CAR). There are several disadvantages to this method: a) autologous TCR T cells have to be generated anew each time for treatment of a new patient, b) the TCR can only be used in humans that express the correct MHC allele, c) since the TCR is highly specific for a given peptide, mutations in the peptide sequence results in escape from TCR recognition. With respect to a) there are numerous efforts in industry and academia to generate "off the shelf" heterologous T cells, *i.e.*, T cell lines that would not be rejected when given to another person. However, there is currently no solution for b) and c).

[0006] Occasionally it has been observed that a single TCR can recognize more than one peptide presented by the same or different MHC molecules. However, up to now, no method existed that can specifically generate single TCRs with multiple (unrelated) specificities.

BRIEF SUMMARY ~~OF THE INVENTION~~

[0007] The present ~~invention~~ disclosure relates to a method of generating CD8+ T cells comprising multi-specific TCRs, the method comprising: (a) administering to a subject a recombinant cytomegalovirus (CMV) vector comprising a nucleic acid sequence that encodes a first heterologous antigen, in an amount effective to generate a first set of CD8+ T cells that recognize a first MHC/heterologous antigen-derived peptide complex, wherein the CMV vector does not express an active UL128, UL130, UL146 and UL147 protein or orthologs thereof; (b) identifying a first CD8+ TCR from the first set of CD8+ T cells, wherein the first CD8+ TCR recognizes the first MHC/heterologous antigen-derived peptide complex; (c) administering to the subject a second heterologous antigen in an amount effective to generate a second set of CD8+ T cells that recognizes a second MHC/heterologous antigen-derived peptide complex; (d) isolating one or more CD8+ T cells from the second set of CD8+ T cells; (e) identifying a second CD8+ TCR from the second set of CD8+ T cells, wherein the second CD8+ TCR recognizes the first MHC/heterologous antigen-derived peptide complex and the second MHC/heterologous antigen-derived peptide complex; (f) transfecting a third set of CD8+ T cells with an expression vector, wherein the expression vector comprises a nucleic acid sequence encoding a third CD8+ TCR and a promoter operably linked to the nucleic acid sequence encoding the third CD8+ TCR, wherein the third CD8+ TCR comprises CDR3α and CDR3β of the second CD8+ TCR, thereby generating one or more CD8+ T cells that

recognize the first MHC/heterologous antigen-derived peptide complex and the second MHC/heterologous antigen-derived peptide complex; and (g) selecting one or more of the third CD8+ TCRs with the highest avidity for a specific peptide of interest. The present disclosure further relates to a CD8 + T cell comprising the multi-specific TCR generated by the method disclosed herein. In another aspect, the present disclosure relates to the CD8 + T cell disclosed herein for use in treating or preventing cancer or treating a pathogenic infection in a subject in need thereof.

[0008] In one ~~embodiment~~example, the recombinant CMV vector does not express an active UL18 protein. In one ~~embodiment~~example, the recombinant CMV vector expresses an active UL40 protein, or ortholog thereof, and an active US28 protein, or ortholog thereof.

[0009] In one ~~embodiment~~example, the first MHC/heterologous antigen-derived peptide complex is a MHC-II/heterologous antigen-derived peptide complex, a MHC-E/heterologous antigen-derived peptide complex, or a MHC-I/heterologous antigen-derived peptide complex. In one ~~embodiment~~example, the second MHC/ heterologous antigen-derived peptide complex is a MHC-II/heterologous antigen-derived peptide complex or a MHC-E/ heterologous antigen-derived peptide complex.

[0010] In one ~~embodiment~~example, the subject is a human or non-human primate. In one ~~embodiment~~example, the recombinant CMV vector is a recombinant human CMV vector or a recombinant rhesus macaque CMV vector.

[0011] In one ~~embodiment~~example, the first and/or second heterologous antigen comprises a tumor antigen, pathogen-specific antigen, a tissue specific antigen, or a host-self antigen. In one example~~embodiment~~, the tumor antigen is related to a cancer selected from the group consisting of prostate cancer, kidney cancer, lung cancer, pancreatic cancer, mesothelioma, breast cancer, acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, non-Hodgkin's lymphoma, multiple myeloma, malignant melanoma, ovarian cancer, colon cancer, renal cell carcinoma, and cervical cancer. In one example~~embodiment~~, the pathogen-specific antigen is related to a pathogen selected from the group consisting of human immunodeficiency virus, herpes simplex virus type 1, herpes simplex virus type 2, hepatitis B virus, hepatitis C virus, papillomavirus, *Plasmodium* parasites, Epstein-barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), Human T-lymphotropic virus type 1 (HTLV1), merkel virus (MCV), cytomegalovirus, and *Mycobacterium tuberculosis*.

[0012] In one example~~embodiment~~, the first CD8+ TCR recognizes specific MHC-II subtopes or supertopes. In one example~~embodiment~~, the first CD8+ TCR recognizes specific MHC-E

subtopes or supertopes. In one example~~embodiment~~, wherein the first CD8+ TCR recognizes specific MHC-I subtopes or supertopes.

[0013] In one example~~embodiment~~, the first CD8+ TCR is identified by DNA or RNA sequencing. In another example~~embodiment~~, the first CD8+ TCR is identified by single cell sequencing.

[0014] In one example~~embodiment~~, the first heterologous antigen and second heterologous antigens are the same. In one example~~embodiment~~, the first heterologous antigen and second heterologous antigen are different.

[0015] In one example~~embodiment~~, the one or more isolated CD8+ T cells from the second set of CD8+ T cells express CD69 and TNFα.

[0016] In one example~~embodiment~~, the second CD8+ TCR recognizes one or more specific MHC-II supertopes. In one example~~embodiment~~, the second CD8+ TCR recognizes one or more specific MHC-E supertopes. In one example~~embodiment~~, the second CD8+ TCR recognizes one or more specific MHC-I supertopes.

[0017] In one example~~embodiment~~, the second CD8+ TCR recognizes a MHC-II supertope and a MHC-E supertope. In one example~~embodiment~~, the second CD8+ TCR recognizes a MHC-I supertope and a MHC-E supertope. In one example~~embodiment~~, the second CD8+ TCR recognizes a MHC-I supertope and a MHC-II supertope.

[0018] In one example~~embodiment~~, the second CD8+ TCR recognizes one or more specific MHC-II subtopes. In one example~~embodiment~~, the second CD8+ TCR recognizes one or more specific MHC-E subtopes. In one example~~embodiment~~, wherein the second CD8+ TCR recognizes one or more specific MHC-I subtopes.

[0019] In one example~~embodiment~~, the second CD8+ TCR recognizes a MHC-II subtope and a MHC-E subtope. In one example~~embodiment~~, the second CD8+ TCR recognizes a MHC-II subtope and a MHC-I subtope. In one example~~embodiment~~, the second CD8+ TCR recognizes a MHC-E subtope and a MHC-I subtope.

[0020] In one example~~embodiment~~, the second CD8+ TCR recognizes a MHC-II subtope or supertope and a MHC-E subtope or supertope. In one example~~embodiment~~, the second CD8+ TCR recognizes a MHC-II subtope or supertope and a MHC-I subtope or supertope. In one example~~embodiment~~, the second CD8+ TCR recognizes a MHC-E subtope or supertope and a MHC-I subtope or supertope.

[0021] In one ~~embodiment~~example, the second CD8+ TCR recognizes specific MHC-II supertopes and MHC-II subtopes. In one ~~embodiment~~example, the second CD8+ TCR recognizes specific MHC-E supertopes and MHC-E subtopes. In one ~~embodiment~~example, the second CD8+ TCR recognizes specific MHC-I supertopes and MHC-I subtopes.

[0022] In one ~~embodiment~~example, the second CD8+ TCR recognizes more than one MHC-II supertope from the same antigen. In one ~~embodiment~~example, the second CD8+ TCR recognizes more than one MHC-E supertope from the same antigen. In one ~~embodiment~~example, the second CD8+ TCR recognizes more than one MHC-I supertope from the same antigen.

