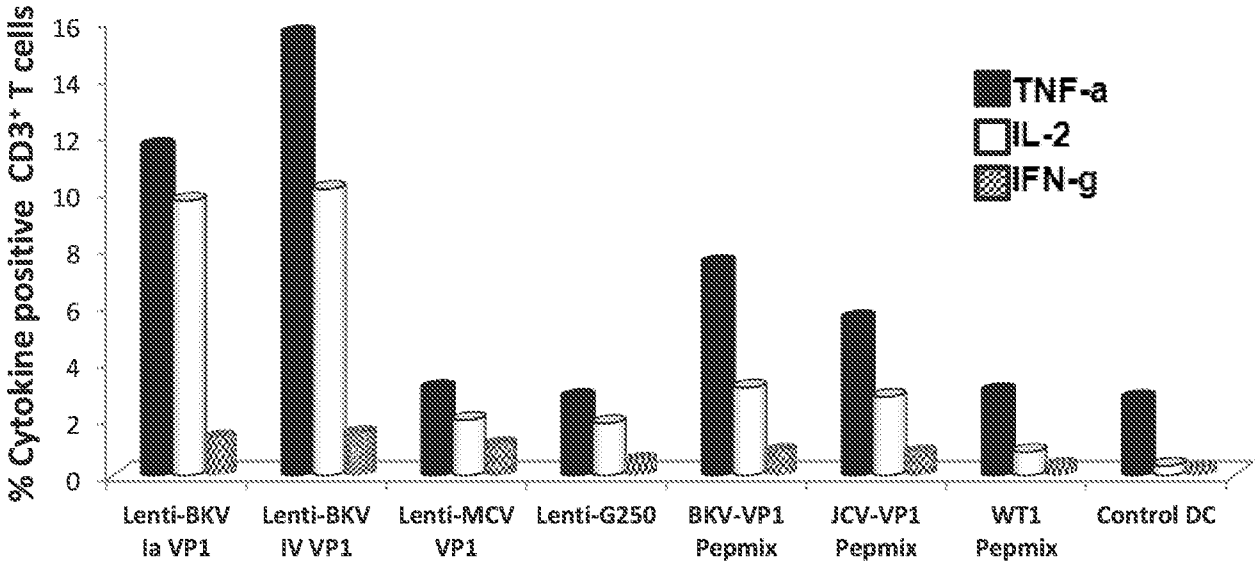

Lenti-BKV LT
Lenti-MCV LT
Lenti-G250
BKV-LT Pepmix
JCV-LT Pepmix
WT1 Pepmix
Control DC
TNF-α
IFN-γ
IL-2+

FIG. 4B

FIG. 5A


% Cytokine positive CD3+ T cells
16
14
12
10
8
6
4
2
0
TNF-a
IL-2
IFN-g
Lenti-BKV Ia VP1
Lenti-BKV IV VP1
Lenti-MCV VP1
Lenti-G250
BKV-VP1 Pepmix
JCV-VP1 Pepmix
WT1 Pepmix
Control DC

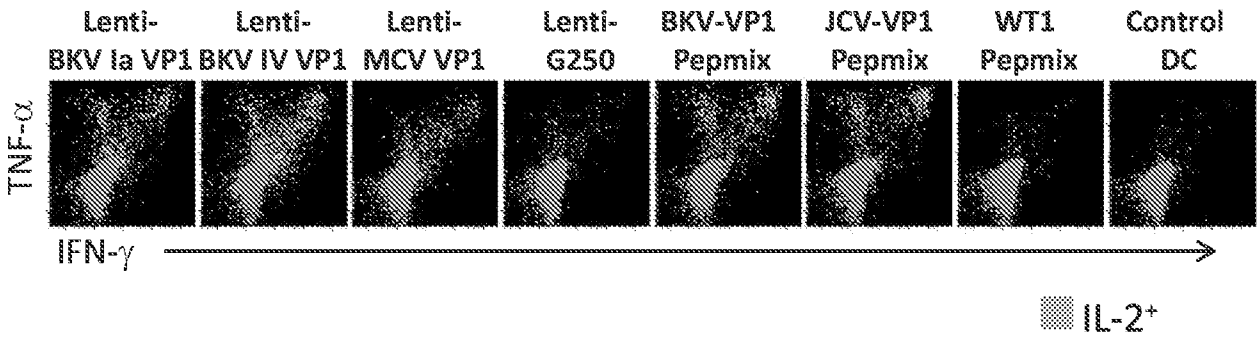
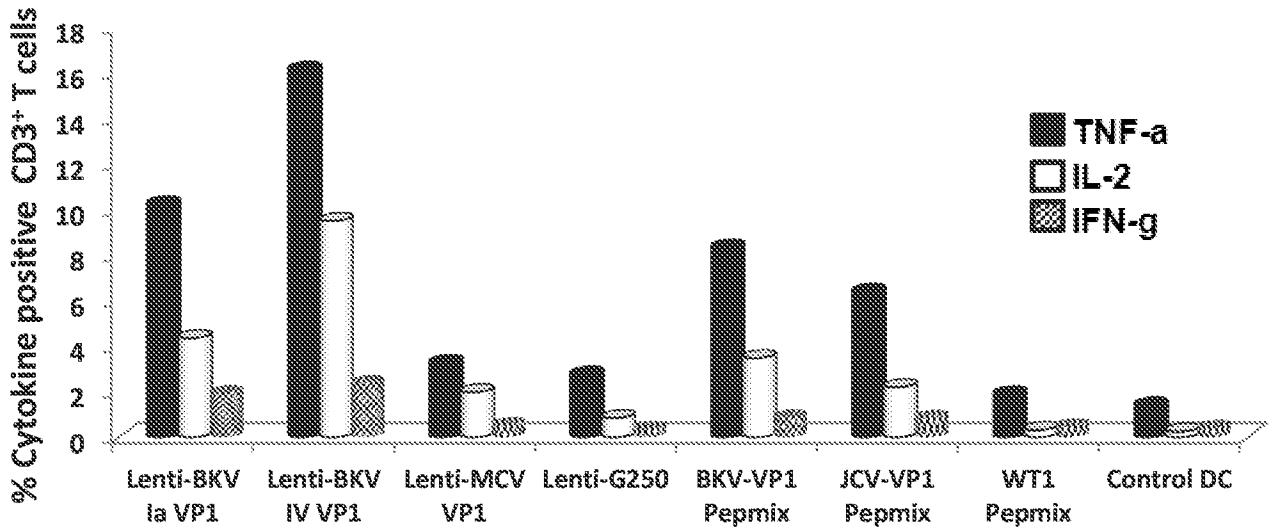

Lenti-BKV Ia VP1
Lenti-BKV IV VP1
Lenti-MCV VP1
Lenti-G250
BKV-VP1 Pepmix
JCV-VP1 Pepmix
WT1 Pepmix
Control DC
TNF-α
IFN-γ
IL-2+

