

5 **COMPOSITIONS AND METHODS FOR VIRAL VECTORS**

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application No. 63/331,903, filed on April 18, 2022; the disclosure of which is hereby incorporated by reference in its entirety for all purposes.

10 **FIELD OF THE INVENTION**

[0002] The disclosure relates generally to HSV-1 vectors, and, more particularly, the invention relates to replication defective HSV-1 vectors and their use to deliver one or more genes encoding transgenic proteins that stimulate immune destruction of tumors.

BACKGROUND

15 [0003] Developments in immuno-oncology have provided new therapies directed to treating cancers that have evaded successful treatment because the cancers have evolved mechanisms for evading a subject's immune system. However, despite the significant advances made to date in treating various cancers with immune-stimulating therapies such as checkpoint inhibitors and CAR T cells, significant numbers of patients remain unresponsive to
20 immunotherapy treatment. Furthermore, solid tumors may be harder to treat using immune-oncologic methods, given the complexity of the tumor microenvironment (TME) of a solid tumor, which contains epithelial cells, endothelial cells, mesenchymal cells, stromal cells, cancer cells and immune cells. In certain solid tumors, the cancer cells influence of the activity or inactivity of the immune cells in the TME.

25 [0004] Viral vectors have been developed to deliver therapeutics to cells. However, many such vectors exhibit insufficient persistence in target cells, preventing sufficient expression of therapeutics. Furthermore, many existing vectors cause non-specific inflammation and tissue damage that makes delivery unsafe. Accordingly, despite the advances made to date, there is still a need for methods and compositions that can stimulate immune activity against tumor
30 cells which, either alone or in combination with other cancer drugs, may be effective in treating a tumor in a given subject.

SUMMARY OF THE INVENTION

[0005] The disclosure is based, in part, upon the discovery that HSV-1 vectors comprising an alteration (such as a gene deletion) that prevents expression of one or more functional

5 infected cell polypeptide 4 (ICP4) and infected cell polypeptide 47 (ICP47) proteins have reduced replicative capacity, are oncolytic, have an extended period of payload expression, are cytotoxic to proliferating cells (*e.g.*, cancer cells), and exhibit enhanced immunogenicity. Accordingly, it has been discovered that such vectors are useful for delivering a therapeutic payload (*e.g.*, one or more therapeutic polypeptides) that can be useful in reducing the size of
10 a tumor, for example, to treat cancer in a subject in need thereof. The vectors can induce delayed oncolysis, allowing for sustained expression of a therapeutic payload (*e.g.*, the one or more therapeutic polypeptides). Further, the vectors exhibit increased immunogenicity (*e.g.*, by increasing the expression of human leucocyte antigen (HLA) on the surface of proliferating cells (*e.g.*, cancer cells), increasing immune activity toward tumor cells. Given
15 the unpredictable nature of replicative capacity, oncolytic activity and persistence in tissue in viruses, particularly in viruses of varying strains, these features were unexpected.

[0006] The disclosure also is based, in part, upon the discovery that particular combinations of therapeutic polypeptides can be used to disrupt one or more pathways (*e.g.*, parallel pathways) affecting the ability of immune cells in the recipient to kill cancer cells, thereby
20 reducing the size of a tumor. For example, certain therapeutic polypeptides that target the stroma of a tumor, support T cell (*e.g.*, CAR T cell) and/or NK cell survival in a tumor microenvironment, induce tertiary lymphoid structures (TLS) in a tumor bed and/or promote phagocytic innate immune surveillance can be expressed from a viral vector, *e.g.*, a viral vector lacking functional ICP4 and ICP47 proteins to increase the ability of immune cells to
25 kill tumor cells, for example, to treat cancer in a subject in need thereof. The vectors can be designed to include a specific combination of therapeutic polypeptides that will be effective to treat a given tumor types, including choosing one or more therapeutic polypeptides the reduce inhibitory features of a specific tumor microenvironment that may prevent the immune system from effectively attacking the tumor.

30 [0007] Accordingly, in one aspect, the disclosure relates to a vector comprising a herpes simplex virus (HSV) genome, wherein the vector comprises an alteration that prevents expression of one or more functional ICP4 and ICP47 proteins. In certain embodiments, the functional ICP4 protein is characterized by the amino acid sequences of SEQ ID NO: 3 or SEQ ID NO: 4 and the functional ICP47 protein is characterized by the amino acid sequences
35 of SEQ ID NO: 5. In certain embodiments, the HSV genome is an HSV-1 genome, *e.g.*, a McKrae strain genome. In certain embodiments, the vector, when administered to a subject,

- 5 results in one or more of (a) delayed oncolysis; (b) increased immunogenicity; and (c) increased immune activation.

[0008] In certain embodiments, the vector comprises a nucleic acid sequence encoding one or more therapeutic polypeptides. In certain embodiments, the therapeutic polypeptide can have one or more of the following attributes:

- 10 (a) targets the stroma of a tumor;
 (b) supports T cell (*e.g.*, CAR T cell) and/or NK cell survival in a tumor microenvironment;
 (c) induces tertiary lymphoid structures (TLS) in a tumor bed; and/or
 (d) promotes phagocytic innate immune surveillance.
- 15 **[0009]** In certain embodiments, the therapeutic polypeptide targets the stroma of a tumor by degrading an extracellular matrix protein. Examples of such therapeutic polypeptides include without limitation, hyaluronidase (SEQ ID NO: 6), MMP-9 (SEQ ID NO: 7), or an inhibitor of lysyl oxidase. In certain embodiments, the therapeutic polypeptide targets the stroma of a tumor by activating local endothelium to increase T cell infiltration. Examples of such
- 20 therapeutic polypeptides include without limitation a proinflammatory cytokine, *e.g.*, TNF (SEQ ID NO: 11), IL-1 β (SEQ ID NO: 8), IL-6 (SEQ ID NO: 9), or IL-18 (SEQ ID NO: 18).
- [0010]** In certain embodiments, the therapeutic polypeptide supports T cell survival in the tumor microenvironment by enhancing recruitment of T cells to a site of a tumor. Examples of such therapeutic polypeptides include, without limitation, CCL19 (SEQ ID NO: 12) or
- 25 CCL21 (SEQ ID NO: 13). In certain embodiments, the therapeutic polypeptide supports T cell survival in the tumor microenvironment by supporting T cell function. Examples of such therapeutic polypeptide include without limitation a T cell trophic factor, *e.g.*, a T cell trophic factor selected from IL-7 (SEQ ID NO: 14), IL-12 (SEQ ID NO: 15 and 16), IL-15 (SEQ ID NO: 17), IL-18 (SEQ ID NO: 18), and IFN γ (SEQ ID NO: 19).
- 30 **[0011]** Depending upon the circumstances, for example, the indication to be treated, the T cell is a CAR T cell. In such an embodiment, the therapeutic polypeptide can comprise soluble TGF β RII (SEQ ID NO: 20 or SEQ ID NO: 21). Alternatively, the therapeutic polypeptide comprises an antigen recognized by the CAR T cell, *e.g.*, mesothelin. Alternatively or in addition, the therapeutic polypeptide can comprise a co-stimulatory
- 35 molecule, *e.g.*, a co-stimulatory molecule selected from CD40L (SEQ ID NO: 22) and OX40L (SEQ ID NO: 23).

- 5 **[0012]** In certain embodiments, the therapeutic polypeptide supports NK cell survival in the tumor microenvironment by enhancing recruitment of NK cells to a site of a tumor. Examples of such therapeutic polypeptides include, without limitation, the chemokine ligands CCL2 (SEQ ID NO: 24), CX3CL1 (SEQ ID NO: 25), CXCL16 (SEQ ID NO: 26), CCL5 (SEQ ID NO: 27), CXCL9 (SEQ ID NO: 28), CXCL10 (SEQ ID NO: 29), CXCL11 (SEQ ID NO: 30). In certain embodiments, the therapeutic polypeptide supports NK cell survival in the tumor microenvironment by supporting NK cell function. Examples of such therapeutic polypeptide include without limitation an NK cell trophic factor, *e.g.*, an NK cell trophic factor selected from IL-2 (SEQ ID NO: 31), IL-15 (SEQ ID NO: 17), IL-18 (SEQ ID NO: 18), and IFN α (SEQ ID NO: 32).
- 10 **[0013]** In certain embodiments, the therapeutic polypeptide induces tertiary lymphoid structures (TLS) in a tumor bed. Such a therapeutic polypeptide can comprise CCL19 (SEQ ID NO: 12), lymphotoxin β (SEQ ID NO: 33), CXCL13 (SEQ ID NO: 34), or TNF.
- 15 **[0014]** In certain embodiments, the therapeutic polypeptide promotes phagocytic innate immune surveillance, which can include, without limitation, a therapeutic polypeptide that disrupts the Sirp α /CD47 axis such as a Sirp α -IgG fusion transgene (SIRPalpha SEQ ID NO: 37).
- 20 **[0015]** In certain embodiments, the nucleic acid further comprises a therapeutic polypeptide that supports anti-tumor macrophage polarization. In certain embodiments, the therapeutic polypeptide that supports anti-tumor macrophage polarization comprises TNF, IL-1 (SEQ ID NO: 35), IL-12 (SEQ ID NO: 15 and 16), IL-17 (SEQ ID NO: 36), or IFN γ (SEQ ID NO: 19).
- 25 **[0016]** In another aspect, the disclosure relates to a method of expressing a polypeptide in a subject comprising administering to the subject a vector comprising a variant of a herpes simplex virus (HSV) strain whose genome contains an alteration such that the variant fails to express functional ICP4 and ICP47 proteins. In certain embodiments, the functional ICP4 protein is characterized by the amino acid sequences of SEQ ID NO: 3 or SEQ ID NO: 4 and the functional ICP47 protein is characterized by the amino acid sequences of SEQ ID NO: 5. In certain embodiments, the HSV strain is an HSV-1 strain, *e.g.*, a McKrae strain. In certain embodiments, the vector, when administered to the subject, results in one or more of (a)
- 30 delayed oncolysis; (b) increased immunogenicity; and (c) increased immune activation.
- 35

5 [0017] In certain embodiments, the HSV strain comprises a nucleic acid encoding a therapeutic polypeptide. In certain embodiments, the therapeutic polypeptide the therapeutic polypeptide can have one or more of the following attributes:

- (a) targets the stroma of a tumor;
- (b) supports T cell (*e.g.*, CAR T cell) and/or NK cell survival in a tumor

10 microenvironment;

- (c) induces tertiary lymphoid structures (TLS) in a tumor bed; and/or
- (d) promotes phagocytic innate immune surveillance.

[0018] In certain embodiments, the therapeutic polypeptide targets the stroma of a tumor by degrading an extracellular matrix protein. Examples of such therapeutic polypeptides include
15 without limitation, hyaluronidase, MMP-9, or an inhibitor of lysyl oxidase. In certain embodiments, the therapeutic polypeptide targets the stroma of a tumor by activating local endothelium to increase T cell infiltration. Examples of such therapeutic polypeptides include without limitation a proinflammatory cytokine, *e.g.*, TNF, IL-1 β , IL-6 or IL-18.

[0019] In certain embodiments, the therapeutic polypeptide supports T cell survival in the
20 tumor microenvironment by enhancing recruitment of T cells to a site of a tumor. Examples of such therapeutic polypeptides include without limitation CCL19 or CCL21. In certain embodiments, the therapeutic polypeptide supports T cell survival in the tumor microenvironment by supporting T cell function. Examples of such therapeutic polypeptide include without limitation a T cell trophic factor, *e.g.*, a T cell trophic factor selected from IL-
25 7, IL-12, IL-15, IL-18, and IFN γ .

[0020] Depending upon the circumstances, for example, the indication to be treated, the T cell is a CAR T cell. In such an embodiment, the therapeutic polypeptide can comprise soluble TGF β RII. Alternatively, the therapeutic polypeptide comprises an antigen recognized by the CAR T cell, *e.g.*, mesothelin. Alternatively or in addition, the therapeutic
30 polypeptide can comprise a co-stimulatory molecule, *e.g.*, a co-stimulatory molecule selected from CD40L and OX40L.

[0021] In certain embodiments, the therapeutic polypeptide supports NK cell survival in the tumor microenvironment by enhancing recruitment of NK cells to a site of a tumor. Examples of such therapeutic polypeptides include, without limitation, the chemokine ligands
35 CCL2, CX3CL1, CXCL16, CCL5, CXCL9, CXCL10, CXCL11. In certain embodiments,

5 the therapeutic polypeptide supports NK cell survival in the tumor microenvironment by supporting NK cell function. Examples of such therapeutic polypeptide include without limitation an NK cell trophic factor, *e.g.*, an NK cell trophic factor selected from IL-2, IL-15, IL-18, and IFN α .

[0022] In certain embodiments, the therapeutic polypeptide induces tertiary lymphoid
10 structures (TLS) in a tumor bed. Such a therapeutic polypeptide can comprise CCL19, lymphotoxin β , CXCL13, or TNF.

[0023] In certain embodiments, the therapeutic polypeptide promotes phagocytic innate immune surveillance, which can include, without limitation, a therapeutic polypeptide that disrupts the Sirp α /CD47 axis such as a Sirp α -IgG fusion transgene.

15 [0024] In certain embodiments, the nucleic acid further comprises a therapeutic polypeptide that supports anti-tumor macrophage polarization. In certain embodiments, the therapeutic polypeptide that supports anti-tumor macrophage polarization comprises TNF, IL-1, IL-12, IL-17, or IFN γ .