[0023] In one ~~embodiment~~example, the second CD8+ TCR recognizes more than one MHC-II subtope from the same antigen. In one ~~embodiment~~example, the second CD8+ TCR recognizes more than one MHC-E subtope from the same antigen. In one ~~embodiment~~example, the second CD8+ TCR recognizes more than one MHC-I subtope from the same antigen.

[0024] In one ~~embodiment~~example, the second CD8+ TCR recognizes one or more MHC-II supertopes and one or more MHC-II subtopes from the same antigen. In one ~~embodiment~~example, the second CD8+ TCR recognizes one or more MHC-E supertopes and one or more MHC-E subtopes from the same antigen. In one ~~embodiment~~example, the second CD8+ TCR recognizes one or more MHC-I supertopes and one or more MHC-I subtopes from the same antigen.

[0025] In one ~~embodiment~~example, the second CD8+ TCR recognizes more than one MHC-II supertope from more than one antigen. In one ~~embodiment~~example, the second CD8+ TCR recognizes more than one MHC-E supertope from more than one antigen. In one ~~embodiment~~example, the second CD8+ TCR recognizes more than one MHC-I supertope from more than one antigen.

[0026] In one ~~embodiment~~example, the second CD8+ TCR recognizes more than one MHC-II subtope from more than one antigen. In one ~~embodiment~~example, the second CD8+ TCR recognizes more than one MHC-E subtope from more than one antigen. In one ~~embodiment~~example, the second CD8+ TCR recognizes more than one MHC-I subtope from more than one antigen.

[0027] In one ~~embodiment~~example, the second CD8+ TCR recognizes one or more MHC-II supertopes and one or more MHC-II subtopes from different antigens. In one ~~embodiment~~example, the second CD8+ TCR recognizes one or more MHC-E supertopes and one or more MHC-E subtopes from different antigens. In one ~~embodiment~~example, the second CD8+

TCR recognizes one or more MHC-I supertopes and one or more MHC-I subtopes from different antigens.

[0028] In one ~~embodiment~~example, the third CD8+ TCR recognizes one or more specific MHC-II supertopes. In one ~~embodiment~~example, the third CD8+ TCR recognizes one or more specific MHC-E supertopes. In one ~~embodiment~~example, the third CD8+ TCR recognizes one or more specific MHC-I supertopes.

[0029] In one ~~embodiment~~example, the third CD8+ TCR recognizes one or more specific MHC-II subtopes. In one ~~embodiment~~example, the third CD8+ TCR recognizes one or more specific MHC-E subtopes. In one ~~embodiment~~example, the third CD8+ TCR recognizes one or more specific MHC-I subtopes.

[0030] In one ~~embodiment~~example, the third CD8+ TCR recognizes specific MHC-II supertopes and MHC-II subtopes. In one ~~embodiment~~example, the third CD8+ TCR recognizes specific MHC-E supertopes and MHC-E subtopes. In one ~~embodiment~~example, the third CD8+ TCR recognizes specific MHC-I supertopes and MHC-I subtopes.

[0031] In one ~~embodiment~~example, the third CD8+ TCR recognizes more than one MHC-II supertope from one antigen. In one ~~embodiment~~example, the third CD8+ TCR recognizes more than one MHC-E supertope from one antigen. In one ~~embodiment~~example, the third CD8+ TCR recognizes more than one MHC-I supertope from one antigen.

[0032] In one ~~embodiment~~example, the third CD8+ TCR recognizes more than one MHC-II subtope from one antigen. In one ~~embodiment~~example, the third CD8+ TCR recognizes more than one MHC-E subtope from one antigen. In one ~~embodiment~~example, wherein the third CD8+ TCR recognizes more than one MHC-I subtope from one antigen.

[0033] In one ~~embodiment~~example, the third CD8+ TCR recognizes one or more MHC-II supertopes and one or more MHC-II subtopes from one antigen. In one ~~embodiment~~example, the third CD8+ TCR recognizes one or more MHC-E supertopes and one or more MHC-E subtopes from one antigen. In one ~~embodiment~~example, the third CD8+ TCR recognizes one or more MHC-I supertopes and one or more MHC-I subtopes from one antigen.

[0034] In one ~~embodiment~~example, the third CD8+ TCR recognizes more than one MHC-II supertope from more than one antigen. In one ~~embodiment~~example, the third CD8+ TCR recognizes more than one MHC-E supertope from more than one antigen. In one ~~embodiment~~example, the third CD8+ TCR recognizes more than one MHC-E supertope from more than one antigen.

[0035] In one ~~embodiment~~example, the third CD8+ TCR recognizes more than one MHC-II subtope from more than one antigen. In one ~~embodiment~~example, the third CD8+ TCR recognizes more than one MHC-E subtope from more than one antigen. In one ~~embodiment~~example, the third CD8+ TCR recognizes more than one MHC-I subtope from more than one antigen.

[0036] In one ~~embodiment~~example, the third CD8+ TCR recognizes specific MHC-E subtopes or supertopes and MHC-II subtopes or supertopes. In one ~~embodiment~~example, the third CD8+ TCR recognizes specific MHC-E subtopes or supertopes and MHC-I subtopes or supertopes. In one ~~embodiment~~example, the third CD8+ TCR recognizes specific MHC-II subtopes or supertopes and MHC-I subtopes or supertopes.

[0037] In one ~~embodiment~~example, the third CD8+ TCR recognizes more than one MHC-II subtope from the same antigen. In one ~~embodiment~~example, the third CD8+ TCR recognizes more than one MHC-E subtope from the same antigen. In one ~~embodiment~~example, the third CD8+ TCR recognizes more than one MHC-I subtope from the same antigen.

[0038] In one ~~embodiment~~example, the third CD8+ TCR recognizes one or more MHC-II supertopes and one or more MHC-II subtopes from different antigens. In one ~~embodiment~~example, the third CD8+ TCR recognizes one or more MHC-E supertopes and one or more MHC-E subtopes from different antigens. In one ~~embodiment~~example, the third CD8+ TCR recognizes one or more MHC-I supertopes and one or more MHC-I subtopes from different antigens.

[0039] In one ~~embodiment~~example, the nucleic acid sequence encoding the third CD8+ TCR is identical to the nucleic acid sequence encoding the second CD8+ TCR.

[0040] In one ~~embodiment~~example, one or more CD8+ T cells are isolated from a second subject and transfecting the one or more CD8+ T cells with a nucleic acid sequence encoding the selected third CD8+ TCR and a promoter operably linked to the nucleic acid sequence encoding the third CD8+ TCR, thereby generating one or more CD8+ T cells that recognize the first MHC/heterologous antigen-derived peptide complex and the second MHC/heterologous antigen-derived peptide complex.

[0041] In one ~~embodiment~~example, the first MHC-heterologous antigen-derived peptide complex is a MHC-II/heterologous antigen-derived peptide complex, a MHC-E/heterologous antigen-derived peptide complex, or a MHC-I/heterologous antigen-derived peptide complex. In one ~~embodiment~~example, the second MHC-heterologous antigen-derived peptide

complex is a MHC-II/heterologous antigen-derived peptide complex, a MHC-E/heterologous antigen-derived peptide complex, or a MHC-I/heterologous antigen-derived peptide complex.

[0042] In one ~~embodiment~~example, the transfected CD8+ T cells are administered to the second subject to treat or prevent cancer. In another ~~embodiment~~example, the cancer is selected from the group consisting of prostate cancer, kidney cancer, lung cancer, pancreatic cancer, mesothelioma, breast cancer, acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, non-Hodgkin's lymphoma, multiple myeloma, malignant melanoma, ovarian cancer, colon cancer, renal cell carcinoma, and cervical cancer.

[0043] In one ~~embodiment~~example, the transfected CD8+ T cells are administered to the second subject to treat a pathogenic-infection

[0044] In another ~~embodiment~~example, the pathogenic infection is selected from the group consisting of human immunodeficiency virus, herpes simplex virus type 1, herpes simplex virus type 2, hepatitis B virus, hepatitis C virus, papillomavirus, *Plasmodium* parasites, Epstein-barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), Human T-lymphotropic virus type 1 (HTLV1), merkel virus (MCV), cytomegalovirus, and *Mycobacterium tuberculosis*.

