

[0004] *In vitro* expanded donor-derived and third party virus-specific T cells targeting Adv, EBV, CMV, BK, HHV6 have shown to be safe when adoptively transferred to stem cell transplant patients with viral infections. Virus-specific T cells reconstituted antiviral immunity for Adv, EBV, CMV, BK and HHV6, were effective in clearing disease, and exhibited considerable expansion *in vivo*. Adoptively transferred *in vitro* expanded virus-specific T cells have also been shown to be safe and associated with clinical benefit when adoptively transferred to patients.

[0005] Embodiments of the present disclosure satisfy a long-felt need in the art by providing therapies for certain viruses by administering ex vivo-expanded, non-genetically modified, virus-specific T cells to control viral infection and ameliorate/eliminate one or more disease symptoms.

SUMMARY OF THE EMBODIMENTS

[0006] In some embodimentexamples, the present disclosure provides a composition comprising a polyclonal population of virus specific T-lymphocytes (VSTs) that recognize a plurality of viral antigens, wherein the plurality of viral antigens comprise at least one first antigen from PIV and at least one second antigen from one or more second viruses. In one aspect, the present disclosure refers to a composition comprising a polyclonal population of cytotoxic T-lymphocytes (CTLs) that recognize a plurality of viral antigens, wherein the plurality of viral antigens comprise at least one first antigen from PIV and at least one second antigen from one or more second viruses. In some embodimentexamples, the VSTs are generated by contacting peripheral blood mononuclear cells (PBMCs) with a plurality of pepmix libraries, each pepmix library containing a plurality of overlapping peptides spanning at least a portion of a viral antigen, wherein at least one of the plurality of pepmix libraries spans a first antigen from PIV-3 and wherein at least one additional pepmix library of the plurality of pepmix libraries spans each second antigen. In some embodimentexamples, the VSTs are generated by contacting T cells with dendritic cells (DCs) primed with a plurality of pepmix libraries, each pepmix library containing a plurality of overlapping peptides spanning at least a portion of a viral antigen, wherein at least one of the plurality of pepmix libraries spans a first antigen from PIV-3 and wherein at least one additional pepmix library of the plurality of pepmix libraries spans each second antigen. In some embodimentexamples, the VSTs are generated by contacting T cells with dendritic cells (DCs) nucleofected with at least one DNA plasmid encoding the PIV-3 antigen and at least one DNA plasmid encoding each second antigen. In some embodimentexamples, the plasmid encodes at least one PIV-3 antigen and at least one of the second antigens. In some embodimentexamples, the VSTs comprise CD4+ T-lymphocytes and CD8+ T- lymphocytes. In some embodimentexamples, the VSTs express $\alpha\beta$ T cell receptors. In some embodimentexamples, the VSTs comprise MHC-restricted T lymphocytes. In some embodimentexamples, the one or more second viruses are selected from the group consisting of respiratory syncytial virus (RSV), Influenza, human metapneumovirus (hMPV) and a combination thereof. In some embodimentexample, the one or more second viruses comprise respiratory syncytial virus (RSV), Influenza, human metapneumovirus, and