[0025] In another aspect, the disclosure relates to a method of preparing a vector comprising
20 a variant herpes simplex virus (HSV) genome which contains an alteration such that the variant fails to express functional ICP4 and ICP47 proteins. The vector expresses at least one therapeutic polypeptide. The method includes incubating cells transfected with (a) a first nucleic acid molecule that comprises (i) a portion of HSV genome but does not encode functional ICP4 and ICP47 proteins and (ii) a sequence that encodes a marker element,
25 wherein the sequence that encodes the marker element is flanked by a first homology region (HR1) and a second homology region (HR2); and (b) a second nucleic acid molecule which comprises a sequence that encodes a therapeutic polypeptide, wherein the sequence encoding the therapeutic polypeptide is flanked by a first homology region (HR1_) and a second homology region (HR2_). The HR1 is homologous to HR1_ and HR2 is homologous to
30 HR2_ such that the sequence encoding the therapeutic polypeptide is integrated into the first nucleic acid molecule via homologous recombination. In certain embodiments, the cells are ICP4 and/or ICP47 complementing cells. In certain embodiments, the method further comprises a step of purifying viral plaques that do not express the marker element.

[0026] In certain embodiments, the HSV genome is an HSV-1 genome, *e.g.*, a McKrae strain
35 genome.

5 **[0027]** In certain embodiments, the therapeutic polypeptide comprises one or more of the following attributes:

- (a) targets the stroma of a tumor;
- (b) supports T cell (*e.g.*, CAR T cell) and/or NK cell survival in a tumor microenvironment;
- 10 (c) induces tertiary lymphoid structures (TLS) in a tumor bed; and/or
- (d) promotes phagocytic innate immune surveillance.

[0028] In certain embodiments, the therapeutic polypeptide targets the stroma of a tumor by degrading an extracellular matrix protein. Examples of such therapeutic polypeptides include without limitation, hyaluronidase, MMP-9, or an inhibitor of lysyl oxidase. In certain

15 embodiments, the therapeutic polypeptide targets the stroma of a tumor by activating local endothelium to increase T cell infiltration. Examples of such therapeutic polypeptides include without limitation a proinflammatory cytokine, *e.g.*, TNF, IL-1 β , IL-6 or IL-18.

[0029] In certain embodiments, the therapeutic polypeptide supports T cell survival in the tumor microenvironment by enhancing recruitment of T cells to a site of a tumor. Examples

20 of such therapeutic polypeptides include without limitation CCL19 or CCL21. In certain embodiments, the therapeutic polypeptide supports T cell survival in the tumor microenvironment by supporting T cell function. Examples of such therapeutic polypeptide include without limitation a T cell trophic factor, *e.g.*, a T cell trophic factor selected from IL-7, IL-12, IL-15, IL-18, and IFN γ .

25 **[0030]** Depending upon the circumstances, for example, the indication to be treated, the T cell is a CAR T cell. In such an embodiment, the therapeutic polypeptide can comprise soluble TGF β RII. Alternatively, the therapeutic polypeptide comprises an antigen recognized by the CAR T cell, *e.g.*, mesothelin. Alternatively or in addition, the therapeutic polypeptide can comprise a co-stimulatory molecule, *e.g.*, a co-stimulatory molecule selected

30 from CD40L and OX40L.

[0031] In certain embodiments, the therapeutic polypeptide supports NK cell survival in the tumor microenvironment by enhancing recruitment of NK cells to a site of a tumor. Examples of such therapeutic polypeptides include, without limitation, the chemokine ligands CCL2, CX3CL1, CXCL16, CCL5, CXCL9, CXCL10, CXCL11. The therapeutic

35 polypeptide can support NK cell survival in the tumor microenvironment by supporting NK

5 cell function. Examples of such therapeutic polypeptide include without limitation an NK cell trophic factor, *e.g.*, an NK cell trophic factor selected from IL-2, IL-15, IL-18, and IFN α .

[0032] In certain embodiments, the therapeutic polypeptide induces tertiary lymphoid structures (TLS) in a tumor bed. Such a therapeutic polypeptide can comprise CCL19, lymphotoxin β , CXCL13, or TNF.

10 [0033] In certain embodiments, the therapeutic polypeptide promotes phagocytic innate immune surveillance, which can include, without limitation, a therapeutic polypeptide that disrupts the Sirp α /CD47 axis such as a Sirp α -IgG fusion transgene.

[0034] In certain embodiments, the nucleic acid further comprises a therapeutic polypeptide that supports anti-tumor macrophage polarization, which can include, without limitation,

15 TNF, IL-1, IL-12, IL-17, or IFN γ .

[0035] In another aspect, the disclosure relates to a variant HSV strain comprising a vector as described herein.

[0036] In another aspect, the disclosure relates to a cell transduced with a vector as described herein.

20 [0037] In another aspect, the disclosure relates to a pharmaceutical composition comprising a vector as described herein and a pharmaceutically acceptable carrier.

[0038] In another aspect, the disclosure relates to a method of reducing the size of a tumor in a subject in need thereof. The method includes administering to the subject a vector comprising a variant of a herpes simplex virus (HSV) strain whose genome contains an

25 alteration such that the variant fails to express functional ICP4 and ICP47 proteins. Furthermore, the vector comprises a nucleic acid encoding one or more therapeutic polypeptides that function to reduce the size of the tumor.

[0039] In certain embodiments, the variant fails to express functional ICP4 and ICP47 proteins characterized by the amino acid sequences of SEQ ID NO: 3 and 4 and SEQ ID NO:

30 5, respectively. In certain embodiments, the HSV strain is an HSV-1 strain, *e.g.*, a McKrae strain.

[0040] In certain embodiments, the therapeutic polypeptide comprises one or more of the following attributes:

(a) targets the stroma of a tumor;

- 5 (b) supports T cell (*e.g.*, CAR T cell) and/or NK cell survival in a tumor microenvironment;
- (c) induces tertiary lymphoid structures (TLS) in a tumor bed; and/or
- (d) promotes phagocytic innate immune surveillance.

[0041] In certain embodiments, the therapeutic polypeptide targets the stroma of a tumor by
 10 degrading an extracellular matrix protein. Examples of such therapeutic polypeptides include without limitation, hyaluronidase, MMP-9, or an inhibitor of lysyl oxidase. In certain embodiments, the therapeutic polypeptide targets the stroma of a tumor by activating local endothelium to increase T cell infiltration. Examples of such therapeutic polypeptides include without limitation a proinflammatory cytokine, *e.g.*, TNF, IL-1 β , IL-6 or IL-18.

15 [0042] In certain embodiments, the therapeutic polypeptide supports T cell survival in the tumor microenvironment by enhancing recruitment of T cells to a site of a tumor. Examples of such therapeutic polypeptides include, without limitation, CCL19 or CCL21. In certain embodiments, the therapeutic polypeptide supports T cell survival in the tumor microenvironment by supporting T cell function. Examples of such therapeutic polypeptide
 20 include without limitation a T cell trophic factor, *e.g.*, a T cell trophic factor selected from IL-7, IL-12, IL-15, IL-18, and IFN γ .

[0043] Depending upon the circumstances, for example, the indication to be treated, the T cell is a CAR T cell. In such an embodiment, the therapeutic polypeptide can comprise soluble TGF β RII. Alternatively, the therapeutic polypeptide comprises an antigen
 25 recognized by the CAR T cell, *e.g.*, mesothelin. Alternatively or in addition, the therapeutic polypeptide can comprise a co-stimulatory molecule, *e.g.*, a co-stimulatory molecule selected from CD40L and OX40L.

[0044] In certain embodiments, the therapeutic polypeptide supports NK cell survival in the tumor microenvironment by enhancing recruitment of NK cells to a site of a tumor.
 30 Examples of such therapeutic polypeptides include, without limitation, the chemokine ligands CCL2, CX3CL1, CXCL16, CCL5, CXCL9, CXCL10, CXCL11. In certain embodiments, the therapeutic polypeptide supports NK cell survival in the tumor microenvironment by supporting NK cell function. Examples of such therapeutic polypeptide include without limitation an NK cell trophic factor, *e.g.*, an NK cell trophic factor selected from IL-2, IL-15,
 35 IL-18, and IFN α .

5 **[0045]** In certain embodiments, the therapeutic polypeptide induces tertiary lymphoid structures (TLS) in a tumor bed. Such a therapeutic polypeptide can comprise CCL19, lymphotoxin β , CXCL13, or TNF.

[0046] In certain embodiments, the therapeutic polypeptide promotes phagocytic innate immune surveillance, which can include, without limitation, a therapeutic polypeptide that
10 disrupts the Sirp α /CD47 axis such as a Sirp α -IgG fusion transgene.

[0047] In certain embodiments, the nucleic acid further comprises a therapeutic polypeptide that supports anti-tumor macrophage polarization. In certain embodiments, the therapeutic polypeptide that supports anti-tumor macrophage polarization comprises TNF, IL-1, IL-12, IL-17, or IFN γ .

15 **[0048]** In another aspect, the disclosure relates to a vector comprising a herpes simplex virus (HSV) genome, wherein the vector comprises an alteration that prevents expression of one or more functional ICP4 and ICP47 proteins, and wherein the vector encodes one or more therapeutic polypeptides that support NK cell survival in the tumor microenvironment (TME).

20 **[0049]** In certain embodiments, the NK cell is a native NK cell or a CAR NK cell. In certain embodiments, the one or more therapeutic polypeptides includes an NK cell recruitment factor. In certain embodiments, the NK cell recruitment factor is a chemokine ligand. In certain embodiments, the chemokine ligand is selected from CCL2, CX3CL1, CXCL16, CCL5, CXCL9, CXCL10, and CXCL11. In certain embodiments, the one or more
25 therapeutic polypeptides includes one or more NK cell trophic factors selected from IL-2, IL-15, IL-18, and IFN α . In certain embodiments, the one or more therapeutic polypeptides includes soluble TGF β RII.

[0050] These and other aspects and features of the invention are described in the following detailed description and claims.

30 **BRIEF DESCRIPTION OF THE DRAWINGS**

[0051] The foregoing and other objects, features and advantages of the disclosure will become apparent from the following description of preferred embodiments, as illustrated in the accompanying drawings. Like referenced elements identify common features in the

5 corresponding drawings. The drawings are not necessarily to scale, with emphasis instead being placed on illustrating the principles of the present disclosure.

[0052] **FIGs. 1A-C** are schematic illustrations of the McKrae strain HSV-1 genome. **FIG. 1A** depicts a wild type McKrae strain HSV-1 genome showing essential genes (encoding UL27 (gB), UL48 (VP16), ICP27, and ICP4) and non-essential genes (encoding ICP0, LAT, 10 UL37, UL38, UL41 (vhs), LAT, ICP0, ICP22, and ICP47). The genome is organized into two segments, unique long (UL) and unique short (US) connected via a joint region. ICP4 is encoded at two loci and ICP47 at one locus. TR indicates a terminal repeat; IR indicates an inverted repeat. **FIG. 1B** depicts a schematic illustration of a McKrae strain HSV-1 genome with deleted ICP4 loci. **FIG. 1C** depicts a schematic illustration of a McKrae strain HSV-1 15 genome with both ICP4 and ICP47 loci deleted.

[0053] **FIG. 2** illustrates an exemplary vector design for delivering multiple payloads (*e.g.*, Genes 1-5) to cancer cells. The upper panel shows a general design of a suitable promoter that drives expression of a payload cassette. The lower panel shows an exemplary design for the payload cassette with a HCMV immediate early promoter (HCMV IEp), Payload genes 1- 20 5 and a poly A tail (pA). Thosea asigna virus 2A (T2) and porcine teschovirus-1 2A (P2) depict exemplary ribosomal skipping sites.

[0054] **FIG. 3A** is a graph showing the viability of cancer cells infected with a McKrae HSV-1 strain with an ICP4 deletion at three MOIs (0, 3, and 10) monitored over 8 days. As shown, infection with the ICP4-deleted virus resulted in delayed oncolysis. **FIG. 3B** 25 provides a graph showing payload expression in cancer cells infected with a McKrae HSV-1 strain with an ICP4 deletion at three MOIs (0, 3, and 10) monitored over 8 days. As shown, infection with the ICP4-deleted virus resulted in sustained payload expression. **FIG. 3C** is a graph showing the viability of Hs578T cancer cells infected with an mutICP4mutICP47 McKrae HSV-1 strain at three MOIs (0, 3, and 10) monitored over 6 days. **FIG. 3D** is a 30 graph showing IFN γ payload expression in Hs578T cancer cells infected with an mutICP4mutICP47 McKrae HSV-1 strain at MOI 10 at day 2, 4 and 6.