[0045] In one ~~embodiment~~example, the first subject is a nonhuman primate and the second subject is a human, and wherein the transfected CD8+ T cells comprises a chimeric nonhuman primate-human CD8+ TCR comprising the non-human primate CDR3α and CDR3β of the second CD8+ TCR.

[0046] In one ~~embodiment~~example, the third CD8+ TCR comprises the non-human primate CDR1α, CDR2α, CDR3α, CDR1β, CDR2β, and CDR3β of the second CD8+ TCR. In one ~~embodiment~~example, the third CD8+ TCR comprises the CDR1α, CDR2α, CDR3α, CDR1β, CDR2β, and CDR3β of the second CD8+ TCR.

[0047] In one ~~embodiment~~example, the first subject is a nonhuman primate and the second subject is a human, and wherein the second CD8+ TCR is a chimeric nonhuman primate-human CD8+ TCR comprising the non-human primate CDR3α and CDR3β of the first CD8+ TCR.

[0048] In one ~~embodiment~~example, the third CD8+ TCR is a chimeric CD8+ TCR.

[0049] In one ~~embodiment~~example, administering the recombinant CMV vector to the first subject comprises intravenous, intramuscular, intraperitoneal, or oral administration.

[0050] In one ~~embodiment~~example, a CD8 + T cell comprising the multi-specific TCR is generated by the method.

[0051] In one ~~embodiment~~example, the CD8+ T cell is administered to a subject in need thereof to treat or prevent cancer. In another ~~embodiment~~example, the cancer is selected from the group consisting of prostate cancer, kidney cancer, lung cancer, pancreatic cancer, mesothelioma, breast cancer, acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, non-Hodgkin's lymphoma, multiple myeloma, malignant melanoma, ovarian cancer, colon cancer, renal cell carcinoma, and cervical cancer.

[0052] In one ~~embodiment~~example, the CD8+ T cell is administered to a subject in need thereof to treat a pathogenic infection. In another ~~embodiment~~example, the pathogenic infection is selected from the group consisting of human immunodeficiency virus, herpes simplex virus type 1, herpes simplex virus type 2, hepatitis B virus, hepatitis C virus, papillomavirus, *Plasmodium* parasites, Epstein-barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), Human T-lymphotropic virus type 1 (HTLV1), merkel virus (MCV), cytomegalovirus, and *Mycobacterium tuberculosis*.

[0053] In one ~~embodiment~~example, the recombinant CMV vector to the first subject comprises intravenous, intramuscular, intraperitoneal, or oral administration.

[0054] The present ~~invention~~ disclosure also relates to a method of generating CD8+ T cells comprising a multi-specific T cell receptor (TCR) comprising: (a) administering to a subject a recombinant cytomegalovirus (CMV) vector comprising a nucleic acid sequence that encodes a first heterologous antigen, in an amount effective to generate a first set of CD8+ T cells that recognize a first MHC-E/ heterologous antigen-derived peptide complex, wherein the CMV vector does not express an active UL128, UL130, UL146 and UL147 protein or orthologs thereof, and wherein the recombinant CMV vector further comprises a microRNA recognition element (MRE); (b) identifying a first CD8+ TCR from the first set of CD8+ T cells, wherein the first CD8+ TCR recognizes the first MHC-E/heterologous antigen-derived peptide complex; (c) administering to the subject a second heterologous antigen in an amount effective to generate a second set of CD8+ T cells that recognizes a second MHC-E/heterologous antigen-derived peptide complex; (d)

isolating one or more CD8+ T cells from the second set of CD8+ T cells; (e) identifying a second CD8+ TCR from the second set of CD8+ T cells, wherein the second CD8+ TCR recognizes the first MHC-E/heterologous antigen-derived peptide complex and the second MHC-E/heterologous antigen-derived peptide complex; (f) transfecting a third set of CD8+ T cells with an expression vector, wherein the expression vector comprises a nucleic acid sequence encoding a third CD8+ TCR and a promoter operably linked to the nucleic acid sequence encoding the third CD8+ TCR, wherein the third CD8+ TCR comprises CDR3α and CDR3β of the second CD8+ TCR, thereby generating one or more CD8+ T cells that recognize the first MHC-E/heterologous antigen-derived peptide complex and the second MHC-E/heterologous antigen-derived peptide complex; and (g) selecting one or more of the third CD8+ TCRs with the highest avidity for the specific peptide of interest.

[0055] In one ~~embodiment~~example, the subject is a human or non-human primate. In one ~~embodiment~~example, the recombinant CMV vector is a recombinant human CMV vector or a recombinant rhesus macaque CMV vector.

[0056] In one ~~embodiment~~example, the first heterologous antigen comprises a tumor antigen, pathogen-specific antigen, a tissue specific antigen, or a host-self antigen. In one ~~embodiment~~example, the tumor antigen is related to a cancer selected from the group consisting of prostate cancer, kidney cancer, lung cancer, pancreatic cancer, mesothelioma, breast cancer, acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, non-Hodgkin's lymphoma, multiple myeloma, malignant melanoma, ovarian cancer, colon cancer, renal cell carcinoma, and cervical cancer. In one ~~embodiment~~example, the pathogen-specific antigen is related to a pathogen selected from the group consisting of human immunodeficiency virus, herpes simplex virus type 1, herpes simplex virus type 2, hepatitis B virus, hepatitis C virus, papillomavirus, *Plasmodium* parasites, Epstein-barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), Human T-lymphotropic virus type 1 (HTLV1), merkel virus (MCV), cytomegalovirus, and *Mycobacterium tuberculosis*.

[0057] In one ~~embodiment~~example, the MRE contains target sites for microRNAs expressed in endothelial cells. In another ~~embodiment~~example, the MRE is specific for the miRNA selected from the group consisting of miR126, miR-126-3p, miR-130a, miR-210, miR-221/222, miR-378, miR-296, and miR-328.

[0058] In one ~~embodiment~~example, the first CD8+ TCR recognizes specific MHC-E subtopes or supertopes.

[0059] In one ~~embodiment~~example, the first CD8+ TCR is identified by DNA or RNA sequencing. In one ~~embodiment~~example, the first CD8+ TCR is identified by single cell sequencing.

[0060] In one ~~embodiment~~example, the second heterologous antigen comprises a tumor antigen, pathogen-specific antigen, a tissue specific antigen, or a host-self antigen. In one ~~embodiment~~example, the tumor antigen is related to a cancer selected from the group consisting of prostate cancer, kidney cancer, lung cancer, pancreatic cancer, mesothelioma, breast cancer, acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, non-Hodgkin's lymphoma, multiple myeloma, malignant melanoma, ovarian cancer, colon cancer, renal cell carcinoma, and cervical cancer. In one ~~embodiment~~example, the pathogen-specific antigen is related to a pathogen selected from the group consisting of human immunodeficiency virus, herpes simplex virus type 1, herpes simplex virus type 2, hepatitis B virus, hepatitis C virus, papillomavirus, *Plasmodium* parasites, Epstein-barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), Human T-lymphotropic virus type 1 (HTLV1), merkel virus (MCV), cytomegalovirus, and *Mycobacterium tuberculosis*.

[0061] In one ~~embodiment~~example, the first heterologous antigen and second heterologous antigens are the same. In one ~~embodiment~~example, the first heterologous antigen and second heterologous antigen are different.

[0062] In one ~~embodiment~~example, the one or more isolated CD8+ T cells from the second set of CD8+ T cells express CD69 and TNFα.

[0063] In one ~~embodiment~~example, the second CD8+ TCR is identified by DNA or RNA sequencing. In one ~~embodiment~~example, the second CD8+ TCR is identified by single cell sequencing.

[0064] In one ~~embodiment~~example, the second CD8+ TCR recognizes one or more specific MHC-E supertopes. In one ~~embodiment~~example, the second CD8+ TCR recognizes one or more specific MHC-E subtopes. In one ~~embodiment~~example, the second CD8+ TCR recognizes specific MHC-E supertopes and MHC-E subtopes.

[0065] In one ~~embodiment~~example, the second CD8+ TCR recognizes more than one MHC-E supertope from the same antigen. In one ~~embodiment~~example, the second CD8+ TCR recognizes more than one MHC-E subtope from the same antigen.