a combination thereof. In some [embodimentexamples](#), the one or more second viruses consists of respiratory syncytial virus (RSV), Influenza, human metapneumovirus, and a combination thereof. In some [embodimentexamples](#), the composition comprises 1, 2, 3, or 4 first antigens. In some [embodimentexamples](#), the first antigen is selected from the group consisting of PIV-3 antigen M, PIV-3 antigen HN, PIV-3 antigen N, PIV-3 antigen F, and combinations thereof. In some [embodimentexamples](#), the 4 first antigens are as follows: PIV-3 antigen M, PIV-3 antigen HN, PIV-3 antigen N, and PIV-3 antigen F. In some [embodimentexamples](#), the composition comprises two or three second viruses. In some [embodimentexamples](#), the composition comprises three second viruses. In some [embodimentexamples](#), the three second viruses are influenza, RSV, and hMPV. In some [embodimentexamples](#), the composition comprises at least two second antigens per each second virus. In some [embodimentexamples](#), the composition comprises 1, 2, 3, 4, 5, 6, 7, or 8 second antigens. In some [embodimentexamples](#), the second antigen is selected from the group consisting of influenza antigen NP1, influenza antigen MP1, RSV antigen N, RSV antigen F, hMPV antigen M, hMPV antigen M2-1, hMPV antigen F, hMPV antigen N, and combinations thereof. In some [embodimentexamples](#), the second antigen comprises influenza antigen NP1, influenza antigen MP1, or both. In some [embodimentexamples](#), the second antigen comprises RSV antigen N, RSV antigen F, or both. In some [embodimentexamples](#), the second antigen comprises hMPV antigen M, hMPV antigen M2-1, hMPV antigen F, hMPV antigen N, and combinations thereof. In some [embodimentexamples](#), the second antigen comprises each of influenza antigen NP1, influenza antigen MP1, RSV antigen N, RSV antigen F, hMPV antigen M, hMPV antigen M2-1, hMPV antigen F, hMPV antigen N. In some [embodimentexamples](#), the plurality of antigens comprise PIV-3 antigen M, PIV-3 antigen HN, PIV-3 antigen N, PIV-3 antigen F, influenza antigen NP1, influenza antigen MP1, RSV antigen N, RSV antigen F, hMPV antigen M, hMPV antigen M2-1, hMPV antigen F, and hMPV antigen N. In some [embodimentexamples](#), the plurality of antigens consist of, or consist essentially of, PIV-3 antigen M, PIV-3 antigen HN, PIV-3 antigen N, PIV-3 antigen F, influenza antigen NP1, influenza antigen MP1, RSV antigen N, RSV antigen F, hMPV antigen M, hMPV antigen M2-1, hMPV antigen F, and hMPV antigen N. In some [embodimentexamples](#), the VSTs are cultured ex vivo in the presence of both IL-7 and IL-4. In some [embodimentexamples](#), the multivirus VSTs have expanded sufficiently within 9-18 days of culture such that they are ready for administration to a subject. In some [embodimentexamples](#), the VSTs exhibit one or more properties selected from (a) negligible alloreactivity; (b) less activation induced cell death of antigen-specific T cells harvested from a subject than corresponding antigen-specific T cells harvested from the same subject, but not cultured in the presence of both IL-7 and IL-4; and (c) viability of greater than 70%. In some [embodimentexamples](#), the composition is negative for bacteria and fungi for at least 7 days in culture; exhibit less than 5 EU/ml of endotoxin, and are negative for mycoplasma. In some [embodimentexamples](#), the pepmixes were chemically synthesized and are, optionally >90% pure. In some

embodimentexamples, the VSTs are Th1 polarized. In some embodimentexamples, the VSTs are able to lyse viral antigen-expressing target cells. In some embodimentexamples, the VSTs do not significantly lyse non-infected autologous or allogenic target cells.

[0007] In one aspect, ~~T~~the present disclosure also provides a pharmaceutical composition comprising any one of the compositions disclosed herein, formulated for intravenous delivery, wherein the composition is negative for bacteria and fungi for at least 7 days in culture; exhibit less than 5 EU/ml of endotoxin, and are negative for mycoplasma. For example, in some embodimentexamples, the present disclosure provides a pharmaceutical composition comprising a polyclonal population of virus specific T-lymphocytes (VSTs) that recognize a plurality of viral antigens, wherein the plurality of viral antigens comprise at least one first antigen from PIV and at least one second antigen from one or more second viruses. In some embodimentexamples, the second virus comprises respiratory syncytial virus (RSV), Influenza, and human metapneumovirus. In some embodimentexamples, the VSTs are generated by contacting peripheral blood mononuclear cells (PBMCs) with a plurality of pepmix libraries, each pepmix library containing a plurality of overlapping peptides spanning at least a portion of a viral antigen, wherein at least one of the plurality of pepmix libraries spans a first antigen from PIV-3 and wherein at least one additional pepmix library of the plurality of pepmix libraries spans each second antigen, wherein the pharmaceutical composition is formulated for intravenous delivery, wherein the composition is negative for bacteria and fungi for at least 7 days in culture; exhibit less than 5 EU/ml of endotoxin, and are negative for mycoplasma.