[0055] **FIG. 4A** is a graph showing payload (GFP) expression in cells infected with one of two exemplary oncolytic viruses, mutICP4 McKrae HSV-1 and mutICP4mutICP47 McKrae HSV-1, at three MOIs (0.3, 1, and 3) after 24 hours. “mutICP4” is a McKrae HSV-1 strain in 35 which the genes encoding ICP4 are deleted. “mutICP4mutICP47” is a McKrae HSV-1 strain in which the genes encoding ICP4 and ICP47 are deleted. These data indicate that

- comparable infection with mutICP4 and mutICP4mutICP47 were obtained. **FIG. 4B** is a graph showing GFP and HLA expression in cells infected with one of two exemplary oncolytic viruses mutICP4 McKrae HSV-1 and mutICP4mutICP47 McKrae HSV-1 at three MOIs (0.3, 1, and 3) after 24 hours. These data indicate that nearly all cells were HLA positive. **FIG. 4C** is a flow cytometry graph of HLA expression in cells infected with one of two exemplary oncolytic viruses mutICP4 McKrae HSV-1 and mutICP4mutICP47 McKrae HSV-1 at three MOIs (0.3, 1, and 3) after 24 hours by flow cytometry. These data show that increased cell surface expression of HLA, a component of MHC class I molecules, is observed at all MOI for viruses deleted for both ICP4 and ICP47 compared to ICP4 only. **FIG. 4D** is a graph showing Tap1 expression in Hs578T cells infected with one of two exemplary oncolytic viruses mutICP4mutICP47 McKrae HSV-1 and mutICP27 McKrae HSV-1 as measured by RNAseq. **FIG. 4E** is a graph showing MHC class I expression in Hs578T cells infected with one of two exemplary oncolytic viruses mutICP4mutICP47 McKrae HSV-1 and mutICP27 McKrae HSV-1 as measured by flow cytometry. **FIG. 4F** is a graph showing cell signaling pathways in cancer cells upregulated after mutICP4mutICP47 McKrae HSV-1 infection. **FIG. 4G** is a graph showing cell signaling pathways in cancer cells upregulated after mutICP27 McKrae HSV-1 infection. **FIG. 4H** is a graph showing expression of TNF super family members (TNF, LTB, TNFSF14, PYCARD, and CASP10) in cancer cells after infection with mutICP4mutICP47 McKrae HSV-1 or mutICP27 McKrae HSV-1.
- [0056]** **FIGs. 5A-B** are graphs showing the number of CD31+ endothelial cells and PDGFRa+PDPN+ stromal cells in the salivary glands of mice after infection with mutICP4mutICP47 McKrae HSV-1 viruses carrying payloads of murine LTB, CXCL13, CCL19, CCL21, and IL-7. **FIGs. 5C** is a graph showing the expression of TGFβ1 in Hs578T cells after infection with mutICP4mutICP47 (with GFP encoded as a marker) as measured by RNAseq.
- [0057]** **FIG. 6A** is a graph showing viability of Hs578T cells after infection with either viral backbone alone (mutICP4mutICP47-GFP) or with mutICP4mutICP47 vectors encoding human IFNγ, human IL-12, or human IFNγ and human IL-12, or added recombinant rhIL-15 after co-culture with PBMCs. **FIGs. 6B** and **6C** are graphs showing the number of Ki67+Granzyme B+ CD8+ T cells after 24h and 72h of coculture of PBMCs with Hs578T cells after infection with MutICP4mutICP47-IFNγ or MutICP4mutICP47-GFP (backbone).

5 **FIG. 6D** is a graph showing the number of Granzyme B+ Ki67- NK cells after 24h of coculture of PBMCs with Hs578T cells after infection with MutICP4mutICP47-IFN γ or MutICP4mutICP47-GFP (backbone). **FIG. 6E** is a graph showing the number of antigen-presenting cells (CD11c+CD16+CD14-Ki67+MHCII++) after 24h of coculture of PBMCs with Hs578T cells after infection with MutICP4mutICP47-IFN γ or MutICP4mutICP47-GFP (backbone). **FIG. 6F** is a graph showing viability of Hs578T cells with or without PBMC coculture after infection with MutICP4mutICP47-IFN γ or MutICP4mutICP47-GFP (backbone). **FIG. 6G** is a graph showing expression of multiple T and NK cell chemokines (CCL2, CXCL9, CXCL10, CXCL11, CCL5, CX3CL1, and CXCL16) by Hs578T cells after infection with mutICP4mutICP47-GFP or mutICP4mutICP47-IFN γ for 24 h as measured by
10
15 RNAseq.

[0058] FIG. 7A is a graph showing the percentage (%) of CD45+ cells in salivary glands of mice harvested 15 days after delivery of mutICP4mutICP-GFP HSV-1 vector or vehicle to the mouse. **FIG. 7B** shows pictures of cells isolated from salivary glands of mice harvested 15 days after delivery of mutICP4mutICP-GFP HSV-1 vector to the mouse collected by
20 microscope at 40x resolution. Cells are stained for CD4+ (T cell marker), B220 (B cell marker), CD11c (dendritic cell marker), and DAPI (nuclei marker). **FIG. 7C** shows pictures from salivary glands of mice harvested 15 days after delivery of a combination of mutICP4mutICP47 HSV-1 vectors which express murine TNF, CCL19, IL-17a, and IL-7 to a mouse collected by microscope at 10x and 40x resolution. Cells are stained for CD4+ (T cell
25 marker), B220 (B cell marker), CD11c (dendritic cell marker), and DAPI (nuclei marker).

[0059] FIG. 8 is a graph showing the percent (%) phagocytosis of labelled Raji cells by macrophages. Raji cells were pre-treated with conditioned medium collected from mutICP4mutICP47-SIRP α -IgG infected Hs578T cells (MOI of 3 or 10), conditioned medium collected from mutICP4mutICP47-GFP cells, IgG, or an anti-CD47 antibody.

30 DETAILED DESCRIPTION

[0060] The invention is based, in part, upon the discovery that HSV-1 vectors comprising an alteration (such as a gene deletion or an inactivating mutation) that prevents expression of one or more functional ICP4 and ICP47 proteins have reduced replicative capacity, are oncolytic, have an extended period of payload expression, are cytotoxic to proliferating cells
35 (*e.g.*, cancer cells), and exhibit enhanced immunogenicity. Accordingly, it has been discovered that the vectors are useful for delivering a therapeutic payload (*e.g.*, a therapeutic

5 polypeptide) that can be useful in reducing the size of a tumor, for example, to treat cancer in a subject in need thereof. The vectors can induce delayed oncolysis, allowing for sustained expression of a therapeutic payload (*e.g.*, a therapeutic polypeptide). Furthermore, the vectors exhibit increased immunogenicity, increasing immune activity toward tumor cells.

[0061] The invention also is based, in part, upon the discovery that particular combinations
 10 of therapeutic polypeptides can be used to disrupt one or more pathways (*e.g.*, parallel pathways) affecting the ability of immune cells to kill cancer cells, thereby reducing the size of a tumor. For example, therapeutic polypeptides that target the stroma of a tumor, support T cell survival (*e.g.*, CAR T cell) and/or NK cell in a tumor microenvironment, induce tertiary lymphoid structures (TLS) in a tumor bed and/or promote phagocytic innate immune
 15 surveillance can be expressed from a viral vector, *e.g.*, a viral vector lacking functional ICP4 and ICP47 proteins to increase the ability of immune cells to kill tumor cells, for example, to treat cancer in a subject in need thereof. Vectors can be designed to include a specific combination of therapeutic polypeptides that will be effective to treat a given tumor types, including choosing one or more therapeutic polypeptides the reduce inhibitory features of a
 20 specific tumor microenvironment that may prevent the immune system from effectively attacking the tumor.

I. Definitions

[0062] In this application, unless otherwise clear from context, (i) the term “a” may be understood to mean “at least one”; (ii) the term “or” may be understood to mean “and/or”;
 25 (iii) the terms “comprising” and “including” may be understood to encompass itemized components or steps whether presented by themselves or together with one or more additional components or steps; and (iv) where ranges are provided, endpoints are included.

[0063] As used herein, the term “administration” refers to the administration of a composition to a subject or system. Administration to an animal subject (*e.g.*, to a human)
 30 may be by any appropriate route. For example, in some embodiments, administration may be bronchial (including by bronchial instillation), buccal, enteral, interdermal, intra-arterial, intradermal, intragastric, intramedullary, intramuscular, intranasal, intraperitoneal, intrathecal, intratumoral, intravenous, intraventricular, within a specific organ (*e.g.*, intrahepatic), mucosal, nasal, oral, rectal, subcutaneous, sublingual, topical, tracheal
 35 (including by intratracheal instillation), transdermal, vaginal and vitreal. Depending upon the

5 circumstances, administration may involve intermittent dosing and/or may involve continuous dosing (*e.g.*, perfusion) for at least a selected period of time.

[0064] As used herein, the term “agent” refers to a compound or entity of any chemical class including, for example, polypeptides, nucleic acids, saccharides, lipids, small molecules, or combinations thereof. In some embodiments, an agent is or comprises a natural product in
10 that it is found in and/or is obtained from nature. In some embodiments, an agent is or comprises one or more entities that is man-made in that it is designed, engineered, and/or produced through action of the hand of man and/or is not found in nature. Some particular embodiments of agents that may be utilized in accordance with the present invention include small molecules, antibodies, antibody fragments, aptamers, nucleic acids (*e.g.*, siRNAs,
15 shRNAs, DNA/RNA hybrids, antisense oligonucleotides, ribozymes), peptides, peptide mimetics, etc.

[0065] As used herein, the term “amelioration” refers to the prevention, reduction or palliation of a state, or improvement of the state of a subject. Amelioration includes, but does not require complete recovery or complete prevention of a disease, disorder or condition.

20 [0066] As used herein, the term “animal” refers to any member of the animal kingdom. In some embodiments, “animal” refers to humans, of either sex and at any stage of development. In some embodiments, “animal” refers to non-human animals, at any stage of development. In certain embodiments, the non-human animal is a mammal (*e.g.*, a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, and/or a pig). In
25 some embodiments, animals include, but are not limited to, mammals, birds, reptiles, amphibians, fish, insects, and/or worms. In some embodiments, an animal may be a transgenic animal, genetically engineered animal, and/or a clone. Depending upon the context, the terms “human,” “patient” and “subject” are used interchangeably herein.

[0067] The terms “about” and “approximately” may be understood to permit standard
30 variation as would be understood by those of ordinary skill in the art. For example, as used herein, the term “about” refers to a $\pm 10\%$ variation from the nominal value unless otherwise indicated or inferred.

[0068] As used herein, the term “combination therapy” refers to those situations in which a subject is simultaneously exposed to two or more therapeutic regimens (*e.g.*, two or more
35 therapeutic agents). In some embodiments, two or more agents or may be administered

- 5 simultaneously; in some embodiments, such agents may be administered sequentially; in some embodiments, such agents are administered in overlapping dosing regimens.

[0069] As used herein, the term “engineered” refers to the aspect of having been manipulated by the hand of man. For example, a polynucleotide is considered to be “engineered” when two or more sequences, that are not linked together in that order in nature, are manipulated by the hand of man to be directly linked to one another in the engineered polynucleotide. For example, in some embodiments of the present disclosure, an engineered polynucleotide comprises a regulatory sequence that is found in nature in operative association with a first coding sequence but not in operative association with a second coding sequence, is linked by the hand of man so that it is operatively associated with the second coding sequence.

10

15 Comparably, a cell or organism is considered to be “engineered” if it has been manipulated so that its genetic information is altered (*e.g.*, new genetic material not previously present has been introduced, for example by transformation, mating, somatic hybridization, transfection, transduction, or other mechanism, or previously present genetic material is altered or removed, for example by substitution or deletion mutation, or by mating protocols). As is

20 common practice and is understood by those in the art, progeny of an engineered polynucleotide or cell are typically still referred to as “engineered” even though the actual manipulation was performed on a prior entity.

[0070] As used herein, “expression” of a nucleic acid sequence refers to one or more of the following events: (1) production of an RNA template from a DNA sequence (*e.g.*, by transcription); (2) processing of an RNA transcript (*e.g.*, by splicing, editing, 5' cap formation, and/or 3' end formation); (3) translation of an RNA into a polypeptide or protein; and/or (4) post-translational modification of a polypeptide or protein.

25

[0071] As used herein, the term “homology” refers to the overall relatedness between polymeric molecules, *e.g.*, between nucleic acid molecules (*e.g.*, DNA molecules and/or RNA molecules) and/or between polypeptide molecules. In some embodiments, polymeric molecules are considered to be “homologous” to one another if their sequences are at least 75%, 80%, 85%, 90%, 95%, or 99% identical. In some embodiments, polymeric molecules are considered to be “homologous” to one another if their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% similar.

30

[0072] As used herein, the term “isolated” refers to a substance and/or entity that has been (1) separated from at least some of the components with which it was associated when initially

35

5 produced (whether in nature and/or in an experimental setting), and/or (2) designed, produced, prepared, and/or manufactured by the hand of man. Isolated substances and/or entities may be separated from about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% of
10 the other components with which they were initially associated. In some embodiments, isolated agents are about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is “pure” if it is substantially free of other components. In some embodiments, as will be understood by those skilled in the art, a
15 substance may still be considered “isolated” or even “pure”, after having been combined with certain other components such as, for example, one or more carriers or excipients (*e.g.*, buffer, solvent, water, etc.); in such embodiments, percent isolation or purity of the substance is calculated without including such carriers or excipients. To give but one example, in some embodiments, a biological polymer such as a polypeptide or polynucleotide that occurs in
20 nature is considered to be “isolated” when, a) by virtue of its origin or source of derivation is not associated with some or all of the components that accompany it in its native state in nature; b) it is substantially free of other polypeptides or nucleic acids of the same species from the species that produces it in nature; c) is expressed by or is otherwise in association with components from a cell or other expression system that is not of the species that
25 produces it in nature. Thus, for instance, in some embodiments, a polypeptide that is chemically synthesized or is synthesized in a cellular system different from that which produces it in nature is considered to be an “isolated” polypeptide. Alternatively or additionally, in some embodiments, a polypeptide that has been subjected to one or more purification techniques may be considered to be an “isolated” polypeptide to the extent that it
30 has been separated from other components a) with which it is associated in nature; and/or b) with which it was associated when initially produced.

[0073] As used herein, the term “marker element” refers to a detectable or selectable agent. In some embodiments, a “marker element” is a detectable or selectable nucleic acid sequence. In some embodiments a “marker element” is an expression product (*e.g.*, RNA or protein)
35 whose presence or absence is detectable and/or selectable in cells. In some embodiments, an expression product is or comprises an enzyme. In some embodiments, an expression product is a fluorophore.