[0066] In one ~~embodiment~~example, the second CD8+ TCR recognizes one or more MHC-E supertopes and one or more MHC-E subtopes from the same antigen. In one ~~embodiment~~example, the second CD8+ TCR recognizes more than one MHC-E supertope from more than one antigen. In one ~~embodiment~~example, the second CD8+ TCR recognizes more than one MHC-E subtope from more than one antigen. In one ~~embodiment~~example, the second CD8+ TCR recognizes one or more MHC-E supertopes and one or more MHC-E subtopes from different antigens.

[0067] In one ~~embodiment~~example, the third CD8+ TCR recognizes one or more specific MHC-E supertopes. In one ~~embodiment~~example, the third CD8+ TCR recognizes one or more specific MHC-E subtopes. In one ~~embodiment~~example, the third CD8+ TCR recognizes specific MHC-E supertopes and MHC-E subtopes.

[0068] In one ~~embodiment~~example, the third CD8+ TCR recognizes more than one MHC-E supertope from one antigen. In one ~~embodiment~~example, the third CD8+ TCR recognizes more than one MHC-E subtope from one antigen.

[0069] In one ~~embodiment~~example, the third CD8+ TCR recognizes one or more MHC-E supertopes and one or more MHC-E subtopes from one antigen. In one ~~embodiment~~example, the third CD8+ TCR recognizes more than one MHC-E supertope from more than one antigen.

[0070] In one ~~embodiment~~example, the third CD8+ TCR recognizes more than one MHC-E subtope from more than one antigen. In one ~~embodiment~~example, the third CD8+ TCR recognizes specific MHC-E supertopes and MHC-E subtopes.

[0071] In one ~~embodiment~~example, the third CD8+ TCR recognizes more than one MHC-E subtope from the same antigen. In one ~~embodiment~~example, the third CD8+ TCR recognizes one or more MHC-E supertopes and one or more MHC-E subtopes from different antigens.

[0072] In one ~~embodiment~~example, the nucleic acid sequence encoding the third CD8+ TCR is identical to the nucleic acid sequence encoding the second CD8+ TCR.

[0073] In one ~~embodiment~~example, one or more CD8+ T cells are isolated from a second subject and transfecting the one or more CD8+ T cells with a nucleic acid sequence encoding the selected third CD8+ TCR and a promoter operably linked to the nucleic acid sequence

encoding the third CD8+ TCR, thereby generating one or more CD8+ T cells that recognize the first MHC-E/heterologous antigen-derived peptide complex and the second MHC-E/heterologous antigen-derived peptide complex.

[0074] In one ~~embodiment~~example, the transfected CD8+ T cells are administered to the second subject to treat or prevent cancer. In another ~~embodiment~~example, the cancer is selected from the group consisting of prostate cancer, kidney cancer, lung cancer, pancreatic cancer, mesothelioma, breast cancer, acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, non-Hodgkin's lymphoma, multiple myeloma, malignant melanoma, ovarian cancer, colon cancer, renal cell carcinoma, and cervical cancer.

[0075] In one ~~embodiment~~example, the transfected CD8+ T cells are administered to the second subject to treat a pathogenic-infection. In another ~~embodiment~~example, the pathogenic infection is selected from the group consisting of human immunodeficiency virus, herpes simplex virus type 1, herpes simplex virus type 2, hepatitis B virus, hepatitis C virus, papillomavirus, *Plasmodium* parasites, Epstein-barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), Human T-lymphotropic virus type 1 (HTLV1), merkel virus (MCV), cytomegalovirus, and *Mycobacterium tuberculosis*.

[0076] In one ~~embodiment~~example, the first subject is a nonhuman primate and the second subject is a human, and wherein the transfected CD8+ T cells comprises a chimeric nonhuman primate-human CD8+ TCR comprising the non-human primate CDR3α and CDR3β of the second CD8+ TCR.

[0077] In one ~~embodiment~~example, the third CD8+ TCR comprises the non-human primate CDR1α, CDR2α, CDR3α, CDR1β, CDR2β, and CDR3β of the second CD8+ TCR. In one ~~embodiment~~example, the third CD8+ TCR comprises the CDR1α, CDR2α, CDR3α, CDR1β, CDR2β, and CDR3β of the second CD8+ TCR.

[0078] In one ~~embodiment~~example, the first subject is a nonhuman primate and the second subject is a human, and wherein the second CD8+ TCR is a chimeric nonhuman primate-human CD8+ TCR comprising the non-human primate CDR3α and CDR3β of the first CD8+ TCR.

[0079] In one ~~embodiment~~example, the third CD8+ TCR is a chimeric CD8+ TCR.

[0080] In one ~~embodiment~~example, administering the recombinant CMV vector to the first subject comprises intravenous, intramuscular, intraperitoneal, or oral administration.

[0081] In one ~~embodiment~~example, a CD8 + T cell comprising the multi-specific TCR is generated by the method.

[0082] In one ~~embodiment~~example, the CD8+ T cell is administered to a subject in need thereof to treat or prevent cancer. In one ~~embodiment~~example, the cancer is selected from the group consisting of prostate cancer, kidney cancer, lung cancer, pancreatic cancer, mesothelioma, breast cancer, acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, non-Hodgkin's lymphoma, multiple myeloma, malignant melanoma, ovarian cancer, colon cancer, renal cell carcinoma, and cervical cancer.

[0083] In one ~~embodiment~~example, the CD8+ T cell is administered to a subject in need thereof to treat a pathogenic infection. In one ~~embodiment~~example, the pathogenic infection is selected from the group consisting of human immunodeficiency virus, herpes simplex virus type 1, herpes simplex virus type 2, hepatitis B virus, hepatitis C virus, papillomavirus, *Plasmodium* parasites, Epstein-barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), Human T-lymphotropic virus type 1 (HTLV1), merkel virus (MCV), cytomegalovirus, and *Mycobacterium tuberculosis*.

[0084] In one ~~embodiment~~example, administering the recombinant CMV vector to the first subject comprises intravenous, intramuscular, intraperitoneal, or oral administration.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0085] FIGs. 1A-B show an overview of the primary study cohort. FIG. 1A is a timeline showing the vaccination dates and sampling window used in this study. FIG. 1B shows the overlapping 15 SIVgag peptides recognized by rhesus macaques (RM) using intracellular cytokine staining (ICS) with each T cell-targeted peptide "box" colored based on their MHC restriction as determined by differentially blocking analysis. Green = MHC-E, red = MHC-1a, blue = MHC-II, purple = indeterminate. The MHC-E and MHC-II restricted supertopes are labeled.

[0086] FIG. 2A-F show TCR clonotypic hierarchies of MHC-E supertope responses. FIG. 2A shows peripheral blood mononuclear cells (PBMC) from RhCMV 68-1/SIVgag-vaccinated RM (Rh-1) that were stimulated with EK9 peptide in the presence of the secretion inhibitor Brefeldin A and intracellular cytokine (TNF-α v. IFN-γ) analysis (ICS) was performed to identify EK9-specific CD8+ T cells (left). In parallel, the same PBMC

WHAT IS CLAIMED IS:

1. An *ex vivo* method of generating CD8+ T cells comprising a multi-specific T cell receptor (TCR), wherein the method comprises:

~~(a) administering to a subject a recombinant cytomegalovirus (CMV) vector comprising a nucleic acid sequence that encodes a first heterologous antigen, in an amount effective to generate a first set of CD8+ T cells that recognize a first MHC/heterologous antigen-derived peptide complex, wherein the CMV vector does not express an active UL128, UL130, UL146 and UL147 protein or orthologs thereof;~~

(~~b~~a) identifying a first CD8+ TCR from ~~the~~ a first set of CD8+ T cells, wherein the first CD8+ TCR recognizes ~~the~~ a first MHC/heterologous antigen-derived peptide complex, wherein the first set of CD8+ T cells was obtained from a subject which had been administered a recombinant cytomegalovirus (CMV) vector comprising a nucleic acid sequence that encodes a first heterologous antigen, in an amount effective to generate the first set of CD8+ T cells that recognize the first MHC/heterologous antigen-derived peptide complex, wherein the CMV vector does not express an active UL128, UL130, UL146 and UL147 protein or orthologs thereof;