FIG. 5B

FIG. 6A


% Cytokine positive CD3+ T cells
18
16
14
12
10
8
6
4
2
0
TNF-a
IL-2
IFN-g
Lenti-BKV Ia VP1
Lenti-BKV IV VP1
Lenti-MCV VP1
Lenti-G250
BKV-VP1 Pepmix
JCV-VP1 Pepmix
WT1 Pepmix
Control DC

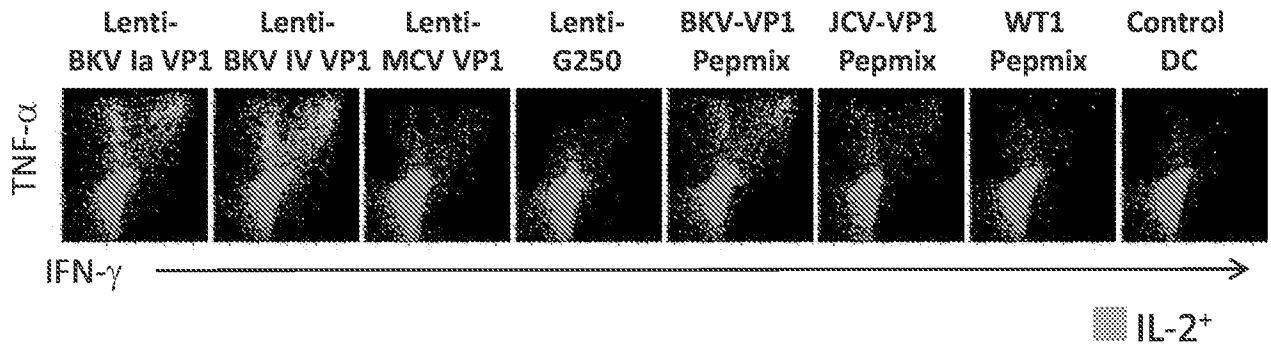
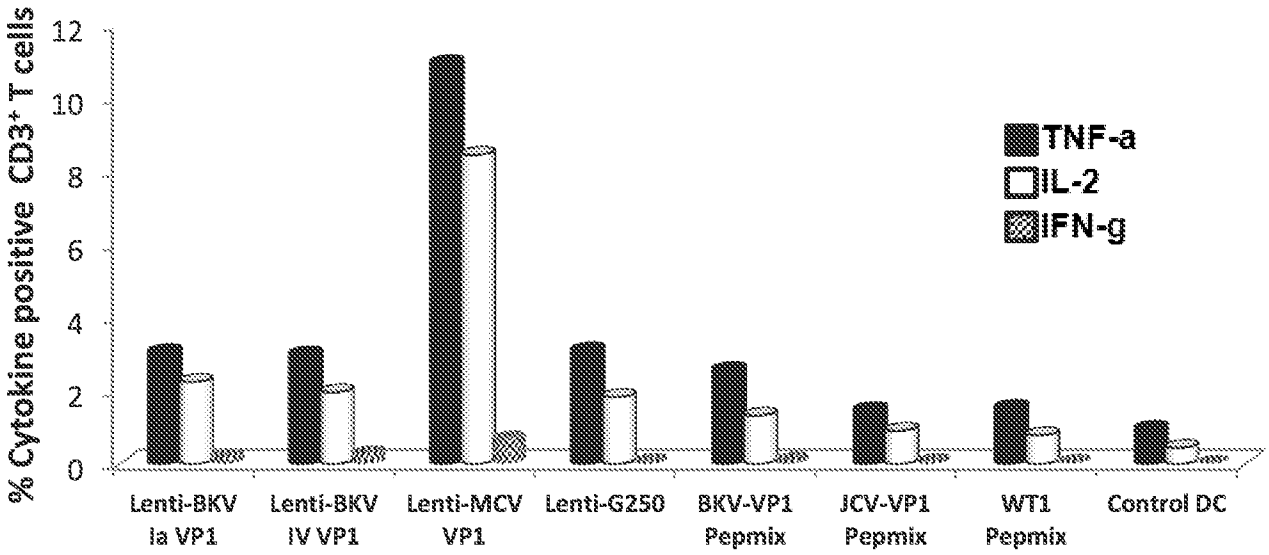

Lenti-BKV Ia VP1
Lenti-BKV IV VP1
Lenti-MCV VP1
Lenti-G250
BKV-VP1 Pepmix
JCV-VP1 Pepmix
WT1 Pepmix
Control DC
TNF-α
IFN-γ
IL-2+

FIG. 6B

FIG. 7A


% Cytokine positive CD3+ T cells
12
10
8
6
4
2
0
TNF-a
IL-2
IFN-g
Lenti-BKV Ia VP1
Lenti-BKV IV VP1
Lenti-MCV VP1
Lenti-G250
BKV-VP1 Pepmix
JCV-VP1 Pepmix
WT1 Pepmix
Control DC

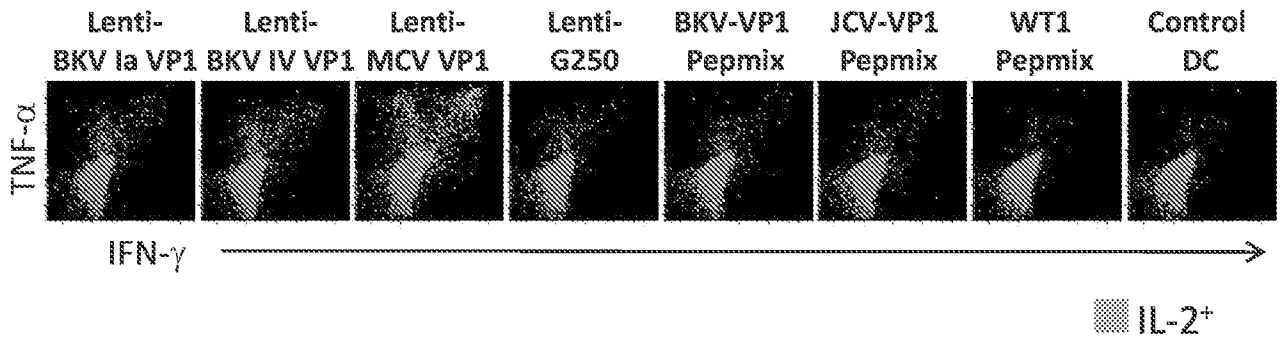

Lenti-BKV Ia VP1
Lenti-BKV IV VP1
Lenti-MCV VP1
Lenti-G250
BKV-VP1 Pepmix
JCV-VP1 Pepmix
WT1 Pepmix
Control DC
TNF-α
IFN-γ
IL-2+