5 [0074] As used herein, the term “nucleic acid” refers to any compound and/or substance that is or can be incorporated into an oligonucleotide chain. In some embodiments, a nucleic acid is a compound and/or substance that is or can be incorporated into an oligonucleotide chain via a phosphodiester linkage. As will be clear from context, in some embodiments, “nucleic acid” refers to individual nucleic acid residues (*e.g.*, nucleotides and/or nucleosides); in some
10 embodiments, “nucleic acid” refers to an oligonucleotide chain comprising individual nucleic acid residues. In some embodiments, a “nucleic acid” is or comprises RNA; in some embodiments, a “nucleic acid” is or comprises DNA. In some embodiments, a nucleic acid is, comprises, or consists of one or more natural nucleic acid residues. In some embodiments, a nucleic acid is, comprises, or consists of one or more nucleic acid analogs. In some
15 embodiments, a nucleic acid analog differs from a nucleic acid in that it does not utilize a phosphodiester backbone. For example, in some embodiments, a nucleic acid is, comprises, or consists of one or more “peptide nucleic acids”, which are known in the art and have peptide bonds instead of phosphodiester bonds in the backbone, are considered within the scope of the present disclosure. Alternatively or additionally, in some embodiments, a
20 nucleic acid has one or more phosphorothioate and/or 5'-N-phosphoramidite linkages rather than phosphodiester bonds. In some embodiments, a nucleic acid is, comprises, or consists of one or more natural nucleosides (*e.g.*, adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxy guanosine, and deoxycytidine). In some embodiments, a nucleic acid is, comprises, or consists of one or more nucleoside analogs
25 (*e.g.*, 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3 -methyl adenosine, 5-methylcytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5 -propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, 0(6)-methylguanine, 2-thiocytidine, methylated bases, intercalated bases, and combinations thereof). In some embodiments, a nucleic acid
30 comprises one or more modified sugars (*e.g.*, 2'-fluororibose, ribose, 2'-deoxyribose, arabinose, and hexose) as compared with those in natural nucleic acids. In some embodiments, a nucleic acid has a nucleotide sequence that encodes a functional gene product such as an RNA or protein. In some embodiments, a nucleic acid includes one or
35 more introns. In some embodiments, nucleic acids are prepared by one or more of isolation from a natural source, enzymatic synthesis by polymerization based on a complementary template (*in vivo* or *in vitro*), reproduction in a recombinant cell or system, and chemical

5 synthesis. In some embodiments, a nucleic acid is at least 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1,000, 1,500, 2,000, 2,500, 3,000, 3,500, 4,000, 4,500, 5,000 or more residues long. In some
10 double stranded. In some embodiments a nucleic acid has a nucleotide sequence comprising at least one element that encodes, or is the complement of a sequence that encodes, a polypeptide. In some embodiments, a nucleic acid has enzymatic activity.

[0075] As used herein, the terms “patient” or “subject” are used interchangeably and refer to any organism to which a provided composition is or may be administered, *e.g.*, for
15 experimental, diagnostic, prophylactic, cosmetic, and/or therapeutic purposes. Typical subjects include animals (*e.g.*, mammals such as mice, rats, rabbits, non-human primates, and/or humans). In some embodiments, a subject is a human. In some embodiments, a subject is suffering from or susceptible to one or more disorders or conditions. In some
20 embodiments, a subject displays one or more symptoms of a disorder or condition. In some embodiments, a subject has been diagnosed with one or more disorders or conditions. In some embodiments, a subject is receiving or has received certain therapy to diagnose and/or to treat a disease, disorder, or condition.

[0076] As used herein, the term “pharmaceutical composition” refers to an active agent (*e.g.*, a vector), formulated together with one or more pharmaceutically acceptable carriers. In
25 some embodiments, active agent is present in unit dose amount appropriate for administration in a therapeutic regimen that shows a statistically significant probability of achieving a predetermined therapeutic effect when administered to a relevant population. In some embodiments, pharmaceutical compositions may be specially formulated for administration in solid or liquid form, including those adapted for the following: oral administration, for
30 example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, *e.g.*, those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; topical application, for example, as a cream,
35 ointment, or a controlled-release patch or spray applied to the skin, lungs, or oral cavity;

5 intravaginally or intrarectally, for example, as a pessary, cream, or foam; sublingually;
ocularly; transdermally; or nasally, pulmonary, and to other mucosal surfaces.

[0077] As used herein, the term “pharmaceutically acceptable” applied to the carrier, diluent,
or excipient used to formulate a composition as disclosed herein means that the carrier,
diluent, or excipient must be compatible with the other ingredients of the composition and not
10 deleterious to the recipient thereof.

[0078] As used herein, the term “pharmaceutically acceptable carrier” means a
pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler,
diluent, excipient, or solvent encapsulating material, involved in carrying or transporting the
subject compound from one organ, or portion of the body, to another organ, or portion of the
15 body. Each carrier must be “acceptable” in the sense of being compatible with the other
ingredients of the formulation and not injurious to the patient. Some examples of materials
which can serve as pharmaceutically-acceptable carriers include: sugars, such as lactose,
glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its
derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate;
20 powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository
waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and
soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol
and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents,
such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water;
25 isotonic saline; Ringer's solution; ethyl alcohol; pH buffered solutions; polyesters,
polycarbonates and/or polyanhydrides; and other non-toxic compatible substances employed
in pharmaceutical formulations.

[0079] As used herein, the terms “prevent” or “prevention”, when used in connection with
the occurrence of a disease, disorder, and/or condition, refers to reducing the risk of
30 developing the disease, disorder and/or condition and/or to delaying onset of one or more
characteristics or symptoms of the disease, disorder or condition. Prevention may be
considered complete when onset of a disease, disorder or condition has been delayed for a
predefined period of time.

[0080] As used herein, the term “treatment” (also “treat” or “treating”) refers to any
35 administration of a substance that partially or completely alleviates, ameliorates, relieves,
inhibits, delays onset of, reduces severity of, and/or reduces incidence of one or more

5 symptoms, features, and/or causes of a particular disease, disorder, and/or condition (*e.g.*, cancer). Such treatment may be of a subject who does not exhibit signs of the relevant disease, disorder and/or condition and/or of a subject who exhibits only early signs of the disease, disorder, and/or condition. Alternatively or additionally, such treatment may be of a subject who exhibits one or more established signs of the relevant disease, disorder and/or
10 condition. In some embodiments, treatment may be of a subject who has been diagnosed as suffering from the relevant disease, disorder, and/or condition. In some embodiments, treatment may be of a subject known to have one or more susceptibility factors that are statistically correlated with increased risk of development of the relevant disease, disorder, and/or condition.

15 **[0081]** As used herein, the term “vector” refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a “plasmid”, which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated to a viral genome or portion thereof. Certain vectors are capable of
20 autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication, episomal mammalian vectors, herpes simplex virus (HSV) vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the
25 expression of genes to which they are operatively linked. Such vectors are referred to herein as “expression vectors.”

[0082] Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (*e.g.*, electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer’s specifications or
30 as commonly accomplished in the art or as described herein. The foregoing techniques and procedures may be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See *e.g.*, Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor,
35 N.Y. (1989)).

5 **[0083]** The use of sections is not meant to limit the invention. Each section can apply to any aspect of the invention. In this application, the use of “or” means “and/or” unless stated otherwise or clear from context to be disjunctive.

[0084] Throughout the description, where compositions are described as having, including, or comprising specific components, or where processes and methods are described as having,
10 including, or comprising specific steps, it is contemplated that, additionally, there are compositions of the present invention that consist essentially of, or consist of, the recited components, and that there are processes and methods according to the present invention that consist essentially of, or consist of, the recited processing steps.

[0085] In the application, where an element or component is said to be included in and/or
15 selected from a list of recited elements or components, it should be understood that the element or component can be any one of the recited elements or components, or the element or component can be selected from a group consisting of two or more of the recited elements or components.

[0086] Further, it should be understood that elements and/or features of a composition or a
20 method described herein can be combined in a variety of ways without departing from the spirit and scope of the present invention, whether explicit or implicit herein. For example, where reference is made to a particular compound, that compound can be used in various embodiments of compositions of the present invention and/or in methods of the present invention, unless otherwise understood from the context. In other words, within this
25 application, embodiments have been described and depicted in a way that enables a clear and concise application to be written and drawn, but it is intended and will be appreciated that embodiments may be variously combined or separated without parting from the present teachings and invention(s). For example, it will be appreciated that all features described and depicted herein can be applicable to all aspects of the invention(s) described and depicted
30 herein.

[0087] It should be understood that the expression “at least one of” includes individually each of the recited objects after the expression and the various combinations of two or more of the recited objects unless otherwise understood from the context and use. The expression “and/or” in connection with three or more recited objects should be understood to have the
35 same meaning unless otherwise understood from the context.

- 5 **[0088]** The use of the term “include,” “includes,” “including,” “have,” “has,” “having,” “contain,” “contains,” or “containing,” including grammatical equivalents thereof, should be understood generally as open-ended and non-limiting, for example, not excluding additional unrecited elements or steps, unless otherwise specifically stated or understood from the context.
- 10 **[0089]** It should be understood that the order of steps or order for performing certain actions is immaterial so long as the present invention remain operable. Moreover, two or more steps or actions may be conducted simultaneously.
- 15 **[0090]** The use of any and all examples, or exemplary language herein, for example, “such as” or “including,” is intended merely to illustrate better the present invention and does not pose a limitation on the scope of the invention unless claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the present invention.

II. Viral Vectors and HSV-1 (McKrae)

- 20 **[0091]** Viral vectors can be used to facilitate the transfer of nucleic acids into cells. HSV-1 vectors can typically accommodate up to 25 kb of foreign DNA sequences. HSV-1 has an approximate 152-kb double-stranded linear DNA genome that can be maintained episomally in the nucleus of cells. The HSV-1 virion is enveloped and approximately 110 nm in diameter.
- 25 **[0092]** At least 17 strains of HSV-1 have been isolated, including, but not limited to, McKrae, strain 17, strain F, H129, HF10, MacIntyre, Strain HF, ATCC 2011 and KOS (for review, see Watson *et al.*, (2012) *VIROLOGY* 433(2):528-537).

A. McKrae

- 30 **[0093]** A McKrae strain was isolated from a patient with herpes simplex keratitis and subsequently passaged in tissue culture. A partial genome sequence of McKrae is provided at SEQ ID NO: 1 (GenBank Accession No.: JQ730035.1).
- [0094]** Inter-strain differences in HSV-1 peripheral replication and virulence are observed after injection into animals. McKrae undergoes spontaneous or induced reactivation at a higher frequency than other known strains and is among the most virulent HSV-1 strains.

5 [0095] HSV genes influence viral characteristics and phenotype. There are at least 9 genes and several non-coding sequences unique to McKrae strain. In addition to those associated with pathogenesis and latency reactivations, such as RL1, RSI, and RL2, three UL genes (UL36, UL49A, UL56) and three US genes (US7, US10, and US11) are unique for McKrae strain. In addition to gene variations, non-coding sequences such as LAT, 'a' sequence, and
10 miRNAs contain variations unique to McKrae.

[0096] One or more of following gene and non-coding sequences can be considered characteristic of McKrae strain. In McKrae, RL1 (ICP34.5) has an extended P-A-T repeat between residues 159 and 160 that results in 8 iterations, while other strains contain only 3-5 iterations. The P-A-T repeat is thought to influence cellular localization of the ICP34.5
15 protein. (Mao *et al.* (2012) J. BIOL. CHEM. 277(13): 11423-31). ICP34.5 is thought to be a neurovirulence factor involved in viral replication and anti-host response.

[0097] McKrae strain also contains an extended repeat element of six iterations of the internal tandem repeat STPSTTT (SEQ ID NO: 38) located within the coding sequence of US07 (gl). Additionally in McKrae, UL 36 contains a premature stop codon introduced due
20 to a G nucleotide deletion in a mononucleotide string encoding amino acid residue 2453 (nt 72,535) and UL 56 (180 aa) contains a single base pair insertion at nucleotide 115,992 (amino acid 97). McKrae strain also contains an extended ORF in US 10 resulting from a single bp insertion at nucleotide 143,416 and the frameshift causes a stop codon loss in McKrae and a unique C-terminal protein sequence. McKrae has amino acid differences at
25 UL49A at residues 28 and 51 compared to other strains. McKrae has histidine and threonine at residues 28 and 51, respectively, whereas strain 17 has arginine and threonine and other strains (*e.g.*, KOS) have histidine and alanine. Also, McKrae strain contains reduced tandem repeats found at the UL-RL junction (49 bp in McKrae as opposed to 181 bp in strain 17 and KOS) and approximately 330 nucleotides missing immediately following the UL-RL junction
30 repeat. McKrae also contains unique variation within the 'a' sequence direct repeat 2 (DR2) array. Instead of a series of unbroken tandem repeats, the McKrae DR2 repeats are interrupted twice by identical guanine-rich sequences.

[0098] Major variation within the LAT intron between strains is due to differences in a repeat element (GCACCCCACTCCAC) (SEQ ID NO: 39) that varies in iteration number
35 beginning at nucleotide 119,482 in McKrae strain, with McKrae containing 13 repeats while strains F, H129 and 17 contain 9 repeats and KOS contains 15 repeats. Also, tandem repeat

5 variation between strains is found beginning in McKrae at base 125,520. McKrae repeat elements include twelve iterations of CCCCAGCCCCTCCCCAG (SEQ ID NO: 40) and eight iterations of CCCCTCGCCCCCTCCCG (SEQ ID NO: 41). The first repeat unit is unique from other strains in that it contains a G-A transition, and strain McKrae contains three iterations more than any other strain. The McKrae strain second repeat element is collapsed,
 10 missing 188 nucleotides relative to all other strains, and separated from the upstream repeat by a 100% conserved sequence of 105 bp containing miR-H5.