~~(c) administering to the subject a second heterologous antigen in an amount effective to generate a second set of CD8+ T cells that recognizes a second MHC/heterologous antigen-derived peptide complex;~~

(~~d~~b) isolating one or more CD8+ T cells from ~~the~~ a second set of CD8+ T cells obtained from the subject which had been administered a second heterologous antigen in an amount effective to generate a second set of CD8+ T cells that recognizes a second MHC/heterologous antigen-derived peptide complex;

(~~e~~c) identifying a second CD8+ TCR from the second set of CD8+ T cells, wherein the second CD8+ TCR recognizes the first MHC/heterologous antigen-derived peptide complex and the second MHC/heterologous antigen-derived peptide complex;

(~~f~~d) transfecting a third set of CD8+ T cells with an expression vector, wherein the expression vector comprises a nucleic acid sequence encoding a third CD8+ TCR and a promoter operably linked to the nucleic acid sequence encoding the third CD8+ TCR, wherein the third CD8+ TCR comprises CDR3α and CDR3β of the second CD8+ TCR, thereby generating one or more CD8+ T cells that recognize the first MHC/heterologous

antigen-derived peptide complex and the second MHC/heterologous antigen-derived peptide complex; and

(~~g~~e) selecting one or more of the third CD8+ TCRs with the highest avidity for a specific peptide of interest.

2. The method of claim 1, wherein the recombinant CMV vector does not express an active UL18 protein.

3. The method of claims 1-2, wherein the recombinant CMV vector expresses an active UL40 protein, or ortholog thereof, and an active US28 protein, or ortholog thereof.

4. The method of claims 1-3, wherein the first MHC/heterologous antigen-derived peptide complex is a MHC-II/heterologous antigen-derived peptide complex, a MHC-E/heterologous antigen-derived peptide complex, or a MHC-I/heterologous antigen-derived peptide complex.

5. The method of claims 1-4, wherein the second MHC/ heterologous antigen-derived peptide complex is a MHC-II/heterologous antigen-derived peptide complex or a MHC-E/ heterologous antigen-derived peptide complex.

6. The method of any one of claims 1-5, wherein:

the recombinant CMV vector further comprises a microRNA recognition element (MRE);

the first MHC/heterologous antigen-derived peptide complex is a MHC-E/ heterologous antigen-derived peptide complex; and

the second MHC-E/heterologous antigen-derived peptide complex is a MHC-E/heterologous antigen-derived peptide complex.

~~6.~~7. The method of claims 1-~~5~~6, wherein the subject is a human or non-human primate.

~~7.~~8. The method of claims 1-~~6~~7, wherein the recombinant CMV vector is a recombinant human CMV vector or a recombinant rhesus macaque CMV vector.

~~8.~~9. The method of claims 1-~~7~~8, wherein the first and/or second heterologous antigen comprises a tumor antigen, pathogen-specific antigen, a tissue specific antigen, or a host-self antigen.

~~9.~~10. The method of claim ~~8~~9, wherein the tumor antigen is related to a cancer selected from the group consisting of prostate cancer, kidney cancer, lung cancer, pancreatic cancer, mesothelioma, breast cancer, acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, non-Hodgkin's lymphoma, multiple myeloma, malignant melanoma, ovarian cancer, colon cancer, renal cell carcinoma, and cervical cancer.

~~10.~~11. The method of claim ~~8~~9, wherein the pathogen-specific antigen is related to a pathogen selected from the group consisting of human immunodeficiency virus, herpes simplex virus type 1, herpes simplex virus type 2, hepatitis B virus, hepatitis C virus, papillomavirus, *Plasmodium* parasites, Epstein-barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), Human T-lymphotropic virus type 1 (HTLV1), merkel virus (MCV), cytomegalovirus, and *Mycobacterium tuberculosis*.

~~11. The method of claims 1-10, wherein the first CD8+ TCR recognizes specific MHC-II subtopes or supertopes.~~

~~12. The method of claims 1-10, wherein the first CD8+ TCR recognizes specific MHC-E subtopes or supertopes.~~

~~13. The method of claims 1-10, wherein the first CD8+ TCR recognizes specific MHC-I subtopes or supertopes.~~

~~14. The method of claims 1-13, wherein the first CD8+ TCR is identified by DNA or RNA sequencing.~~

~~15. The method of claim 14, wherein the first CD8+ TCR is identified by single cell sequencing.~~

~~16.~~12. The method of claims 1-~~15~~11, wherein the first heterologous antigen and second heterologous antigens are the same.

~~17.~~13. The method of claims 1-~~15~~11, wherein the first heterologous antigen and second heterologous antigen are different.

~~18.~~14. The method of claims 1-~~17~~13, wherein the one or more isolated CD8+ T cells from the second set of CD8+ T cells express CD69 and TNFα.

15. The method of any one of claims 6-14, wherein the MRE contains target sites for microRNAs expressed in endothelial cells.

16. The method of claim 15, wherein the MRE is specific for the miRNA selected from the group consisting of miR126, miR-126-3p, miR-130a, miR-210, miR-221/222, miR-378, miR-296, and miR-328.

~~19. The method of claims 1-18, wherein the second CD8+ TCR recognizes one or more specific MHC-II supertopes.~~

~~20. The method of claims 1-18, wherein the second CD8+ TCR recognizes one or more specific MHC-E supertopes.~~

~~21. The method of claims 1-18, wherein the second CD8+ TCR recognizes one or more specific MHC-I supertopes.~~

~~22. The method of claims 19-20, wherein the second CD8+ TCR recognizes a MHC-II supertope and a MHC-E supertope.~~

~~23. The method of claims 20-21, wherein the second CD8+ TCR recognizes a MHC-I supertope and a MHC-E supertope.~~

~~24. The method of claims 19 and 21, wherein the second CD8+ TCR recognizes a MHC-I supertope and a MHC-II supertope.~~

~~25. The method of claims 1-18, wherein the second CD8+ TCR recognizes one or more specific MHC-II subtopes.~~

~~26. The method of claims 1-18, wherein the second CD8+ TCR recognizes one or more specific MHC-E subtopes.~~

~~27. The method of claims 1-18, wherein the second CD8+ TCR recognizes one or more specific MHC-I subtopes.~~

~~28. The method of claims 25-26, wherein the second CD8+ TCR recognizes a MHC-II subtope and a MHC-E subtope.~~

~~29. The method of claims 26-27, wherein the second CD8+ TCR recognizes a MHC-II subtope and a MHC-I subtope.~~

~~30. The method of claims 25 and 27, wherein the second CD8+ TCR recognizes a MHC-E subtope and a MHC-I subtope.~~

~~31. The method of claims 19-20 and 25-26, wherein the second CD8+ TCR recognizes a MHC-II subtope or supertope and a MHC-E subtope or supertope.~~

~~32. The method of claims 19, 21, 25 and 27, wherein the second CD8+ TCR recognizes a MHC-II subtope or supertope and a MHC-I subtope or supertope.~~

~~33. The method of claims 20-21 and 26-27, wherein the second CD8+ TCR recognizes a MHC-E subtope or supertope and a MHC-I subtope or supertope.~~

~~34. The method of claims 19 and 25, wherein the second CD8+ TCR recognizes specific MHC-II supertopes and MHC-II subtopes.~~

~~35. The method of claims 20 and 26, wherein the second CD8+ TCR recognizes specific MHC-E supertopes and MHC-E subtopes.~~

~~36. The method of claims 21 and 27, wherein the second CD8+ TCR recognizes specific MHC-I supertopes and MHC-I subtopes.~~

~~37. The method of claim 19, wherein the second CD8+ TCR recognizes more than one MHC-II supertope from the same antigen.~~

~~38. The method of claim 20, wherein the second CD8+ TCR recognizes more than one MHC-E supertope from the same antigen.~~

~~39. The method of claim 21, wherein the second CD8+ TCR recognizes more than one MHC-I supertope from the same antigen.~~