FIG. 7B

FIG. 8A

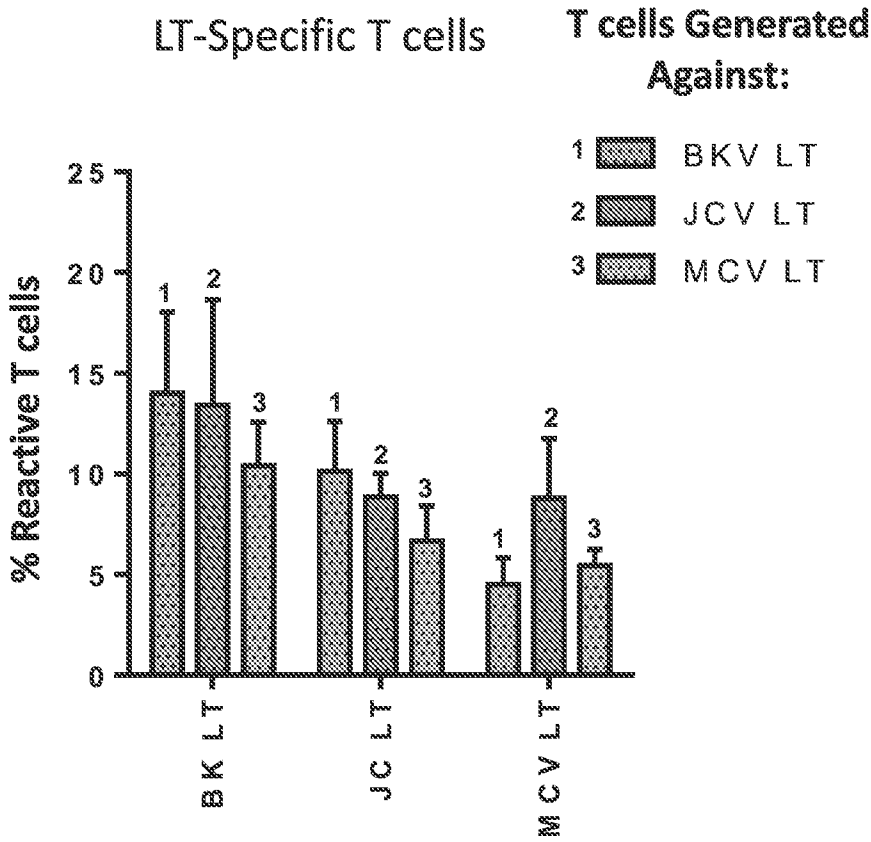

LT-Specific T cells
T cells Generated Against:
1 BKV LT
2 JCV LT
3 MCV LT
% Reactive T cells
25
20
15
10
5
0
BK LT
JC LT
MCV LT

FIG. 8B

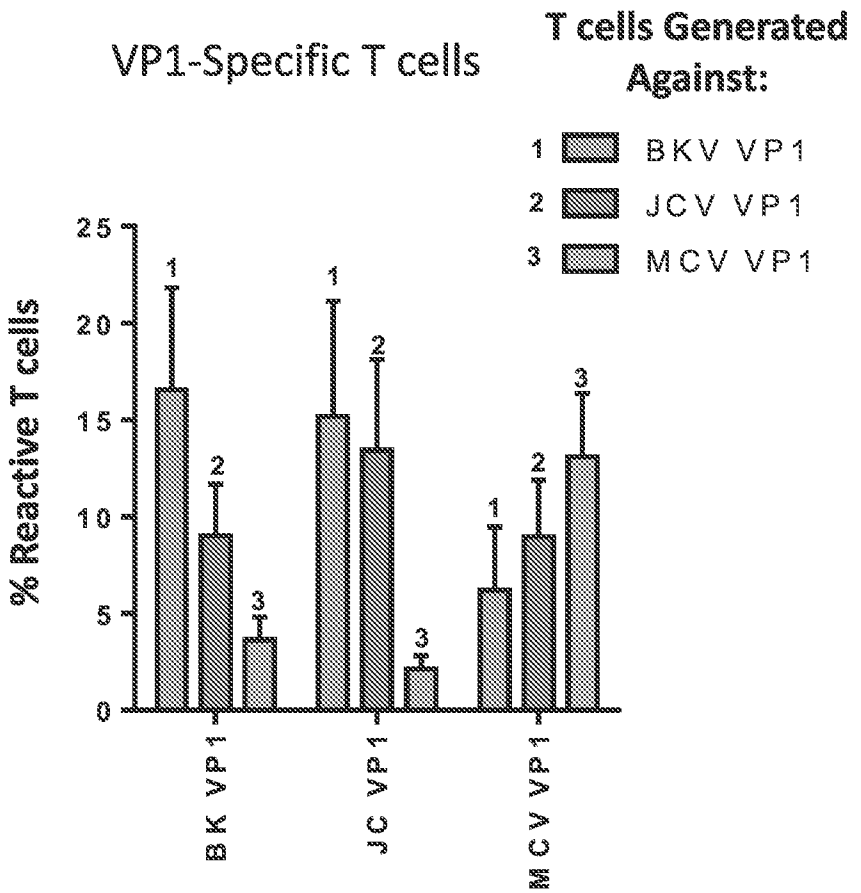

VP1-Specific T cells
T cells Generated Against:
1 BKV VP1
2 JCV VP1
3 MCV VP1
% Reactive T cells
25
20
15
10
5
0
BK VP1
JC VP1
MCV VP1