[0099] McKrae further contains a unique coding sequence for ICP4 that is not found in other known strains (Watson *et al.*, (2012) *supra*). ICP4 is an immediate early transcriptional regulator and has been implicated in reactivation. Whereas other strains contain an alanine
 15 rich region (AASAPDAADALAAA) (SEQ ID NO: 42) between residues 707 and 720, in McKrae the alanine rich region is replaced by a serine rich sequence (GPRSSSSSGVAA) (SEQ ID NO: 43). The serine rich block of substitutions present in McKrae is adjacent to the nuclear localization signal (NLS) (amino acid 728-734). A change in conformation of this region may alter the NLS and in turn affect localization of not only ICP4, but also other viral
 20 proteins (e.g. ICP0, ICP8) that are affected by ICP4 localization (Knipe and Smith, Molecular and Cellular Biology, (1986), 6(7), 2371-2381). Thus, this region may influence viral phenotype in part by altering the localization of proteins to the nucleus.

B. Modifications of HSV-1 Vectors

[00100] Vectors comprising an HSV-1 genome (e.g., from a McKrae strain) may have
 25 one or more HSV genes necessary for replication rendered nonfunctional. For example, a vector comprising an HSV genome may contain an alteration that prevents expression of one or more functional ICP0, ICP4, ICP22, ICP27, and ICP47 genes. HSV genes necessary for replication include, for example, immediate early genes such as ICP4 and ICP47. ICP4 is a viral transcription factor which is expressed soon after infection and sustains the HSV-1 viral
 30 cycle. ICP47 of HSV-1 is an 87-amino acid cytosolic polypeptide, 88 residues if the initiation methionine is included. It binds to the TAP1-TAP2 heterodimer in human but not in mouse cells and prevents transport of peptides through blockade of the peptide binding site of TAP. As a consequence, MHC class I molecules fail to be loaded with peptides. The resultant empty class I molecules are retained in the ER and presentation of epitopes to CTL
 35 is abolished in HSV-infected human cells. An alteration that prevents expression of one or more functional HSV genes (e.g., ICP4 and ICP47) can include a mutation (e.g., a missense

5 mutation, a nonsense mutation, an insertion, a deletion, etc.) in the coding sequence of the gene or in a regulatory sequence affecting expression of the gene (*e.g.*, a promoter). ICP47 mutations include, but are not limited to, mutations in A4, D27, K31, R32, R34, or R41 relative to SEQ ID NO: 5 and combinations thereof (as described in Mozzie *et al.* (2022) MOL BIOL EVOL. 39(7):msac142). ICP4 mutations and deletion variants that disrupt its

10 ability to activate transcription include, but are not limited to, n208, d8-10, nd8-10, ΔSER, d120, n12, ΔSERn7, d3-8, nd3-8, m20, m20n7, m90, m90n7, d143, d143n7, and nd3-10 (as described in Wagner *et al.* (2012) J VIROL. 86(12):6862-74). Deletion of the region between amino acids 30 and 210 in the N-terminus of ICP4 sufficient to eliminate transcriptional activation (as described in Wagner *et al.* (2013) J VIROL. 87(2): 1010–1018). In some

15 embodiments, the disclosure provides replication defective HSV vectors with alterations in one or more of ICP0, ICP4, ICP22, ICP27, and ICP47.

[00101] HSV-1 IE promoters contain one or more copies of an IE-specific regulatory sequence of consensus TAATGARAT (SEQ ID NO: 44) (where R is a purine). These motifs are normally located within a few hundred base pairs of the proximal IE promoter sequences,

20 but in conjunction with their flanking sequences they are discrete functional entities which can confer IE-specific regulation to other proximal promoter elements of different temporal class. In some embodiments, replication-defective viruses are created by deleting nucleotides in an IE-specific regulatory sequence, *e.g.*, in an IE-specific regulatory sequence affecting expression of one or more of ICP0, ICP4, ICP22, ICP27, and ICP47. In some embodiments,

25 an IE-specific regulatory sequence contains an internal deletion. In some embodiments, an IE-specific regulatory sequence contains a terminal deletion. In some embodiments, an IE-specific regulatory sequence is completely deleted.

[00102] In some embodiments, the disclosure provides HSV vectors with a nonfunctional ICP4 and ICP47 genes. In certain embodiments, the ICP4 gene or a regulatory

30 sequence affecting expression of ICP4 comprises a mutation and the ICP47 gene or a regulatory sequence affecting expression of ICP47 comprises a mutation. For example, the ICP4 gene can comprise a mutation and the ICP47 gene can comprise a mutation. In another example, the regulatory sequence affecting expression of ICP4 includes a mutation and a regulatory sequence affecting expression of ICP47 includes a mutation. In one specific

35 example, the ICP4 gene or a regulatory sequence affecting expression of ICP4 includes a deletion (*e.g.*, a complete deletion or a partial deletion sufficient to abolish activity) and the

- 5 ICP47 gene or a regulatory sequence affecting expression of ICP47 includes a deletion (*e.g.*, a complete deletion or a partial deletion sufficient to abolish activity). The ICP4 gene can include a deletion and the ICP47 gene can include a deletion. Furthermore, combinations of the foregoing are contemplated, *e.g.*, deletion of ICP4 and a mutation in a regulatory sequence for ICP47.
- 10 **[00103]** In some embodiments, the disclosure provides HSV vectors or HSV strains with a nonfunctional ICP47 gene. In some embodiments, the disclosure provides HSV vectors or HSV strains with nonfunctional ICP4 and ICP47 genes. In some embodiments, the disclosure provides an HSV vector or an HSV strain with ICP4 and ICP47 deleted. In some embodiments, the gene encoding ICP4 and the gene encoding ICP47 is fully or partially
- 15 deleted, without disrupting expression of any additional immediate early genes.
- [00104]** HSV-1 vectors that have altered (*e.g.*, mutated) HSV genes can be produced in cell lines that express the deficient protein in trans. In some embodiments, HSV-1 vectors are produced in a mammalian cell line, *e.g.*, in a mammalian cell line of Vero lineage. In some embodiments, the cell line expresses ICP4. In some embodiments, the cell line
- 20 expresses ICP47. In some embodiments, the cell line expresses ICP4 and ICP47. In some embodiments, the cell line expresses one or more of ICP0, ICP4, ICP22, ICP27, and ICP47. In some embodiments, the cell line expresses ICP4, ICP22, and ICP47. In some embodiments, the cell line expresses ICP4, ICP22, and UL55. In some embodiments, the cell line expresses ICP4, ICP27, and UL55. In some embodiments, the cell line comprises a
- 25 nucleic acid molecule having a simian virus 40 polyadenylation signal (SV40 pA). Depending upon the circumstances, the viral vectors can be produced in Vero 6-5C cells or Vero D cells.
- [00105]** In certain embodiments, the viral vectors of the disclosure are McKrae HSV-1 viral vectors. Wild type McKrae strain HSV-1 comprises two copies of the gene encoding
- 30 ICP4 and one copy of the gene encoding ICP47. In some embodiments, the disclosure provides HSV vectors with a nonfunctional ICP4 and ICP47 genes. In certain embodiments, at least one ICP4 gene (*i.e.*, one or both copies of the gene) or a regulatory sequence affecting expression of ICP4 comprises a mutation and the ICP47 gene or a regulatory sequence affecting expression of ICP47 comprises a mutation. For example, at least one copy of the
- 35 ICP4 gene (*i.e.*, one or both copies of the ICP4 gene) can comprise a mutation and the ICP47 gene can comprise a mutation. In another example, a regulatory sequence affecting

- 5 expression of at least one ICP4 gene (i.e., one or both copies of the ICP4 gene) includes a mutation and a regulatory sequence affecting expression of ICP47 includes a mutation. In one specific example, at least one ICP4 gene (i.e., one or both copies of the ICP4 gene) or a regulatory sequence affecting expression of at least one ICP4 gene (i.e., one or both copies of the ICP4 gene) includes a deletion and the ICP47 gene or a regulatory sequence affecting
- 10 expression of ICP47 includes a deletion. At least one ICP4 gene (i.e., one or both copies of the ICP4 gene) can include a deletion and the ICP47 gene can include a deletion.

- [00106]** In some embodiments, the disclosure provides HSV vectors or HSV strains with a nonfunctional ICP47 gene. In some embodiments, the disclosure provides HSV vectors or HSV strains with nonfunctional ICP4 and ICP47 genes. In some embodiments, the
- 15 disclosure provides an HSV vector or an HSV strain with one or both copies of ICP4 deleted and ICP47 deleted. In some embodiments, the one or both copies of the gene encoding ICP4 is fully or partially deleted and the gene encoding ICP47 is fully or partially deleted, without disrupting expression of any additional immediate early genes.

C. Applications of HSV-1 Vectors

- 20 **[00107]** The viral vectors (*e.g.*, HSV-1 vectors) described herein can exhibit features that are advantageous for use in treating disease, *e.g.*, in treating cancer.

- [00108]** In certain embodiments viral vectors as described herein exhibit (*e.g.*, when administered to a subject) delayed oncolysis. Delayed oncolysis allows for viral persistence in target (*e.g.*, cancer) cells, sustained payload expression, exposure of tumor-associated
- 25 antigens within a payload-primed tumor microenvironment, and limited non-specific inflammation and tissue damage. Delayed oncolysis allows for viral persistence in target cells, sustained payload expression, exposure of tumor-associated antigens within a payload-primed tumor microenvironment, and limited non-specific inflammation and tissue damage.

- [00109]** In certain embodiments, the viral vector exhibits (*e.g.*, when administered to a
- 30 subject) increased immunogenicity. Immunogenicity can be measured, for example, by detecting increased Tap1 gene expression and/or increased MHC class I expression (HLA). As described in the examples herein, methods for measuring Tap1 gene expression are known in the art and include, for example, RNA seq analysis. Methods for measuring HLA expression are known in the art, and include, for example, flow cytometry.

5 [00110] In certain embodiments, the viral vector exhibits (*e.g.*, when administered to a subject) increased immune activation. Immune activation can be measured, for example, by detecting the increase or decrease in expression of RNAs in pathways relevant to immune cell activation, including, but not limited to, IFN α response pathways, inflammatory response pathways, myc signaling pathways, and/or MtorC1 signaling pathways. Genes involved in
 10 such pathways are described at the GSEA website. See, for example, gsea-msigdb.org/gsea/msigdb/collections.jsp. Immune activation can also be detected by detecting increased expression of TNF super family members, including tumor necrosis factor-alpha (TNF), lymphotoxin beta (LTB), APRIL (TNFSF13), and LIGHT (TNFSF14) and/or detecting altered expression of immunogenic cell death regulators indicative of an increase
 15 pyroptosis and/or apoptosis, such as detecting an increase in expression of PYCARD and Caspase 10 (CASP10).

III. Payload

[00111] Viral vectors in accordance with the present disclosure comprise a nucleic acid molecule comprising the payload of the vector. A payload comprises a nucleic acid molecule that encodes one or more polypeptides. It is contemplated that the payload can comprise a nucleic acid molecule that comprises a sequence complementary to a nucleic acid sequence that encodes a polypeptide. The payload can encode a nucleic acid molecule that has a regulatory function, *e.g.*, a small interfering RNA (siRNA) polynucleotide or a micro RNA
 25 (miRNA) polynucleotide.

[00112] The payload can be a nucleic acid molecule that encodes a protein that is exogenous to the target tissue or subject to which the vector is administered. For example, the payload can be a nucleic acid molecule that encodes a protein that is endogenous to the target tissue or subject to which the vector is administered. In some embodiments, a nucleic acid molecule is codon optimized.
 30

[00113] The nucleic acid comprising the payload of the vector can encode, for example 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 therapeutic polypeptides. In certain embodiments, the payload of the vector encodes 1 to 2, 1 to 3, 1 to 4, 1 to 5, 1 to 6, 1 to 7, 1 to 8, 1 to 9, 1 to 10, 2 to 3, 2 to 4, 2 to 5, 2 to 6, 2 to 7, 2 to 8, 2 to 9, 2 to 10, 3 to 4, 3 to 5, 3 to 6, 3 to 7, 3 to 8, 3 to 9, 3 to
 35 10, 4 to 5, 4 to 6, 4 to 7, 4 to 8, 4 to 9, 4 to 10, 5 to 6, 5 to 7, 5 to 8, 5 to 9, or 5 to 10 therapeutic polypeptides.

5 **[00114]** The therapeutic polypeptides encoded on the vector can have one or more of the following functional attributes:

- (1) target the stroma of a tumor;
- (2) support T cell (e.g., CAR T cell) survival in a tumor microenvironment;
- (3) induce tertiary lymphoid structures (TLS) in a tumor bed; and/or
- 10 (4) promote phagocytic innate immune surveillance.

[00115] One or more therapeutic polypeptides from one or more categories (1-4 above) can be encoded on the same vector to target multiple pathways for increasing immune activity against a tumor. For example, a vector can encode 1, 2, or 3 therapeutic polypeptides from category 1 and 1, 2, or 3 therapeutic polypeptides from category 2, 3, or 4.

15 Alternatively, a vector can encode 1, 2, or 3 therapeutic polypeptides from category 2 and 1, 2, or 3 therapeutic polypeptides from category 3 or 4. Alternatively, a vector can encode 1, 2, or 3 therapeutic polypeptides from category 3 and 1, 2, or 3 therapeutic polypeptides from category 4. Alternatively, a vector can encode 1 or 2 therapeutic polypeptides from category 1 and 1 or 2 therapeutic polypeptides from category 2, 3, and/or 4. Alternatively, a vector

20 can encode 1 or 2 therapeutic polypeptides from category 2 and 1 or 2 therapeutic polypeptides from category 3 and/or 4. Similarly, a vector can encode 1 or 2 therapeutic polypeptides from category 3 and 1 or 2 therapeutic polypeptides from category 4.