~~40. The method of claim 25, wherein the second CD8+ TCR recognizes more than one MHC-II subtope from the same antigen.~~

~~41. The method of claim 26, wherein the second CD8+ TCR recognizes more than one MHC-E subtope from the same antigen.~~

~~42. The method of claim 27, wherein the second CD8+ TCR recognizes more than one MHC-I subtope from the same antigen.~~

~~43. The method of claims 19 and 25, wherein the second CD8+ TCR recognizes one or more MHC-II supertopes and one or more MHC-II subtopes from the same antigen.~~

~~44. The method of claims 20 and 26, wherein the second CD8+ TCR recognizes one or more MHC-E supertopes and one or more MHC-E subtopes from the same antigen.~~

~~45. The method of claims 21 and 27, wherein the second CD8+ TCR recognizes one or more MHC-I supertopes and one or more MHC-I subtopes from the same antigen.~~

~~46. The method of claim 19, wherein the second CD8+ TCR recognizes more than one MHC-II supertope from more than one antigen.~~

~~47. The method of claim 20, wherein the second CD8+ TCR recognizes more than one MHC-E supertope from more than one antigen.~~

~~48. The method of claim 21, wherein the second CD8+ TCR recognizes more than one MHC-I supertope from more than one antigen.~~

~~49. The method of claim 25, wherein the second CD8+ TCR recognizes more than one MHC-II subtope from more than one antigen.~~

~~50. The method of claim 26, wherein the second CD8+ TCR recognizes more than one MHC-E subtope from more than one antigen.~~

~~51. The method of claims 27, wherein the second CD8+ TCR recognizes more than one MHC-I subtope from more than one antigen.~~

~~52. The method of claims 19 and 25, wherein the second CD8+ TCR recognizes one or more MHC-II supertopes and one or more MHC-II subtopes from different antigens.~~

~~53. The method of claims 20 and 26, wherein the second CD8+ TCR recognizes one or more MHC-E supertopes and one or more MHC-E subtopes from different antigens.~~

~~54. The method of claims 21, and 27, wherein the second CD8+ TCR recognizes one or more MHC-I supertopes and one or more MHC-I subtopes from different antigens.~~

~~55. The method of claims 1-18, wherein the third CD8+ TCR recognizes one or more specific MHC-II supertopes.~~

~~56. The method of claims 1-18, wherein the third CD8+ TCR recognizes one or more specific MHC-E supertopes.~~

~~57. The method of claims 1-18, wherein the third CD8+ TCR recognizes one or more specific MHC-I supertopes.~~

~~58. The method of claims 1-18, wherein the third CD8+ TCR recognizes one or more specific MHC-II subtopes.~~

~~59. The method of claims 1-18, wherein the third CD8+ TCR recognizes one or more specific MHC-E subtopes.~~

~~60. The method of claims 1-18, wherein the third CD8+ TCR recognizes one or more specific MHC-I subtopes.~~

~~61. The method of claims 55 and 58, wherein the third CD8+ TCR recognizes specific MHC-II supertopes and MHC-II subtopes.~~

~~62. The method of claims 56 and 59, wherein the third CD8+ TCR recognizes specific MHC-E supertopes and MHC-E subtopes.~~

~~63. The method of claims 57 and 60, wherein the third CD8+ TCR recognizes specific MHC-I supertopes and MHC-I subtopes.~~

~~64. The method of claim 55 , wherein the third CD8+ TCR recognizes more than one MHC-II supertope from one antigen.~~

~~65. The method of claim 56, wherein the third CD8+ TCR recognizes more than one MHC-E supertope from one antigen.~~

~~66. The method of claim 57, wherein the third CD8+ TCR recognizes more than one MHC-I supertope from one antigen~~

~~67. The method of claim 58, wherein the third CD8+ TCR recognizes more than one MHC-II subtope from one antigen.~~

~~68. The method of claim 59, wherein the third CD8+ TCR recognizes more than one MHC-E subtope from one antigen.~~

~~69. The method of claim 60, wherein the third CD8+ TCR recognizes more than one MHC-I subtope from one antigen.~~

~~70. The method of claims 55 and 58, wherein the third CD8+ TCR recognizes one or more MHC-II supertopes and one or more MHC-II subtopes from one antigen.~~

~~71. The method of claims 56 and 59, wherein the third CD8+ TCR recognizes one or more MHC-E supertopes and one or more MHC-E subtopes from one antigen.~~

~~72. The method of claims 57-60, wherein the third CD8+ TCR recognizes one or more MHC-I supertopes and one or more MHC-I subtopes from one antigen.~~

~~73. The method of claim 55, wherein the third CD8+ TCR recognizes more than one MHC-II supertope from more than one antigen.~~

~~74. The method of claim 56, wherein the third CD8+ TCR recognizes more than one MHC-E supertope from more than one antigen.~~

~~75. The method of claims 57, wherein the third CD8+ TCR recognizes more than one MHC-I supertope from more than one antigen~~

~~76. The method of claim 58, wherein the third CD8+ TCR recognizes more than one MHC-II subtope from more than one antigen.~~

~~77. The method of claim 59, wherein the third CD8+ TCR recognizes more than one MHC-E subtope from more than one antigen.~~

~~78. The method of claim 60, wherein the third CD8+ TCR recognizes more than one MHC-I subtope from more than one antigen~~

~~79. The method of claims 55-56 and 58-59, wherein the third CD8+ TCR recognizes specific MHC-E subtopes or supertopes and MHC-II subtopes or supertopes.~~

~~80. The method of claims 56-57 and 59-60, wherein the third CD8+ TCR recognizes specific MHC-E subtopes or supertopes and MHC-I subtopes or supertopes.~~

~~81. The method of claims 55, 57-58, and 60, wherein the third CD8+ TCR recognizes specific MHC-II subtopes or supertopes and MHC-I subtopes or supertopes.~~

~~82. The method of claim 58, wherein the third CD8+ TCR recognizes more than one MHC-II subtope from the same antigen.~~

~~83. The method of claim 59, wherein the third CD8+ TCR recognizes more than one MHC-E subtope from the same antigen.~~

~~84. The method of claim 60, wherein the third CD8+ TCR recognizes more than one MHC-I subtope from the same antigen.~~

~~85. The method of claims 55 and 58, wherein the third CD8+ TCR recognizes one or more MHC-II supertopes and one or more MHC-II subtopes from different antigens.~~

~~86. The method of claims 56 and 59, wherein the third CD8+ TCR recognizes one or more MHC-E supertopes and one or more MHC-E subtopes from different antigens.~~

~~87. The method of claims 57 and 60, wherein the third CD8+ TCR recognizes one or more MHC-I supertopes and one or more MHC-I subtopes from different antigens.~~

~~88.~~17. The method of claims 1-~~87~~16, wherein the nucleic acid sequence encoding the third CD8+ TCR is identical to the nucleic acid sequence encoding the second CD8+ TCR.

~~89.~~18. The method of claims 1-~~88~~17, further comprising isolating one or more CD8+ T cells from a second subject and transfecting the one or more CD8+ T cells with a nucleic acid sequence encoding the selected third CD8+ TCR and a promoter operably linked to the nucleic acid sequence encoding the third CD8+ TCR, thereby generating one or more CD8+ T cells that recognize the first MHC/heterologous antigen-derived peptide complex and the second MHC/heterologous antigen-derived peptide complex.