FIG. 9

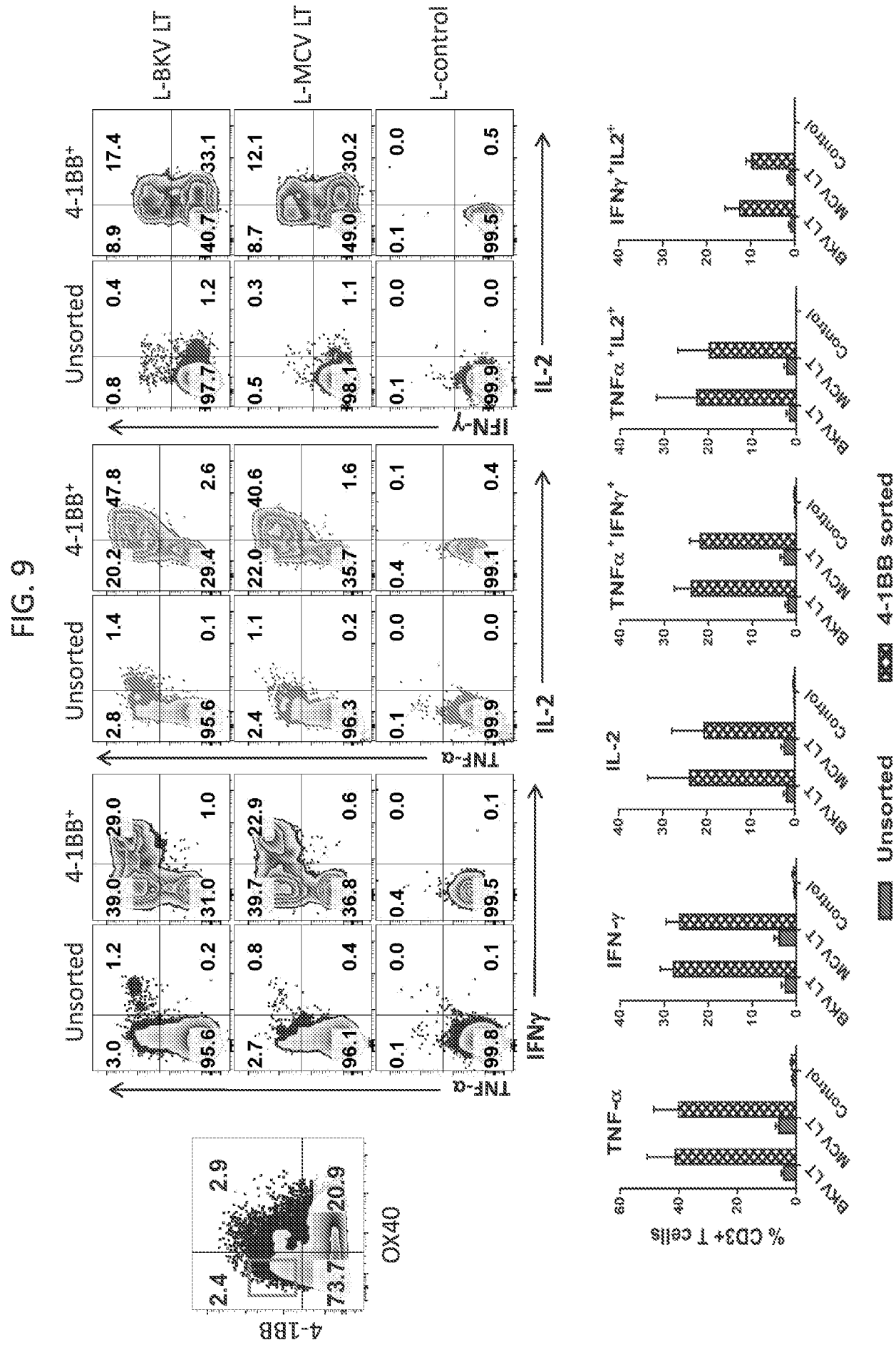

L-BKV LT
L-MCV LT
L-control
Unsorted
4-1BB+
IFNγ
TNF-α
IL-2
4-1BB
OX40
TNF-α
IFN-γ
IL-2
TNFα+IFNγ+
TNFα+IL2+
IFNγ+IL2+
% CD3+ T cells
BKV LT
MCV LT
Control
Unsorted
4-1BB sorted

FIG. 10

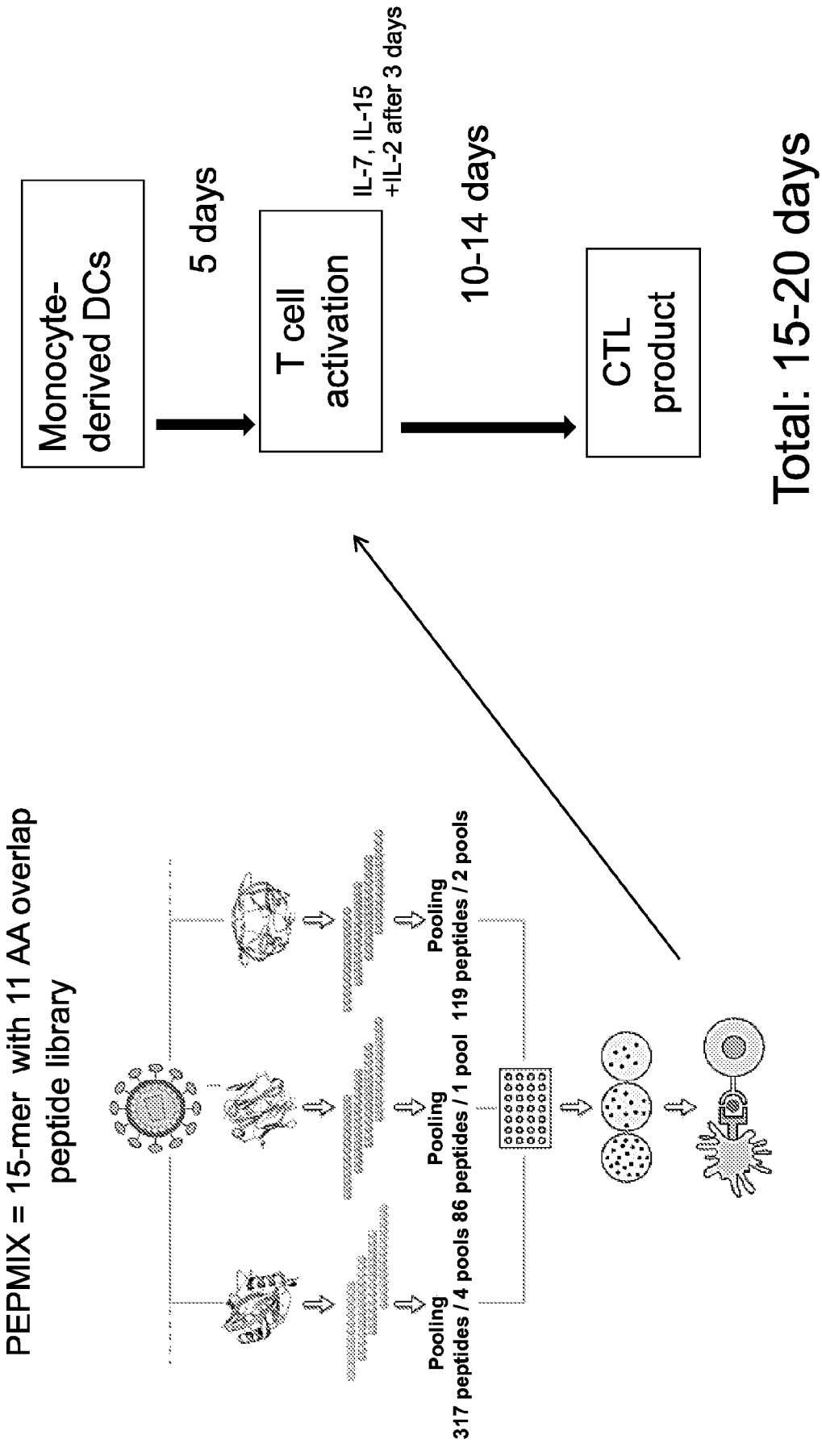

Monocyte-derived DCs
5 days
T cell activation
IL-7, IL-15
+IL-2 after 3 days
10-14 days
CTL product
Total: 15-20 days
PEPMIX = 15-mer with 11 AA overlap peptide library
Pooling
317 peptides / 4 pools
Pooling
86 peptides / 1 pool
Pooling
119 peptides / 2 pools