[00116] The therapeutic polypeptides in the payload can be cloned in tandem and driven from the same promoter, with a linker sequence separating the individual

25 polypeptides. In certain embodiments, the linker sequence comprises a ribosomal skipping site (sequence) such as a 2A peptide. In an expression construct, when such a ribosomal skipping site is included in a linker positioned between two polypeptide sequences, translation of the transcribed mRNA results in production of the two polypeptides separately, rather than as a fusion protein. The 2A peptide sequences share a core sequence motif of

30 DXEXNPGP, wherein X is any amino acid (SEQ ID NO: 45). Non-limiting examples of suitable 2A peptide sequences include T2A (EGRGSLTTCGDVEENPGP; SEQ ID NO: 46), P2A (ATNFSLLKQAGDVEENPGP; SEQ ID NO: 181), E2A (QCTNYALLKLAGDVESNPGP; SEQ ID NO: 47) and F2A (VKQTLNFDLLKLAGDVESNPGP; SEQ ID NO: 48). Additionally, an optional Gly-Ser-

- 5 Gly (GSG) tripeptide can be added to the N-terminal end of a 2A peptide to enhance efficiency.

A. Targeting the Tumor Stroma

[00117] A viral vector can encode one or more therapeutic polypeptides that target the tumor stroma and/or activate the local endothelium to increase T cell infiltration of the tumor,
10 thereby to reduce the size of the tumor.

[00118] The tumor stroma can be targeted by different mechanisms. For example, the extracellular matrix can be dissolved by proteins involved in extracellular matrix (ECM) degradation, including, but not limited to, hyaluronidase, MMP-9 and an inhibitor of lysyl oxidase. Accordingly, in certain embodiments, a viral vector encodes one or more
15 therapeutic polypeptides involved in extracellular matrix (ECM) degradation, including, but not limited to, hyaluronidase, MMP-9 and an inhibitor of lysyl oxidase.

[00119] Additionally, cancer associated fibroblasts can be targeted by proteins interfering with the TGF β pathway. Accordingly, in certain embodiments, a viral vector encodes one or more therapeutic polypeptides that interfere with the TGF β pathway,
20 including, for example, a polypeptide that includes an external domain (*e.g.*, non-transmembrane domain) of TGF β . In certain embodiments, the therapeutic polypeptide includes a fusion protein comprising an external domain of TGF β , an IgG domain (*e.g.*, an Fc domain), and an external domain of TGF β RII.

[00120] The local endothelium can be activated to favor T cell infiltration by
25 expressing proinflammatory cytokines, including, but not limited to, TNF, IL-1 β , IL-6, and IL-18. Expression of enzymes that degrade the tumor extracellular matrix will simultaneously disrupt physical barriers within the tumor to allow for immune cell infiltration. Accordingly, in certain embodiments, a viral vector encodes one or more proinflammatory cytokines, including, for example, TNF, IL-1 β , IL-6, and IL-18.

30 [00121] Tumor stroma targeting and local endothelium activation can be modulated together or separately by localized expression of these therapeutic polypeptides.

[00122] In certain embodiments, one or more genes encoding hyaluronidase, MMP-9, an inhibitor of lysyl oxidase, a therapeutic polypeptide interfering with the TGF β pathway, TNF, IL-1 β , IL-6, or IL-18 are cloned into a non-replicating McKrae strain HSV-1 vector.
35 The McKrae strain HSV-1 vector can be packaged into a McKrae strain HSV-1 virus, *e.g.*,

5 using a packaging strain. In certain embodiments, cancer cells are transduced with the resulting McKrae strain HSV-1 virus.

[00123] In certain embodiments, the effects on the tumor stroma or local endothelium by cancer cells secreting factors that dissolve the tumor stroma or activate the local endothelium for T cell infiltration can be assessed *in vitro* or *in vivo* using methods known in
10 the art.

B. Supporting T Cell (e.g., CAR T Cell) Survival in the Tumor Microenvironment

[00124] In certain embodiments, a viral vector encodes one or more therapeutic polypeptides that support T cell survival in the tumor microenvironment (TME). In the embodiments described herein, the T cell can be a native T cell (*e.g.*, a T cell existing in the
15 subject to whom a viral vector is administered) or a CAR T cell (*e.g.*, a CAR T cell that has been administered to the subject). In certain embodiments, the viral vector is administered with a T cell (*e.g.*, a CAR T cell), for example, to treat a tumor.

[00125] T cells in the tumor microenvironment can be supported by different mechanisms. For example, the recruitment of T cells, such as native or CAR T cells, to the
20 tumor site can be improved by the presence of T cell recruitment factors such as the chemokine ligands CCL19 or CCL21 in the tumor microenvironment.

[00126] The local T cells can be supported to elicit a strong and durable anti-tumor response. For example, the local expression of T cell trophic factors such as IL-7, IL-12, IL-15, IL-18, and IFN γ can elicit a strong and durable anti-tumor response from the local or
25 recruited T cells. Accordingly, in certain embodiments, a viral vector encodes one or more T cell trophic factors, including, for example, IL-7, IL-12, IL-15, IL-18, and IFN γ .

[00127] Additionally, TGF β signaling in cells within the tumor can be suppressed by soluble TGF β RII expression. Accordingly, in certain embodiments, the therapeutic polypeptide comprises soluble TGF β RII.

30 [00128] Local expression of a gene encoding a co-stimulatory molecule such as CD40L or OX40L, depending on the target TME, can stimulate local or recruited T cells. Accordingly, in certain embodiments, the therapeutic polypeptide comprises CD40L or OX40L.

[00129] T cell support or T cell recruitment can be modulated together or separately by
35 these therapeutic polypeptides.

- 5 **[00130]** In certain embodiments, one or more genes encoding CCL19, CCL21, IL-7, IL-12, IL-15, IL-18, IFN γ , soluble TGF β RII, CD40L, or OX40L are cloned into a non-replicating McKrae strain HSV-1 vector. The McKrae strain HSV-1 vector can be packaged into a McKrae strain HSV-1 virus, *e.g.*, using a packaging strain. In certain embodiments, cancer cells are transduced with the resulting McKrae strain HSV-1 virus.
- 10 **[00131]** Effects on the T cell recruitment or T cell function by cancer cells secreting these factors can be assessed *in vitro* or *in vivo* using methods known in the art.

C. Supporting NK Cell (e.g., CAR NK Cell) Survival in the Tumor Microenvironment

- 15 **[00132]** In certain embodiments, a viral vector encodes one or more therapeutic polypeptides that support NK cell survival in the tumor microenvironment (TME). In the embodiments described herein, the NK cell can be a native NK cell (*e.g.*, a NK cell existing in the subject to whom a viral vector is administered) or a CAR NK cell (*e.g.*, a CAR NK cell that has been administered to the subject). In certain embodiments, the viral vector is administered with an NK cell (*e.g.*, a CAR NK cell), for example, to treat a tumor.
- 20 **[00133]** NK cells in the tumor microenvironment can be supported by different mechanisms. For example, the recruitment of NK cells, such as native or CAR NK cells, to the tumor site can be improved by the presence of NK cell recruitment factors such as the chemokine ligands CCL2, CX3CL1, CXCL16, CCL5, CXCL9, CXCL10, CXCL11 in the tumor microenvironment.
- 25 **[00134]** The local NK cells can be supported to elicit a strong and durable anti-tumor response. For example, the local expression of NK cell trophic factors such as IL-2, IL-15, IL-18, and IFN α can elicit a strong and durable anti-tumor response from the local or recruited NK cells. Accordingly, in certain embodiments, a viral vector encodes one or more NK cell trophic factors, including, for example, IL-2, IL-15, IL-18, and IFN α .
- 30 **[00135]** Additionally, TGF β signaling in cells within the tumor can be suppressed by soluble TGF β RII expression. Accordingly, in certain embodiments, the therapeutic polypeptide comprises soluble TGF β RII.
- [00136]** NK cell support or NK cell recruitment can be modulated together or separately by these therapeutic polypeptides.

5 **[00137]** In certain embodiments, one or more genes encoding CCL2, CX3CL1, CXCL16, CCL5, CXCL9, CXCL10, CXCL11 IL-2, IL-15, IL-18, and IFN α , or soluble TGF β RII, are cloned into a non-replicating McKrae strain HSV-1 vector. The McKrae strain HSV-1 vector can be packaged into a McKrae strain HSV-1 virus, *e.g.*, using a packaging strain. In certain embodiments, cancer cells are transduced with the resulting McKrae strain
10 HSV-1 virus.

[00138] Effects on the NK cell recruitment or NK cell function by cancer cells secreting these factors can be assessed *in vitro* or *in vivo* using methods known in the art.

D. Inducing Tertiary Lymphoid Structures in a Tumor Bed

[00139] In certain embodiments, a viral vector encodes one or more therapeutic
15 polypeptides induce tertiary lymphoid structures (TLS) within the tumor bed.

[00140] TLS formation in tumors can play a role in anti-tumor immunity and is associated with response to immune checkpoint inhibition. As such, a viral vector encoding therapeutic polypeptides that induce TLS formation can be used according to the methods described herein. In certain embodiments, the viral vector is used in combination with
20 additional solid tumor therapeutic agents including, but not limited to, CAR T cell therapy and immune checkpoint inhibitors.

[00141] In certain embodiments, one or more genes encoding a therapeutic polypeptide involved in TLS establishment and maintenance, including, but not limited to, CCL19, lymphotoxin β , CXCL13, and TNF, are cloned into a non-replicating McKrae strain HSV-1
25 vector. The McKrae strain HSV-1 vector can be packaged into a McKrae strain HSV-1 virus, *e.g.*, using a packaging strain. In certain embodiments, cancer cells are transduced with the resulting McKrae strain HSV-1 virus.

[00142] Effects on the tertiary lymphoid structures (TLS) within the tumor bed by cancer cells secreting these factors can be assessed *in vitro* or *in vivo* using methods known in
30 the art.

E. Promoting Phagocytic Innate Immune Surveillance

[00143] In certain embodiments, a viral vector encodes one or more therapeutic polypeptides that promote phagocytic innate immune surveillance and elimination of tumor cells.

5 **[00144]** CD47 is an immunoglobulin that is overexpressed on the surface of many types of cancer cells. CD47 forms a signaling complex with signal-regulatory protein α (SIRP α) on macrophages, enabling the escape of these cancer cells from macrophage-mediated phagocytosis. Promotion of the phagocytic innate immune surveillance and elimination of tumor cells can be induced by the disruption of the Sirp α /CD47 axis, for
10 example, by the presence of a Sirp α -IgG fusion transgene. Disruption of the Sirp α /CD47 axis can promote phagocytic innate immune surveillance and elimination of tumor cells by unmasking the tumor 'eat me' signals. Accordingly, in certain embodiments, a viral vector encodes a therapeutic polypeptide that disrupts the Sirp α /CD47 axis, thereby promoting phagocytic innate immune surveillance and elimination of tumor cells. In certain
15 embodiments, the therapeutic polypeptide is an anti-CD47 antibody. In certain embodiments, the viral vector encodes a Sirp α -IgG fusion polypeptide.

[00145] Additionally, the vector can encode therapeutic polypeptides to support anti-tumor macrophage polarization, including, but not limited to, TNF, IL-1, IL-12, IL-17, and IFN γ .

20 **[00146]** In certain embodiments, one or more therapeutic polypeptides involved in TLS establishment and maintenance in the tissue, including, but not limited to, a Sirp α -IgG fusion transgene, TNF, IL-1, IL-12, IL-17, and IFN γ , can be cloned into a non-replicating McKrae strain HSV-1 vector.

[00147] Effects on phagocytic innate immune surveillance by cancer cells secreting
25 these factors can be assessed *in vitro* or *in vivo* using methods known in the art.

IV. Regulatory Elements

[00148] The inclusion of non-native regulatory sequences, gene control sequences, promoters, non-coding sequences, introns, or coding sequences in a nucleic acid of the present disclosure is contemplated herein. The inclusion of nucleic acid tags or signaling
30 sequences, or nucleic acids encoding protein tags or protein signaling sequences, is further contemplated herein. Typically, the coding region is operably linked with one or more regulatory nucleic acid components.

[00149] A promoter included in a nucleic acid of the present disclosure can be a tissue- or cell type-specific promoter, a promoter specific to multiple tissues or cell types, an organ-specific promoter, a promoter specific to multiple organs, a systemic or ubiquitous promoter,
35

- 5 or a nearly systemic or ubiquitous promoter. Promoters having stochastic expression, inducible expression, conditional expression, or otherwise discontinuous, inconstant, or unpredictable expression are also included within the scope of the present disclosure. A promoter of the present disclosure may include any of the above characteristics or other promoter characteristics known in the art.
- 10 **[00150]** Examples of known promoters include, but are not limited to, the cytomegalovirus (CMV) promoter CMV/human beta 3 globin promoter GFAP promoter, chicken beta actin (CBA) promoter the β -glucuronidase (GUSB) promoter and ubiquitin promoters such as those isolated from human ubiquitin A, human ubiquitin B, and human ubiquitin C.
- 15 **[00151]** In some embodiments, a promoter is a neuron specific promoter in that it is a promoter having specific expression in neurons, preferential expression in neurons, or that typically drives expression of an associated coding sequence in neurons or a subset of neurons but not in one or more other tissues or cell types. Examples of such promoters include calcitonin gene-related peptide (CGRP), synapsin I (SYN), calcium/calmodulin-
- 20 dependent protein kinase II, tubulin alpha I, neuron-specific enolase, microtubule-associated protein IB (MAP1B), and platelet-derived growth factor beta chain promoters, as well as derivatives thereof. In some embodiments, the promoter is a calcitonin gene-related peptide (CGRP) promoter or derivative thereof.
- [00152]** Other regulatory elements may additionally be operatively linked to the
- 25 payload, such as an enhancer and a polyadenylation site. In some embodiments, an enhancer comprises a human cytomegalovirus (HCMV) sequence. In some embodiments, a polyadenylation site comprises a bovine growth hormone (BGH) polyadenylation signal.
- [00153]** In some embodiments, a promoter is a chimeric of one or more promoters or regulatory elements found in nature. In some embodiments, the viral vectors comprise a
- 30 payload whose expression is driven by a CGRP promoter with an HCMV enhancer sequence.