~~90. The method of claim 89, wherein the first MHC-heterologous antigen-derived peptide complex is a MHC-II/heterologous antigen-derived peptide complex, a MHC-E/heterologous antigen-derived peptide complex, or a MHC-I/heterologous antigen-derived peptide complex.~~

~~91. The method of claims 89-90, wherein the second MHC-heterologous antigen-derived peptide complex is a MHC-II/heterologous antigen-derived peptide complex, a MHC-E/heterologous antigen-derived peptide complex, or a MHC-I/heterologous antigen-derived peptide complex.~~

~~92. The method of claims 89-91, further comprising administering the transfected CD8+ T cells to the second subject to treat or prevent cancer.~~

~~93. The method of claim 92, wherein the cancer is selected from the group consisting of prostate cancer, kidney cancer, lung cancer, pancreatic cancer, mesothelioma, breast cancer, acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, non-Hodgkin's lymphoma, multiple myeloma, malignant melanoma, ovarian cancer, colon cancer, renal cell carcinoma, and cervical cancer.~~

~~94. The method of claims 89-91, further comprising administering the transfected CD8+ T cells to the second subject to treat a pathogenic infection.~~

~~95. The method of claim 92, wherein the pathogenic infection is selected from the group consisting of human immunodeficiency virus, herpes simplex virus type 1, herpes simplex virus type 2, hepatitis B virus, hepatitis C virus, papillomavirus, *Plasmodium* parasites, Epstein-barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), Human T-lymphotropic virus type 1 (HTLV1), merkel virus (MCV), cytomegalovirus, and *Mycobacterium tuberculosis*.~~

~~96.~~19. The method of ~~claims 89-95~~claim 18, wherein the first subject is a nonhuman primate and the second subject is a human, and wherein (i) the transfected CD8+ T cells comprises a chimeric nonhuman primate-human CD8+ TCR comprising the non-human primate CDR3α and CDR3β of the second CD8+ TCR; (ii) the third CD8+ TCR comprises the non-human primate CDR1α, CDR2α, CDR3α, CDR1β, CDR2β, and CDR3β of the second CD8+ TCR; (iii) the third CD8+ TCR comprises the CDR1α, CDR2α, CDR3α, CDR1β, CDR2β, and CDR3β of the second CD8+ TCR; (iv) the second CD8+ TCR is a

chimeric nonhuman primate-human CD8+ TCR comprising the non-human primate CDR3α and CDR3β of the first CD8+ TCR; and/or (v) the third CD8+ TCR is a chimeric CD8+ TCR .

~~97. The method of claims 89-96, wherein the third CD8+ TCR comprises the non-human primate CDR1α, CDR2α, CDR3α, CDR1β, CDR2β, and CDR3β of the second CD8+ TCR.~~

~~98. The method of claims 89-97, wherein the third CD8+ TCR comprises the CDR1α, CDR2α, CDR3α, CDR1β, CDR2β, and CDR3β of the second CD8+ TCR.~~

~~99. The method of claims 89-98, wherein the first subject is a nonhuman primate and the second subject is a human, and wherein the second CD8+ TCR is a chimeric nonhuman primate-human CD8+ TCR comprising the non-human primate CDR3α and CDR3β of the first CD8+ TCR.~~

~~100. The method of claims 1-99, wherein the third CD8+ TCR is a chimeric CD8+ TCR.~~

~~101.~~20.The method of claims 1-~~100~~19, wherein ~~administering~~ the recombinant CMV vector is to be administered to the first subject ~~comprises~~ via intravenous, intramuscular, intraperitoneal, or oral administration.

~~102.~~21.A CD8 + T cell comprising the multi-specific TCR generated by the method of claims 1-~~101~~20.

~~103.~~22.~~A method of treating or preventing cancer in a subject in need thereof, the method comprising administering the~~ The CD8+ T cell of claim ~~102~~ 21 for use in treating or preventing cancer or treating a pathogenic infection in a subject in need thereof~~to the subject~~.

104. The method of claim 103, wherein the cancer is selected from the group consisting of prostate cancer, kidney cancer, lung cancer, pancreatic cancer, mesothelioma, breast cancer, acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, non-Hodgkin's lymphoma, multiple myeloma, malignant melanoma, ovarian cancer, colon cancer, renal cell carcinoma, and cervical cancer.

105. A method of treating a pathogenic infection in a subject in need thereof, the method comprising administering the CD8+ T cell of claim 102 to the subject.

106. The method of claim 105, wherein the pathogenic infection is selected from the group consisting of human immunodeficiency virus, herpes simplex virus type 1, herpes simplex virus type 2, hepatitis B virus, hepatitis C virus, papillomavirus, *Plasmodium* parasites, Epstein-barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), Human T-lymphotropic virus type 1 (HTLV1), merkel virus (MCV), cytomegalovirus, and *Mycobacterium tuberculosis*.

107. The method of claims 102-106, wherein administering the recombinant CMV vector to the first subject comprises intravenous, intramuscular, intraperitoneal, or oral administration.

108. A method of generating CD8+ T cells comprising a multi-specific T cell receptor (TCR), wherein the method comprises:

(a) administering to a subject a recombinant cytomegalovirus (CMV) vector comprising a nucleic acid sequence that encodes a first heterologous antigen, in an amount effective to generate a first set of CD8+ T cells that recognize a first MHC-E/heterologous antigen-derived peptide complex, wherein the CMV vector does not express an active UL128, UL130, UL146 and UL147 protein or orthologs thereof, and wherein the recombinant CMV vector further comprises a microRNA recognition element (MRE);

(b) identifying a first CD8+ TCR from the first set of CD8+ T cells, wherein the first CD8+ TCR recognizes the first MHC-E/heterologous antigen-derived peptide complex;

(c) administering to the subject a second heterologous antigen in an amount effective to generate a second set of CD8+ T cells that recognizes a second MHC-E/heterologous antigen-derived peptide complex;

(d) isolating one or more CD8+ T cells from the second set of CD8+ T cells;

(e) identifying a second CD8+ TCR from the second set of CD8+ T cells, wherein the second CD8+ TCR recognizes the first MHC-E/heterologous antigen-derived peptide complex and the second MHC-E/heterologous antigen-derived peptide complex;

(f) transfecting a third set of CD8+ T cells with an expression vector, wherein the expression vector comprises a nucleic acid sequence encoding a third CD8+ TCR and a promoter operably linked to the nucleic acid sequence encoding the third CD8+ TCR, wherein the third CD8+ TCR comprises CDR3α and CDR3β of the second CD8+ TCR, thereby generating one or more CD8+ T cells that recognize the first MHC-E/heterologous antigen-derived peptide complex and the second MHC-E/heterologous antigen-derived peptide complex; and

(g) selecting one or more of the third CD8+ TCRs with the highest avidity for the specific peptide of interest.

109. The method of claim 108, wherein the subject is a human or non-human primate.

110. The method of claims 108-109, wherein the recombinant CMV vector is a recombinant human CMV vector or a recombinant rhesus macaque CMV vector.

111. The method of claims 108-110, wherein the first heterologous antigen comprises a tumor antigen, pathogen-specific antigen, a tissue specific antigen, or a host self-antigen.

112. The method of claims 111, wherein the tumor antigen is related to a cancer selected from the group consisting of prostate cancer, kidney cancer, lung cancer, pancreatic cancer, mesothelioma, breast cancer, acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, non-Hodgkin's lymphoma, multiple myeloma, malignant melanoma, ovarian cancer, colon cancer, renal cell carcinoma, and cervical cancer.

113. The method of claims 111, wherein the pathogen-specific antigen is related to a pathogen selected from the group consisting of human immunodeficiency virus, herpes simplex virus type 1, herpes simplex virus type 2, hepatitis B virus, hepatitis C virus, papillomavirus, *Plasmodium* parasites, Epstein-barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), Human T-lymphotropic virus type 1 (HTLV1), merkel virus (MCV), cytomegalovirus, and *Mycobacterium tuberculosis*.

114. The method of claims 108, wherein the MRE contains target sites for microRNAs expressed in endothelial cells.

115. The method of claim 114, wherein the MRE is specific for the miRNA selected from the group consisting of miR126, miR-126-3p, miR-130a, miR-210, miR-221/222, miR-378, miR-296, and miR-328.

116. The method of claims 108-115, wherein the first CD8+ TCR recognizes specific MHC-E subtopes or supertopes.

117. The method of claims 108-116, wherein the first CD8+ TCR is identified by DNA or RNA sequencing.

118. The method of claim 117, wherein the first CD8+ TCR is identified by single cell sequencing.

119. The method of claims 108-118, wherein the second heterologous antigen comprises a tumor antigen, pathogen-specific antigen, a tissue specific antigen, or a host-self antigen.