FIG. 11A

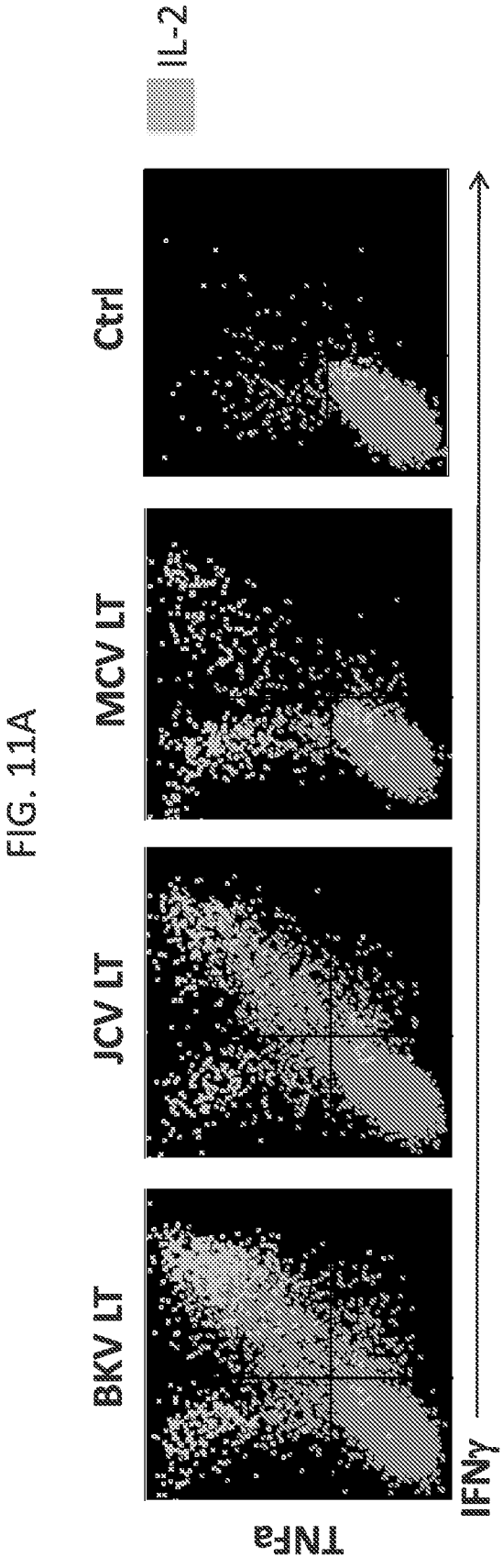
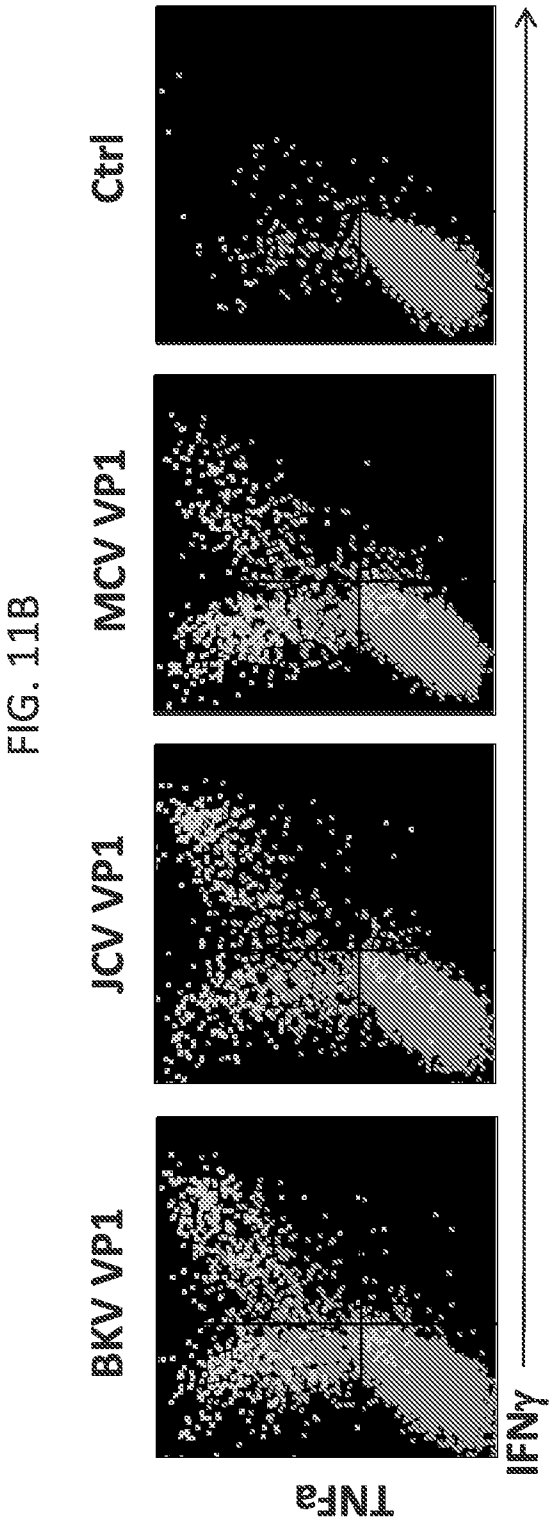


FIG. 11B



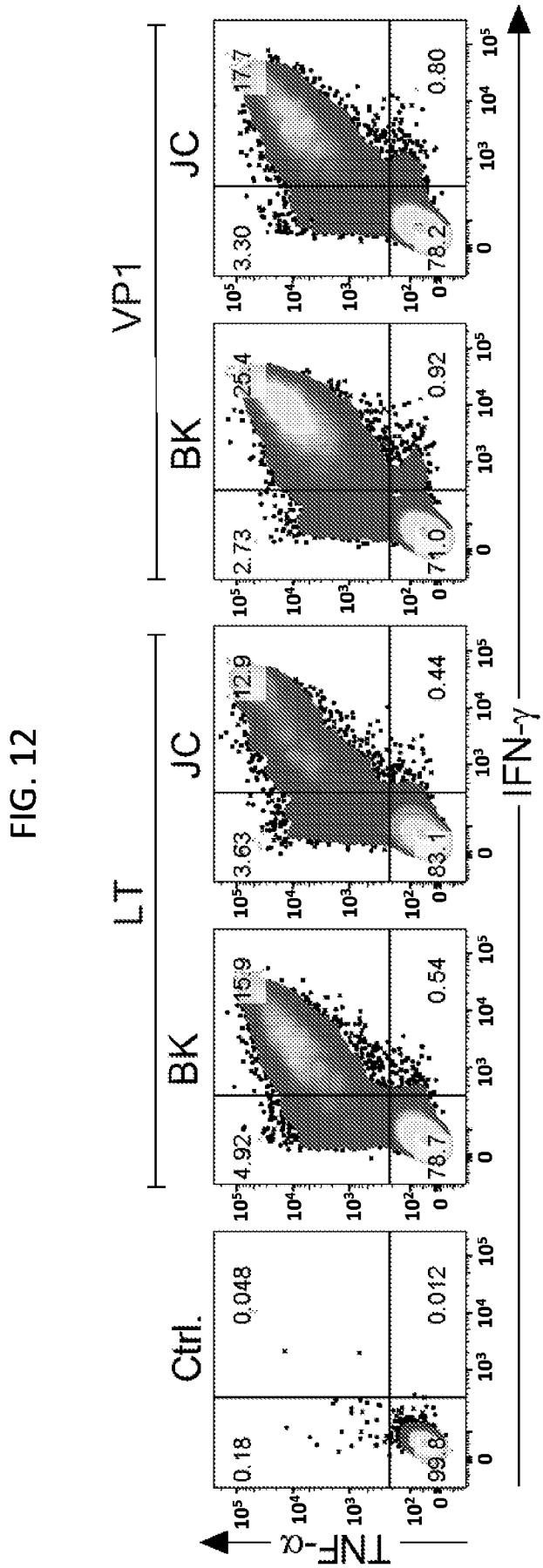

Ctrl.
LT
BK
JC
VP1
BK
JC
TNF-α
IFN-γ
0.18
99.8
0.048
0.012
4.92
78.7
15.9
0.54
3.63
83.1
12.9
0.44
2.73
71.0
25.4
0.92
3.30
78.2
17.7
0.80