[0008] The present disclosure also provides a method of lysing a target cell with any one or more of the compositions or pharmaceutical compositions disclosed herein. For example, in some embodimentexamples, the present disclosure provides a method of lysing a target cell comprising contacting the target cell with a polyclonal population of virus specific T-lymphocytes (VSTs) that recognizes a plurality of viral antigens, wherein the plurality of viral antigens comprise at least one first antigen from PIV and at least one second antigen from one or more second viruses. In some embodimentexamples, the second virus comprises respiratory syncytial virus (RSV), Influenza, and human metapneumovirus. In some embodimentexamples, the VSTs are generated by contacting peripheral blood mononuclear cells (PBMCs) with a plurality of pepmix libraries, each pepmix library containing a plurality of overlapping peptides spanning at least a portion of a viral antigen, wherein at least one of the plurality of pepmix libraries spans a first antigen from PIV-3 and wherein at least one additional pepmix library of the plurality of pepmix libraries spans each second antigen. In some embodimentexamples,

the contacting occurs in vivo in a subject. In some [embodimentexamples](#), the contacting occurs in vivo via administration of the VSTs to a subject.

[0009] The present disclosure also provides a method of treating or preventing a viral infection comprising administering to a subject in need thereof any one or more of the compositions or pharmaceutical compositions disclosed herein. For example, in some [embodimentexamples](#), the present disclosure provides a method of treating or preventing a viral infection comprising administering to a subject in need thereof a polyclonal population of virus specific T-lymphocytes (VSTs) that recognize a plurality of viral antigens, wherein the plurality of viral antigens comprise at least one first antigen from PIV and at least one second antigen from one or more second viruses. In some [embodimentexamples](#), the second virus comprises respiratory syncytial virus (RSV), Influenza, and human metapneumovirus. In some [embodimentexample](#), the VSTs are generated by contacting peripheral blood mononuclear cells (PBMCs) with a plurality of pepmix libraries, each pepmix library containing a plurality of overlapping peptides spanning at least a portion of a viral antigen, wherein at least one of the plurality of pepmix libraries spans a first antigen from PIV-3 and wherein at least one additional pepmix library of the plurality of pepmix libraries spans each second antigen. In some [embodimentexamples](#), between 5×10^6 and 5×10^7 VSTs / m² administered to the subject. In some [embodimentexamples](#), the subject is administered the VSTs in multiple doses. In one [embodimentexample](#), the subject is administered the VSTs and then the subject's viral load is monitored and if the viral load increases the subject is administered a second dose of the VSTs. In some [embodimentexamples](#), the subject is immunocompromised. In some [embodimentexamples](#), the subject has acute myeloid leukemia, acute lymphoblastic leukemia, or chronic granulomatous disease. In some [embodimentexamples](#), the subject, prior to receiving the VSTs, received: (a) a matched related donor transplant with reduced intensity conditioning; (b) a matched unrelated donor transplant with myeloablative conditioning; (c) a haplo-identical transplant with reduced intensity conditioning; or (d) a matched related donor transplant with myeloablative conditioning. In some [embodimentexamples](#), the subject (a) has received a solid organ transplantation; (b) has received chemotherapy; (c) has an HIV infection; (d) has a genetic immunodeficiency; and/or (e) has received an allogeneic stem cell transplant. In some [embodimentexamples](#), the composition is administered to the subject a plurality of times. In some [embodimentexamples](#), the administration of the composition effectively treats or prevents a viral infection in the subject, wherein the viral infection is selected from the group consisting of parainfluenza virus type 3, respiratory syncytial virus, Influenza, human metapneumovirus, and a combination thereof. In some [embodimentexamples](#), the subject is a human.