120. The method of claim 119, wherein the tumor antigen is related to a cancer selected from the group consisting of prostate cancer, kidney cancer, lung cancer, pancreatic cancer, mesothelioma, breast cancer, acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoblastic leukemia, chronic

~~lymphoblastic leukemia, non-Hodgkin's lymphoma, multiple myeloma, malignant melanoma, ovarian cancer, colon cancer, renal cell carcinoma, and cervical cancer.~~

~~121. The method of claim 119, wherein the pathogen-specific antigen is related to a pathogen selected from the group consisting of human immunodeficiency virus, herpes simplex virus type 1, herpes simplex virus type 2, hepatitis B virus, hepatitis C virus, papillomavirus, *Plasmodium* parasites, Epstein-barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), Human T-lymphotropic virus type 1 (HTLV1), merkel virus (MCV), cytomegalovirus, and *Mycobacterium tuberculosis*.~~

~~122. The method of claims 108-121, wherein the first heterologous antigen and second heterologous antigens are the same.~~

~~123. The method of claims 108-121, wherein the first heterologous antigen and second heterologous antigen are different.~~

~~124. The method of claims 108-123, wherein the one or more isolated CD8+ T cells from the second set of CD8+ T cells express CD69 and TNFα.~~

~~125. The method of claims 108-124, wherein the second CD8+ TCR is identified by DNA or RNA sequencing.~~

~~126. The method of claim 125, wherein the second CD8+ TCR is identified by single cell sequencing.~~

~~127. The method of claims 108-126, wherein the second CD8+ TCR recognizes one or more specific MHC-E supertopes.~~

~~128. The method of claims 108-126, wherein the second CD8+ TCR recognizes one or more specific MHC-E subtopes.~~

~~129. The method of claims 127-128, wherein the second CD8+ TCR recognizes specific MHC-E supertopes and MHC-E subtopes.~~

~~130. The method of claims 108-127, wherein the second CD8+ TCR recognizes more than one MHC-E supertope from the same antigen.~~

~~131. The method of claims 108-126 and 128, wherein the second CD8+ TCR recognizes more than one MHC-E subtope from the same antigen.~~

~~132. The method of claim 129, wherein the second CD8+ TCR recognizes one or more MHC-E supertopes and one or more MHC-E subtopes from the same antigen.~~

~~133. The method of claim 108-127, wherein the second CD8+ TCR recognizes more than one MHC-E supertope from more than one antigen.~~

~~134. The method of claim 108-126 and 128, wherein the second CD8+ TCR recognizes more than one MHC-E subtope from more than one antigen.~~

~~135. The method of claim 108-128, wherein the second CD8+ TCR recognizes one or more MHC-E supertopes and one or more MHC-E subtopes from different antigens.~~

~~136. The method of claims 108-127, wherein the third CD8+ TCR recognizes one or more specific MHC-E supertopes.~~

~~137. The method of claims 108-126 and 128, wherein the third CD8+ TCR recognizes one or more specific MHC-E subtopes.~~

~~138. The method of claims 136-137, wherein the third CD8+ TCR recognizes specific MHC-E supertopes and MHC-E subtopes.~~

~~139. The method of claim 136, wherein the third CD8+ TCR recognizes more than one MHC-E supertope from one antigen.~~

~~140. The method of claim 137, wherein the third CD8+ TCR recognizes more than one MHC-E subtope from one antigen.~~

~~141. The method of claim 139-140, wherein the third CD8+ TCR recognizes one or more MHC-E supertopes and one or more MHC-E subtopes from one antigen.~~

~~142. The method of claim 136, wherein the third CD8+ TCR recognizes more than one MHC-E supertope from more than one antigen.~~

~~143. The method of claim 137, wherein the third CD8+ TCR recognizes more than one MHC-E subtope from more than one antigen.~~

~~144. The method of claim 136, wherein the third CD8+ TCR recognizes more than one MHC-E supertopes from the same antigen.~~

~~145. The method of claim 137, wherein the third CD8+ TCR recognizes more than one MHC-E subtope from the same antigen.~~

~~146. The method of claim 142-143, wherein the third CD8+ TCR recognizes one or more MHC-E supertopes and one or more MHC-E subtopes from different antigens.~~

~~147. The method of claims 108-146, wherein the nucleic acid sequence encoding the third CD8+ TCR is identical to the nucleic acid sequence encoding the second CD8+ TCR.~~

~~148. The method of claim 108-147, further comprising isolating one or more CD8+ T cells from a second subject and transfecting the one or more CD8+ T cells with a nucleic acid sequence encoding the selected third CD8+ TCR and a promoter operably linked to the nucleic acid sequence encoding the third CD8+ TCR, thereby generating one or more~~

~~CD8+ T cells that recognize the first MHC-E/heterologous antigen-derived peptide complex and the second MHC-E/heterologous antigen-derived peptide complex.~~

~~149. The method of claim 148, further comprising administering the transfected CD8+ T cells to the second subject to treat or prevent cancer.~~

~~150. The method of claim 149, wherein the cancer is selected from the group consisting of prostate cancer, kidney cancer, lung cancer, pancreatic cancer, mesothelioma, breast cancer, acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, non-Hodgkin's lymphoma, multiple myeloma, malignant melanoma, ovarian cancer, colon cancer, renal cell carcinoma, and cervical cancer.~~

~~151. The method of claim 148, further comprising administering the transfected CD8+ T cells to the second subject to treat a pathogenic infection.~~

~~152. The method of claim 149, wherein the pathogenic infection is selected from the group consisting of human immunodeficiency virus, herpes simplex virus type 1, herpes simplex virus type 2, hepatitis B virus, hepatitis C virus, papillomavirus, *Plasmodium* parasites, Epstein-barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), Human T-lymphotropic virus type 1 (HTLV1), merkel virus (MCV), cytomegalovirus, and *Mycobacterium tuberculosis*.~~

~~153. The method of claim 148-152, wherein the first subject is a nonhuman primate and the second subject is a human, and wherein the transfected CD8+ T cells comprises a chimeric nonhuman primate-human CD8+ TCR comprising the non-human primate CDR3α and CDR3β of the second CD8+ TCR.~~

~~154. The method of claim 148-153, wherein the third CD8+ TCR comprises the non-human primate CDR1α, CDR2α, CDR3α, CDR1β, CDR2β, and CDR3β of the second CD8+ TCR.~~

155. The method of claim 148-154, wherein the third CD8+ TCR comprises the CDR1α, CDR2α, CDR3α, CDR1β, CDR2β, and CDR3β of the second CD8+ TCR.

156. The method of claim 148-155, wherein the first subject is a nonhuman primate and the second subject is a human, and wherein the second CD8+ TCR is a chimeric nonhuman primate-human CD8+ TCR comprising the non-human primate CDR3α and CDR3β of the first CD8+ TCR.

157. The method of claim 148-156, wherein the third CD8+ TCR is a chimeric CD8+ TCR.

158. The method of claims 108-157, wherein administering the recombinant CMV vector to the first subject comprises intravenous, intramuscular, intraperitoneal, or oral administration.

159. A CD8 + T cell comprising the multi-specific TCR generated by the method of claims 108-158.

160. A method of treating or preventing cancer in a subject in need thereof, the method comprising administering the CD8+ T cell of claim 159 to the subject.

161. The method of claim 160, wherein the cancer is selected from the group consisting of prostate cancer, kidney cancer, lung cancer, pancreatic cancer, mesothelioma, breast cancer, acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, non-Hodgkin's lymphoma, multiple myeloma, malignant melanoma, ovarian cancer, colon cancer, renal cell carcinoma, and cervical cancer.

162. A method of treating a pathogenic infection in a subject in need thereof, the method comprising administering the CD8+ T cell of claim 159 to the subject.

~~163. The method of claim 160, wherein the pathogenic infection is selected from the group consisting of human immunodeficiency virus, herpes simplex virus type 1, herpes simplex virus type 2, hepatitis B virus, hepatitis C virus, papillomavirus, *Plasmodium* parasites, Epstein-barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), Human T-lymphotropic virus type 1 (HTLV1), merkel virus (MCV), cytomegalovirus, and *Mycobacterium tuberculosis*.~~

~~The method of claims 160-163, wherein administering the recombinant CMV vector to the first subject comprises intravenous, intramuscular, intraperitoneal, or oral administration.~~