V. Preparation of Vectors

- [00154]** The present disclosure relates particularly to McKrae strain viral vectors that are replication defective. Viral vectors can be generated by mutation (*e.g.*, deletion) of one or more immediate early genes or regulatory sequences that affect the expression thereof. Viral
- 35 genes can be mutated using methods of recombinant technology known in the art. A viral

5 vector of the present disclosure may be rendered replication defective as a result of a homologous recombination event. Replication defective viral vectors can be generated by mutation of an ICP4 gene and mutation of an ICP47 gene. For example, replication defective viral vectors are generated by deletion of an ICP4 gene and deletion of an ICP47 gene.

[00155] In some embodiments, viral vectors of the present disclosure are generated by
10 deletion of loci encoding one or more ICPs (*e.g.*, ICP4 and ICP47) through homologous recombination. In some embodiments, generation of a viral vector of the present disclosure includes a step of homologous recombination of a first plasmid with a second plasmid. In some embodiments, the first plasmid contains nucleic acid sequences homologous to regions of an HSV genome that are adjacent to a nucleic acid region of an HSV genome that is
15 intended to be replaced. In some embodiments, the second plasmid contains an HSV genome, or fragment thereof. In some embodiments, the first plasmid contains nucleic acid sequence encoding a gene of interest between the homologous nucleic acid sequences. In some embodiments, the gene of interest may be or include a marker protein that is detectable by fluorescence, chemiluminescence, or other property, which can be used to select for
20 vectors resulting from successful homologous recombination.

[00156] In some embodiments, a viral vector of the present disclosure is generated by homologous recombination of a first plasmid containing a nucleic acid sequence homologous to regions upstream of the ICP4 promoter including the viral origin contained within the short inverted repeat regions of HSV, with a second plasmid containing an HSV McKrae strain
25 genome.

[00157] In some embodiments, a vector is made by first replacing both copies of the ICP4 loci by homologous recombination using plasmid SASB3 and screening for green fluorescent protein (GFP)-expressing plaques. In some embodiments, a plasmid is constructed by cloning the Sph I to Afl III (Sal I linker) fragment (1928 bp) of the HSV-1
30 KOS strain genome (nucleotides 124485-126413, KT899744, KOS strain) into Sph I/Sal I digested pSP72 followed by insertion of the 695 bp Bgl II to BamH I fragment (GenBank Accession No.: KT899744.1 (SEQ ID NO: 2), KOS strain, nucleotides 131931 to 132626) containing regions upstream of the ICP4 promoter including the viral origin contained within the short inverted repeat regions into the Bgl II to BamH I sites of the vector plasmid. In
35 some embodiments, a plasmid is constructed by cloning a HCMV-eGFP fragment in the BamHI site of a plasmid as described above. In some embodiments, a plasmid as described

5 above is then recombined into a specific locus of a wild-type McKrae virus. In some embodiments, the resulting viral vector is isolated using a stable cell line that expresses one or more genes deleted or disrupted in the HSV genome that are required for replication.

[00158] In some embodiments, a vector is made by first replacing both copies of the ICP4 loci by homologous recombination using plasmid SDAXB and screening for green
 10 fluorescent protein (GFP)-expressing plaques. In some embodiments, a plasmid is constructed by cloning the Sph I to Afl III fragment (1928 bp) of the HSV-1 KOS strain genome (nucleotides 124346 to 126273 of GenBank Accession No.: KT899744.1 (SEQ ID NO: 2), KOS strain) into Sph I/Afl III digested pSP72 to make plasmid SDA followed by changing the Afl III site to a BamHI site (plasmid SDAB). A BamHI to Bgl II DNA PCR
 15 fragment containing regions upstream of the ICP4 promoter including the viral origin (nucleotides 144933 to 145534 of GenBank Accession No.: JQ730035.1 (SEQ ID NO: 1), McKrae strain) contained within the short inverted repeat regions was cloned into the BamHI site of plasmid SDAB to make plasmid SDAXB. In some embodiments, a plasmid is constructed by cloning a HCMV-eGFP fragment in the BamHI site of a plasmid as described
 20 above. In some embodiments, a plasmid as described above is then recombined into a specific locus of a wild-type McKrae virus. In some embodiments, the resulting vector is isolated using a stable cell line that expresses one or more genes deleted or disrupted in the HSV genome that are required for replication.

[00159] In some embodiments, a vector is made comprising a combined ICP4 and
 25 ICP47 deletion. In some embodiments, a plasmid sequence comprising McKrae sequence base pairs 143521 to 144562 and 144640 to 145534 (GenBank Accession No.: JQ730035.1 (SEQ ID NO: 1), McKrae strain) can be synthesized. An SCMV promoter flanked by Pme I sites can be cloned between these flanking sequences. In some embodiments, a marker, for example, a red fluorescent marker, can be placed in the proper orientation with respect to the
 30 SCMV promoter. In some embodiments, a plasmid as described above is then recombined into a specific locus of a McKrae virus with a deletion in the ICP4 locus. In some embodiments, fluorescent clones can be isolated, and the insertion of the construct into the ICP47 locus can be confirmed by PCR. The resultant recombined McKrae virus has deletions of base pairs 144562 to 144640 according to a reference sequence (GenBank
 35 Accession No.: JQ730035.1 (SEQ ID NO: 1), McKrae strain). In some embodiments, the

- 5 resulting viral vector is isolated using a stable cell line that expresses one or more genes deleted or disrupted in the HSV genome that are required for replication.

VI. Characterization of Vectors

- [00160] Viral vectors in accordance with the present disclosure can be characterized by genomic sequencing in order to determine if the expected vector was successfully created.
- 10 Any method of sequencing known in the art is acceptable for this purpose. Methods of sequencing include, for example, nanopore sequencing, single molecule real time sequencing (SMRT), DNA nanoball (DNB) sequencing, pyrosequencing and using DNA arrays.

- [00161] The expression of a payload from a viral vector can be detected by any method known in the art for detecting proteins or nucleic acids. Methods of detecting protein
- 15 expression include immunohistochemistry, flow cytometry, Western blotting, enzyme-linked immunosorbent assay (ELISA), immune-electron microscopy, individual protein immunoprecipitation (IP), protein complex immunoprecipitation (Co-IP), chromatin immunoprecipitation (ChIP), RNA immunoprecipitation (RIP), Immunoelectrophoresis, spectrophotometry, and bicinchoninic acid assay (BCA). Methods of detecting nucleic acid
- 20 expression include Southern blotting, Northern blotting, polymerase chain reaction (PCR), quantitative PCR, and RT-PCR.

VII. Applications/Uses

- [00162] Viral vectors in accordance with the present disclosure are useful for a wide variety of therapeutic applications. Vectors as described herein are useful to deliver one or
- 25 more payloads to one or more target cells. In certain embodiments, the payload persists in target cells for up to 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 2 weeks, 3 weeks, 4 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, a year, or more than a year.
- 30 [00163] In some embodiments, target cells reside in tissues that are poorly vascularized and difficult to reach by systemic circulation. In some embodiments, target cells are cells susceptible to infection by HSV. In some embodiments, target cells are particularly susceptible to infection by a McKrae strain of HSV.

- [00164] Viral vectors in accordance with the present disclosure are useful for
- 35 delivering one or more therapeutic polypeptides to a cell, *e.g.*, a cell in a subject. For

5 example, viral vectors comprising a heterologous nucleic acid segment operably linked to a promoter are useful for any disease or clinical condition associated with reduction or absence of the protein encoded by the heterologous nucleic acid segment, or any disease or clinical condition that can be effectively treated by expression of the encoded protein within the subject. Viral vectors that contain an expression cassette for synthesis of an RNAi agent
 10 (*e.g.*, one or more siRNAs or shRNAs) are useful in treating any disease or clinical condition associated with overexpression of a transcript or its encoded protein in a subject, or any disease or clinical condition that may be treated by causing reduction of a transcript or its encoded protein in a subject. Viral vectors that comprise an expression cassette for synthesis of one or more RNAs that self-hybridize or hybridize with each other to form an RNAi agent
 15 targeted to a transcript encoding a cytokine may be used to regulate immune system responses (*e.g.*, responses responsible for organ transplant rejection, allergy, autoimmune diseases, inflammation, etc.). Viral vectors that provide a template for synthesis of one or more RNAs that self-hybridize or hybridize with each other to form an RNAi agent targeted to a transcript of an infectious agent or targeted to a cellular transcript whose encoded
 20 product is necessary for or contributes to any aspect of the infectious process may be used in the treatment of infectious diseases.

[00165] In one aspect, the disclosure relates to a method of reducing the size of a tumor in a subject in need thereof. The method includes administering to the subject a vector comprising a variant of a herpes simplex virus (HSV) strain whose genome contains an
 25 alteration such that the variant fails to express functional ICP4 and ICP47 proteins. Furthermore, the vector comprises a nucleic acid encoding a therapeutic polypeptide that functions to reduce the size of the tumor. In certain embodiments, the variant fails to express functional ICP4 and ICP47 proteins characterized by the amino acid sequences of SEQ ID NO: 3 and 4 and SEQ ID NO: 5, respectively. In certain embodiments, the HSV strain is an
 30 HSV-1 strain, *e.g.*, a McKrae strain.

[00166] In certain embodiments, the therapeutic polypeptide comprises one or more of the following functional attributes:

- (a) targets the stroma of a tumor;
- (b) supports T cell (*e.g.*, CAR T cell) and/or NK cell survival in a tumor
 35 microenvironment;

- 5 (c) induces tertiary lymphoid structures (TLS) in a tumor bed; and/or
(d) promotes phagocytic innate immune surveillance.

[00167] Exemplary therapeutic polypeptides are discussed above in Section III, and are incorporated herein.

VIII. Administration

- 10 [00168] Compositions comprising viral vectors as described herein may be formulated for delivery by any available route including, but not limited to intratumoral, parenteral (*e.g.*, intravenous), intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, rectal, and vaginal. Preferred routes of delivery include intratumoral. In some
15 embodiments, pharmaceutical compositions include a viral vector in combination with a pharmaceutically acceptable carrier. As used herein the language “pharmaceutically acceptable carrier” includes solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Supplementary active compounds can also be incorporated into the compositions. In some embodiments, viral vectors are formulated in glycerol. In some
20 embodiments, viral vectors are formulated in approximately 10% glycerol in phosphate buffered saline.

- [00169] It is advantageous to formulate compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit
25 containing a predetermined quantity of a viral vector calculated to produce the desired therapeutic effect in association with a pharmaceutical carrier.

- [00170] The pharmaceutical composition can be administered at various intervals and over different periods of time as required, *e.g.*, one time per week for between about 1 to 10 weeks, between 2 to 8 weeks, between about 3 to 7 weeks, about 4, 5, or 6 weeks, etc. The
30 skilled artisan will appreciate that certain factors can influence the dosage and timing required to effectively treat a subject, including, but not limited to, the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Treatment of a subject with a viral vector can include a single treatment or, in many cases, can include a series of treatments.

5 **[00171]** In certain embodiments, the pharmaceutical composition can be administered to a patient more than one time (*e.g.*, two, three, four, five, six, seven, eight, nine, ten, or more times), for example, as a result of the improved safety profile exhibited by the vectors described herein.

10 **[00172]** In some embodiments, the active agents, *i.e.*, a viral vector of the disclosure and/or other agents to be administered together with a viral vector of the disclosure, are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such compositions will be apparent to those skilled in the art. In some embodiments the composition is targeted to particular cell types or to cells that are infected by a virus.

IX. Combination therapy

20 **[00173]** According to the present disclosure, provided compositions may be administered in combination with one or more other active agents and/or therapeutic modalities, such as known therapeutic agents and/or independently active biologically active agents. In some embodiments, provided compositions include one or more such other active agents; in some embodiments, such other active agents are provided as part of distinct compositions. In some embodiments, combination therapy involves simultaneous administration of one or more doses or units of two or more different active agents and/or therapeutic modalities; in some embodiments, combination therapy involves simultaneous exposure to two or more different active agents and/or therapeutic modalities, for example through overlapping dosing regimens.

30 **[00174]** In some embodiments, provided compositions include or are administered in combination with one or more other active agents useful for the treatment of the relevant disease, disorder and/or condition.

35 **[00175]** Throughout the description, where apparatus, devices, and systems are described as having, including, or comprising specific components, or where processes and methods are described as having, including, or comprising specific steps, it is contemplated that, additionally, there are apparatus, devices, and systems of the present invention that

5 consist essentially of, or consist of, the recited components, and that there are processes and methods according to the present invention that consist essentially of, or consist of, the recited processing steps.

[00176] Practice of the invention will be more fully understood from the foregoing examples, which are presented herein for illustrative purposes only, and should not be
10 construed as limiting the invention in any way.

EXAMPLES

[00177] The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion. The present examples, along with the methods described herein are presently representative
15 of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses which are encompassed within the spirit of the invention as defined by the scope of the claims will occur to those skilled in the art.

Example 1: Generation of ICP4 and ICP47 modified McKrae strain HSV-1 Vector

[00178] This example describes the cloning and production of a non-replicating
20 McKrae strain HSV-1 vector comprising alterations in the ICP4 and ICP47 regions.