[0010] The present disclosure also provides a composition comprising a polyclonal population of VSTs that recognize a plurality of viral antigens, wherein the plurality of viral antigens comprises at least one antigen selected from parainfluenza virus type 3 (PIV-3), respiratory syncytial virus, Influenza, human metapneumovirus, and a combination thereof. In one aspect, the present disclosure refers to a composition comprising a polyclonal population of cytotoxic T-lymphocytes (CTLs) that recognize a plurality of viral antigens, wherein the plurality of viral antigens comprise at least one antigen selected from parainfluenza virus type 3 (PIV-3), respiratory syncytial virus, Influenza, and human metapneumovirus. In some embodimentexamples, VSTs recognize a plurality of viral antigens, wherein the plurality of viral antigens comprises at least one antigen from each of parainfluenza virus type 3, respiratory syncytial virus, Influenza, and human metapneumovirus. In some embodimentexamples, the VSTs recognize a plurality of viral antigens, wherein the plurality of viral antigens comprise at least two antigens from each of parainfluenza virus type 3, respiratory syncytial virus, Influenza, and human metapneumovirus. In some embodimentexamples, the plurality of antigens comprise, consist of, or consist essentially of, PIV-3 antigen M, PIV-3 antigen HN, PIV-3 antigen N, PIV-3 antigen F, influenza antigen NP1, influenza antigen MP1, RSV antigen N, RSV antigen F, hMPV antigen M, hMPV antigen M2-1, hMPV antigen F, and hMPV antigen N. In some embodimentexamples, the composition is a pharmaceutical composition formulated for intravenous delivery. In one aspect, the present disclosure refers to a pharmaceutical composition comprising any one of the compositions disclosed herein formulated for intravenous delivery. In some embodimentexamples, the composition is negative for bacteria and fungi for at least 7 days in culture; exhibit less than 5 EU/ml of endotoxin, and are negative for mycoplasma.

[0011] The present disclosure also provides a method of lysing a target cell comprising contacting the target cell with a composition comprising a polyclonal population of VSTs that recognize a plurality of viral antigens, wherein the plurality of viral antigens comprise at least one antigen selected from parainfluenza virus type 3 (PIV-3), respiratory syncytial virus, Influenza, and human metapneumovirus. In some embodimentexamples, the composition is a pharmaceutical composition. In some embodimentexamples, the contacting occurs in vivo in a subject. In some embodimentexamples, the contacting occurs in vivo via administration of the VSTs to a subject.

[0012] The present disclosure also provides a method of treating or preventing a viral infection comprising administering to a subject in need thereof cell a composition comprising a polyclonal population of VSTs that recognize a plurality of viral antigens, wherein the plurality of viral antigens comprise at least one antigen selected from parainfluenza virus type 3 (PIV-3), respiratory syncytial virus, Influenza, and human metapneumovirus. In some embodimentexamples, the composition is a pharmaceutical composition. In some embodimentexamples, the composition is administered to the subject a plurality of times. In some embodimentexamples, the administration of the composition effectively treats or prevents a viral infection in the subject, wherein the viral infection is selected from the group consisting of parainfluenza virus type 3, respiratory syncytial virus, Influenza, and human metapneumovirus. In some embodimentexamples, the subject is a human.

[0012a] In one aspect, the present disclosure refers to a method of lysing a target cell comprising contacting the target cell with any one of the compositions disclosed herein or the pharmaceutical composition disclosed herein *in vitro*.

[0012b] In one aspect, the present disclosure refers to use of any one of the compositions disclosed herein or the pharmaceutical composition disclosed herein, in the manufacture of a medicament for lysing a target cell, wherein the medicament is to be contacted with the target cell *in vivo* in a subject.

[0012c] In one aspect, the present disclosure refers to use of any one of the compositions disclosed herein or the pharmaceutical composition disclosed herein, in the manufacture of a medicament for treating or preventing a viral infection in a subject in need thereof.