FIG. 12

FIG. 13

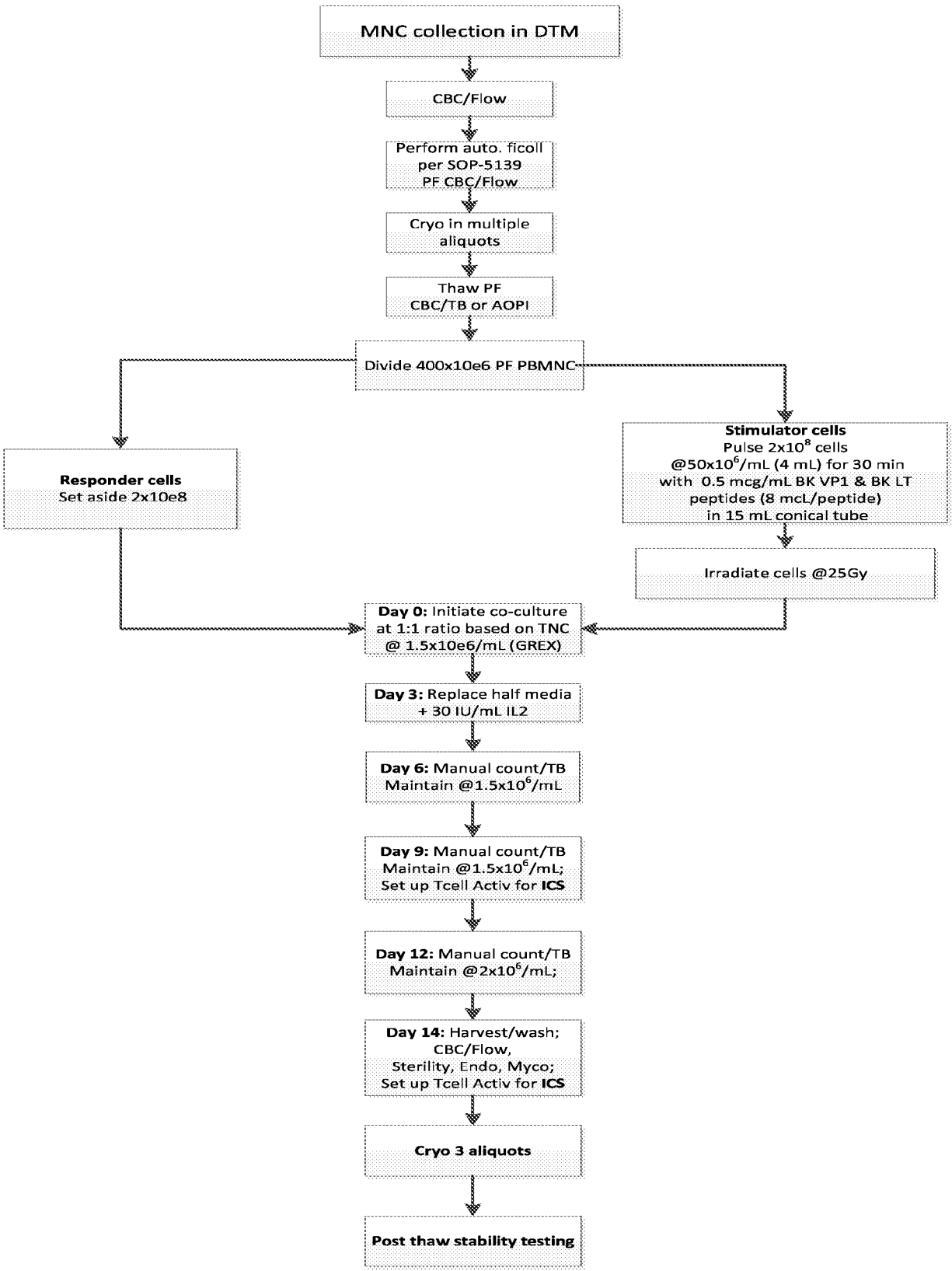

MNC collection in DTM
CBC/Flow
Perform auto. ficoll per SOP-5139 PF CBC/Flow
Cryo in multiple aliquots
Thaw PF CBC/TB or AOPI
Divide 400x10e6 PF PBMNC
Responder cells Set aside 2x10e8
Stimulator cells Pulse 2x10^8 cells @50x10^6/mL (4 mL) for 30 min with 0.5 mcg/mL BK VP1 & BK LT peptides (8 mcL/peptide) in 15 mL conical tube
Irradiate cells @25Gy
Day 0: Initiate co-culture at 1:1 ratio based on TNC @ 1.5x10e6/mL (GREX)
Day 3: Replace half media + 30 IU/mL IL2
Day 6: Manual count/TB Maintain @1.5x10^6/mL
Day 9: Manual count/TB Maintain @1.5x10^6/mL; Set up Tcell Activ for ICS
Day 12: Manual count/TB Maintain @2x10^6/mL;
Day 14: Harvest/wash; CBC/Flow, Sterility, Endo, Myco; Set up Tcell Activ for ICS
Cryo 3 aliquots
Post thaw stability testing

FIG. 14

MCC LT ANTIGEN = LT Exon 1 and 2; truncated):

MDLVLNRKEREALCKLLEIAPNCYGNIPLMKAAFKRSCLKHHPDKGGNPVIMMELNTLWSKFQQ
NIHKLRSDFSMFDEVDEAPIYGTTKFKEWWRSGGFSFGKAYEYGPNPHGTNSRSRKPSSNASRGA
PSGSSPPHSQSSSSGYGSFSASQASDSQSRGPDIPPEHHEEPTSSSGSSSREETTNSGRESSTPNGTS
VPRNSSRTDGTWED**LFCDE**

MCC ST ANTIGEN= LT Exon 1 and ST Exon 1:

MDLVLNRKEREALCKLLEIAPNCYGNIPLMKAAFKRSCLKHHPDKGGNPVIMMELNTLWSKFQQ
NIHKLRSDFSMFDEVSTKFPWEEYGTLKDYMQSGYNARFCRGPGCMLKQLRDSK**CACISC**KLSRQ
HCSLKTLKQKNCLTWGE**CFCYQC**FILWFGFPPTWESFDWWQKTLEETDYCLLHLHLF

FIG. 15

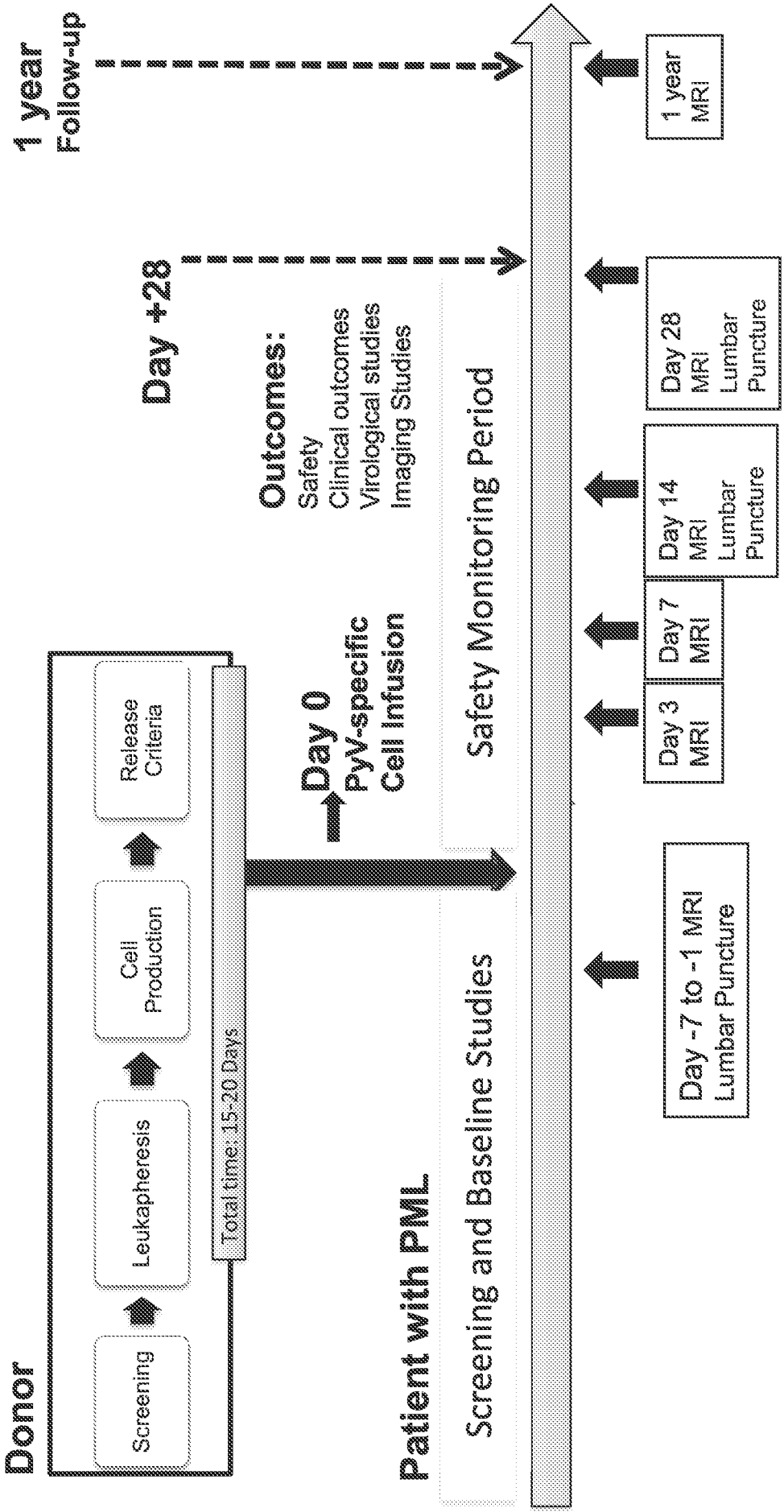
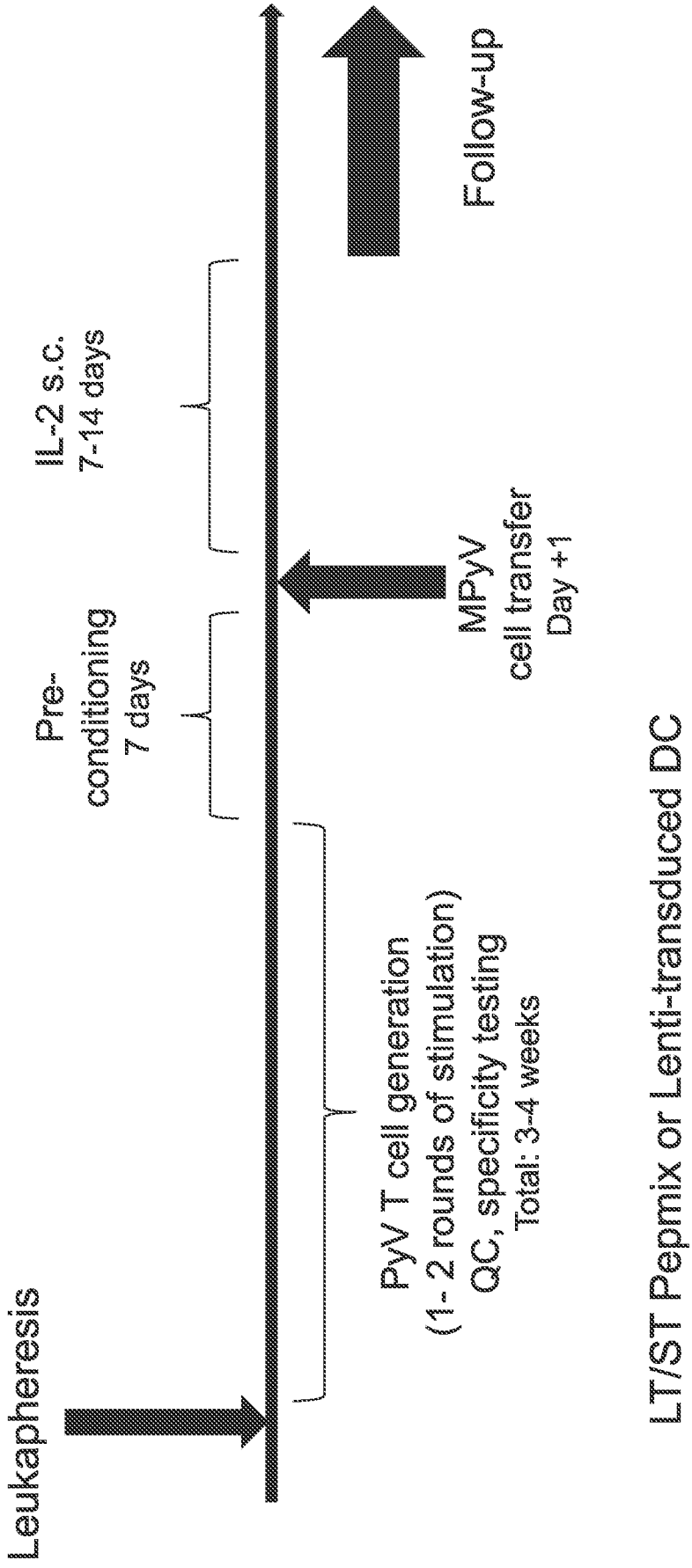

1 year
Follow-up
Day +28
Outcomes:
Safety
Clinical outcomes
Virological studies
Imaging Studies
Donor
Screening
Leukapheresis
Cell Production
Release Criteria
Total time: 15-20 Days
Day 0
PyV-specific
Cell Infusion
Safety Monitoring Period
Patient with PML
Screening and Baseline Studies
Day -7 to -1 MRI
Lumbar Puncture
Day 3 MRI
Day 7 MRI
Day 14 MRI
Lumbar Puncture
Day 28 MRI
Lumbar Puncture
1 year MRI

FIG. 16


Leukapheresis
Pre-conditioning 7 days
IL-2 s.c. 7-14 days
PyV T cell generation (1- 2 rounds of stimulation) QC, specificity testing Total: 3-4 weeks
MPyV cell transfer Day +1
Follow-up
LT/ST Pepmix or Lenti-transduced DC

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/059021

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N5/0784 A61K38/16 A61K39/12 C07K14/01
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SPYRIDONIDIS ALEXANDROS ET AL: "Improved Strategy for Rapid Generation of Quadrivirus-Specific CD8+and CD4+Cytotoxic T Lymphocytes (CTLs) for Adoptive Transfer After Stem Cell Transplantation (SCT)", BLOOD, AMERICAN SOCIETY OF HEMATOLOGY, US, vol. 120, no. 21, 1 December 2012 (2012-12-01), XP009188963, ISSN: 0006-4971	2-5
Y	the whole document	1,8-10, 14
	----- -/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 March 2016	29/03/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Armandola, Elena

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ULRIKE GERDEMANN ET AL: "Nucleofection of DCs to Generate Multivirus-specific T Cells for Prevention or Treatment of Viral Infections in the Immunocompromised Host", MOLECULAR THERAPY, vol. 17, no. 9, 1 September 2009 (2009-09-01), pages 1616-1625, XP055050633, ISSN: 1525-0016, DOI: 10.1038/mt.2009.140	18-20, 22,23
Y	the whole document	8-10,14

X	JONGMING LI ET AL: "T-cell responses to peptide fragments of the BK virus T antigen: implications for cross-reactivity of immune response to JC virus", JOURNAL OF GENERAL VIROLOGY, SOCIETY FOR GENERAL MICROBIOLOGY, SPENCERS WOOD, GB, vol. 87, no. 10, 1 October 2006 (2006-10-01), pages 2951-2960, XP002665598, ISSN: 0022-1317, DOI: 10.1099/VIR.0.82094-0	5
Y	the whole document	2,4

X	EMILY BLYTH ET AL: "BK Virus-Specific T Cells for Use in Cellular Therapy Show Specificity to Multiple Antigens and Polyfunctional Cytokine Responses", TRANSPLANTATION, vol. 92, no. 10, 1 November 2011 (2011-11-01), pages 1077-1084, XP055034458, ISSN: 0041-1337, DOI: 10.1097/TP.0b013e31823328c0	15-17, 24-34
Y	the whole document	1-5

X	PROVENZANO MAURIZIO ET AL: "Characterization of highly frequent epitope-specific CD45RA+/CCR7+/- T lymphocyte responses against p53-binding domains of the human polyomavirus BK large tumor antigen in HLA-A*0201+ BKV-seropositive donors", JOURNAL OF TRANSLATIONAL MEDICINE, BIOMED CENTRAL, LONDON, GB, vol. 4, no. 1, 10 November 2006 (2006-11-10), page 47, XP021024911, ISSN: 1479-5876, DOI: 10.1186/1479-5876-4-47	15
Y	the whole document	2,4

	-/--	

2

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	P. COMOLI: "Dendritic Cells Pulsed with Polyomavirus BK Antigen Induce Ex Vivo Polyoma BK Virus-Specific Cytotoxic T-Cell Lines in Seropositive Healthy Individuals and Renal Transplant Recipients", JOURNAL OF THE AMERICAN SOCIETY OF NEPHROLOGY., vol. 14, no. 12, 1 December 2003 (2003-12-01), pages 3197-3204, XP055255721, US ISSN: 1046-6673, DOI: 10.1097/01.ASN.0000096374.08473.E3	15
Y	the whole document	2-5

X	BALDUZZI A ET AL: "Polyomavirus JC-targeted T-cell therapy for progressive multiple leukoencephalopathy in a hematopoietic cell transplantation recipient", BONE MARROW TRANSPLANTATION, NATURE PUBLISHING GROUP, GB, vol. 46, no. 7, 1 July 2011 (2011-07-01), pages 987-992, XP002729765, ISSN: 0268-3369, DOI: 10.1038/BMT.2010.221 [retrieved on 2010-10-04] the whole document	15

X	PASQUIER DU R A ET AL: "LOW FREQUENCY OF CYTOTOXIC T LYMPHOCYTES AGAINST THE NOVEL HLA-A*0201-RESTRICTED JC VIRUS EPITOPE VP1P36 IN PATIENTS WITH PROVEN OR POSSIBLE PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY", JOURNAL OF VIROLOGY, THE AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 77, no. 22, 1 November 2003 (2003-11-01), pages 11918-11926, XP001181758, ISSN: 0022-538X, DOI: 10.1128/JVI.77.22.11918-11926.2003 the whole document	15

X	KORALNIK I J ET AL: "Association of prolonged survival in HLA-A2<+> progressive multifocal leukoencephalopathy patients with a CTL response specific for a commonly recognized JC virus epitope", THE JOURNAL OF IMMUNOLOGY, THE AMERICAN ASSOCIATION OF IMMUNOLOGISTS, US, vol. 168, no. 1, 1 January 2002 (2002-01-01), pages 499-504, XP002510846, ISSN: 0022-1767 the whole document	15

	-/--	

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J. DYALL ET AL: "Lentivirus-transduced human monocyte-derived dendritic cells efficiently stimulate antigen-specific cytotoxic T lymphocytes", BLOOD, vol. 97, no. 1, 1 January 2001 (2001-01-01), pages 114-121, XP055257113, US ISSN: 0006-4971, DOI: 10.1182/blood.V97.1.114 the whole document	11,21
A	EP 2 548 567 A1 (UNIVERSITAETSKLINIKUM HAMBURG [DE]; UNIV ZUERICH [CH]) 23 January 2013 (2013-01-23) the whole document	1-34

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2015/059021

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 2548567 A1	23-01-2013	AU 2012288955 A1	20-02-2014
		CA 2842543 A1	31-01-2013
		CN 103826653 A	28-05-2014
		EA 201490322 A1	30-05-2014
		EP 2548567 A1	23-01-2013
		EP 2734218 A2	28-05-2014
		HK 1198517 A1	15-05-2015
		JP 2014520877 A	25-08-2014
		KR 20140062702 A	26-05-2014
		US 2014356320 A1	04-12-2014
		WO 2013014134 A2	31-01-2013