[00179] Wild type McKrae strain HSV-1 comprises two copies of the gene encoding ICP4 (**FIG. 1A**). First, a plasmid comprising the full length viral genome was modified using homologous recombination by replacing both copies of the two ICP4 loci with modified loci and screening for green fluorescent protein (GFP)-expressing plaques *e.g.*, as described in
25 WO 2017/165813. Briefly, a plasmid was constructed by cloning the Sph I to Afl III fragment (1928 bp) of the HSV-1 KOS strain genome (nucleotides 124346 to 126273 of GenBank Accession No.: KT899744.1 (SEQ ID NO: 2), KOS strain) into Sph I/Afl III digested pSP72 to make plasmid SDA followed by changing the Afl III site to a BamHI site (plasmid SDAB). A BamHI to Bgl II DNA PCR fragment containing regions upstream of the
30 ICP4 promoter including the viral origin (nucleotides 144933 to 145534 of GenBank Accession No.: JQ730035.1 (SEQ ID NO: 1), McKrae strain) contained within the short inverted repeat regions was cloned into the BamHI site of plasmid SDAB to make plasmid SDAXB. Additionally, a HCMV-eGFP fragment was cloned into the BamHI site of plasmid SDAXB. The SDAXB plasmid was then recombined into the ICP4 locus of a wild-type

5 McKrae virus genome to produce mutICP4 McKrae HSV-1. The resulting viral vector structure is shown in **FIG. 1B**.

[00180] Second, the ICP47 locus of mutICP4 McKrae HSV-1 was modified. Briefly, a plasmid sequence comprising McKrae sequence base pairs 143521 to 144562 and 144640 to 145534 (GenBank Accession No.: JQ730035.1 (SEQ ID NO: 1), McKrae strain) was
 10 synthesized. An SCMV promoter flanked by Pme I sites was cloned between these flanking sequences. Additionally, a red fluorescent marker was placed in the proper orientation with respect to the SCMV promoter and the resultant plasmid was used to recombine into mutICP4 McKrae HSV-1, thereby creating mutICP4mutICP47 McKrae HSV-1. Fluorescent clones were isolated, and PCR was performed to confirm the insertion of the construct into
 15 the ICP47 locus. The resultant viruses have deletions of base pairs 144562 to 144640 according to reference sequence (GenBank Accession No.: JQ730035.1 (SEQ ID NO: 1), McKrae strain). The resulting viral vector structure is shown in **FIG. 1C**.

[00181] Following the creation of the mutICP4mutICP47 vector, vector stock was produced by infecting complementing cells and purifying vector from the supernatant.

20 **Example 2: Cloning, Production, and Assessment of Single or Multi Gene Payload**

[00182] This example describes cloning, production, and assessment of a multi gene payload viral vector.

[00183] Briefly, a mutICP4 McKrae HSV-1 or mutICP4mutICP47 McKrae HSV-1 vector, which contains a marker element (*e.g.*, GFP) in place of the gene encoding ICP4
 25 and/or ICP47, was further modified by replacing the marker element with one or more genes of interest (GOI), *e.g.*, a therapeutic polypeptide, to prepare the construct used in the following examples. To replace the marker element with a gene of interest (GOI) in mutICP4 McKrae HSV-1, mutICP27, or mutICP4mutICP47 McKrae HSV-1, the GOI, flanked by a HCMV immediate early protein gene (IEp) and a polyadenylation signal (HCMV-GOI-pA),
 30 was cloned into the plasmid and transduced into a stable ICP4-expressing Vero cell line. After cell transduction, plaques that do not express the marker element were isolated and tested by ELISA for GOI expression. In the examples that follow, the GOI IFN γ , LTB, CXCL13, CCL19, CCL21, and IL-7 were cloned into vectors and expressed. An exemplary multi gene payload viral vector is shown in **FIG. 2**.

35

5 **Example 3: Assessment of Oncolysis and Payload Expression**

[00184] This example describes the assessment of onset of oncolysis and payload expression of mutICP4 McKrae HSV-1 expressing murine IL-17a and mutICP4mutICP47-IFN γ McKrae HSV-1 expressing IFN γ in human breast cancer cells.

mutICP4 McKrae HSV-1

10 [00185] Hs578T human breast cancer cells were infected with increasing amounts of mutICP4 McKrae HSV-1 expressing murine IL-17a (multiplicity of infection (MOI) of 0, 3, and 10), and then cell viability was assessed by CellTiter-Glo assay (CellTiter-Glo® Luminescent Cell Viability Assay (Promega Corp., Madison, WI)) (**FIG. 3A**) and expression of the murine IL-17a payload (**FIG. 3B**) was assessed by ELISA for 8 days.

15 [00186] These results demonstrate that mutICP4 McKrae HSV-1 mediated changes in cell viability were not observed until several days after infection and a robust expression of the payload was sustained for up to 8 days.

mutICP4mutICP47 McKrae HSV-1

[00187] Hs578T human breast cancer cells were infected with increasing amounts of mutICP4mutICP47-IFN γ McKrae HSV-1 expressing IFN γ (multiplicity of infection (MOI) of 0, 3, and 10), and then cell viability was assessed by RealTime-Glo™ assay (Promega Corp., Madison, WI) (**FIG. 3C**) and expression of the IFN γ payload (**FIG. 3D**) was assessed by ELISA for 6 days.

[00188] These results demonstrate that cell growth was suppressed with mutICP4mutICP47-IFN γ infection in a dose-dependent manner (**FIG. 3C**). IFN γ production was highest in the first 2 days after infection and was detected above baseline 6 days post-infection. # $p < 0.0001$ (**FIG. 3D**). The results show that mutICP4mutICP47-IFN γ mediated changes in viability were not observed until several days after infection and a robust expression of the payload was sustained for up to 6 days (**FIG. 3C** and **FIG. 3D**). Delayed oncolysis allows for viral persistence in target cells, sustained payload expression, exposure of tumor-associated antigens within a payload-primed tumor microenvironment, and limited non-specific inflammation and tissue damage. It is believed that delayed oncolysis will result in an improved safety profile and that the vector will be amenable to repeat dosing.

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30

5 **Example 4: Assessment of Immunogenicity and Immune Activation**

Immunogenicity

[00189] This example describes the assessment of the impact on immunogenicity of
mutICP4 McKrae HSV-1 and mutICP4mutICP47 McKrae HSV-1 in human breast cancer
cells. This example also describes the assessment of the impact on immunogenicity of
10 mutICP4mutICP47 McKrae HSV-1 as compared to another replication-defective McKrae
HSV-1 with mutated ICP27 (mutICP27) in human breast cancer cells. ICP27 is an
immediate early gene (like ICP4) that when disrupted renders the virus replication defective.
The mutICP27 vector allows for the comparison of the mutICP4mutICP47 replication
defective vector with another replication defective vector that has a fully intact ICP47 locus
15 within the same strain. Because the ICP4 and ICP47 loci are in close proximity, it is difficult
to disrupt the ICP4 locus without affecting the ICP47 locus, and the ICP4 control used in the
examples may also disrupt ICP47 function. Thus, the mutICP27 vector provides a good
alternative for a control vector comparison.

[00190] Hs578T human breast cancer cells were infected with increasing amounts
20 (multiplicity of infection (MOI) of 0.3, 1, and 3) of mutICP4 McKrae HSV-1-GFP or
mutICP4mutICP47 McKrae HSV-1-GFP. After 24 h, infection (GFP+ cells) and cell-surface
MHC class I expression (HLA) was assessed by flow cytometry.

[00191] The mutICP4 McKrae HSV-1 and mutICP4mutICP47 McKrae HSV-1 strains
showed a comparable infection rate at all three MOIs tested (**FIG. 4A**). The results further
25 show that nearly all cells were HLA positive (**FIG. 4B**) and cell surface expression of HLA
was increased for mutICP4mutICP47 McKrae HSV-1 compared with mutICP4 McKrae
HSV-1 (**FIG. 4C**).

[00192] These results show that mutICP4mutICP47 McKrae HSV-1 induces increased
immunogenicity in breast cancer cells as compared to mutICP4 McKrae HSV-1.

30 [00193] Next, Hs578T cells were infected with 10 PFU/cell of mutICP4mutICP47 or
mutICP27, with each HSV-1 strain encoding GFP as a marker. After 24 hours of infection,
cells were collected for (1) RNAseq analysis of Tap 1 (a protein that mediates unidirectional
translocation of peptide antigens from cytosol to endoplasmic reticulum (ER) for loading
onto MHC class I) and (2) flow cytometry analysis of cell-surface MHC class I (MHC-I)
35 expression (HLA).

5 [00194] As shown in **FIG. 4D**, mutICP4mutICP47 infection led to increased antigen presentation as measured by increased Tap1 gene expression (**FIG. 4D**) and upregulation of cell surface MHC-I compared with mutICP27 infection (**FIG. 4E**). The results show that cell surface expression of MHC-I was not downregulated with mutICP4mutICP47 vector infection as was observed with mutICP27 vector infection, but it was increased ~50% above
 10 the level for uninfected cells. (**FIG. 4E**; ** $p < 0.01$, # $p < 0.0001$). Thus, infection with a mutICP4mutICP47 vector leads to increased immunogenicity in breast cancer cells as compared to infection with a mutICP27 vector.

Immune Activation – Gene Set Enrichment Analysis

[00195] The cellular response to viral backbones can be measured through gene
 15 expression analysis as determined by RNAseq. Gene set enrichment analysis (GSEA) is a bioinformatics method to identify gene-associated pathways that are altered by treatment using this RNAseq data (see, e.g., the GSEA website at [gsea-msigdb.org/gsea/msigdb/collections.jsp](https://www.broadinstitute.org/gsea/msigdb/collections.jsp)). By identifying pathways that are significantly altered in response to treatment, GSEA provides insights into the cellular mechanisms
 20 underlying the response to viral backbones.

[00196] To determine genes that are upregulated or downregulated by mutICP4mutICP47 or mutICP27 infection, Hs578T cells were infected with 10 PFU/cell of mutICP4mutICP47 (with GFP encoded as a marker) or mutICP27 for 24 hours, and RNA was extracted from the cells for RNAseq analysis. GSEA software was used to analyze the
 25 RNAseq data, which are reported as normalized enrichment scores and p-values.

[00197] The results show that the top upregulated pathways by mutICP4mutICP47 compared with mutICP27 were immune activation pathways, including HALLMARK_INTERFERON_ALPHA_RESPONSE and HALLMARK_INFLAMMATORY_RESPONSE (**FIG. 4F**). The top upregulated pathways
 30 by mutICP27 infection were those involved in cellular responses, including HALLMARK_MYC_TARGETS AND MTORC1_SIGNALING (**FIG. 4G**). Hallmark gene sets are curated and concisely summarize distinct, well-defined biological states or processes, exhibiting consistent expression patterns (see e.g., Liberzon *et al.* (2015) CELL SYST.; 1(6): 417–425). For example, the HALLMARK_INTERFERON_ALPHA_RESPONSE pathway
 35 comprises genes, such as Tap1, that are up-regulated in response to alpha interferon proteins. The mutICP4mutICP47 vector induced greater immune activation than the mutICP27 vector.

5 Immune Activation – TNFSF Expression

[00198] The TNF super family members (TNFSF) are important factors in inducing both the innate and adaptive immune response. These molecules act through direct induction of immunogenic forms of cell death, including apoptosis and pyroptosis, and through direct stimulation of immune cells.

10 [00199] To determine whether TNFSF was involved in mutICP4mutICP47 or mutICP27 cellular responses, Hs578T cells were infected with 10 PFU/cell of mutICP4mutICP47 or mutICP27, with each HSV-1 strain encoding GFP as a marker, for 24 hours, and RNA is extracted from the cells for RNAseq analysis.

15 [00200] The results show that mutICP4mutICP47 induced higher expression of TNF super family members as compared to mutICP27, including tumor necrosis factor-alpha (TNF), lymphotoxin beta (LTB), APRIL (TNFSF13), and LIGHT (TNFSF14) (**FIG. 4H**). Each of these genes can act both as an inducer of immunogenic cell death and a stimulator of the immune response. Furthermore, mutICP4mutICP47 upregulated the expression of PYCARD and Caspase 10 (CASP10), regulators of pyroptosis and apoptosis, respectively, to
20 a greater extent than mutICP27 infection. The mutICP4mutICP47 infection preferentially activated immune responses and immunogenic cell death pathways as compared to mutICP27 viral infection

Example 5: Multi Gene Payload for Targeting the Tumor Stroma

25 [00201] This example describes the design and assessment of viral vectors with a multi gene payload for targeting the tumor stroma and activating the local endothelium to favor T cell infiltration of the tumor to reduce the size of the tumor.

30 [00202] The tumor stroma can be targeted by different mechanisms. For example, the extracellular matrix can be dissolved by proteins involved in extracellular matrix (ECM) degradation, including, but not limited to, hyaluronidase, MMP-9 and an inhibitor of lysyl oxidase. Additionally, cancer associated fibroblasts can be targeted by proteins interfering with the TGFβ pathway.

[00203] The local endothelium can be activated to favor T cell infiltration by proinflammatory cytokines, including, but not limited to, TNF, IL-1β, IL-6, and IL-18. It is contemplated that inclusion of enzymes that degrade the tumor extracellular matrix will

- 5 simultaneously disrupt physical barriers within the tumor to allow for immune cell infiltration.

[00204] Tumor stroma targeting and local endothelium activation can be modulated together or separately by localized expression of these factors.

- [00205] Accordingly, one or more of hyaluronidase, MMP-9, an inhibitor of lysyl
 10 oxidase, gene interfering with the TGF β pathway, TNF, IL-1 β , IL-6, or IL-18 can be cloned into a non-replicating McKrae strain HSV-1 vector as described in **Example 2**, and expressed and purified as described in **Example 1**. Cancer cells can be transduced with one or more
 GOI containing McKrae strain HSV-1 virus at different MOIs as described in **Example 3**,
 and assessed for oncolysis, payload expression and/or secretion, and immunogenicity as
 15 described in **Example 3** and **Example 4**.

- [00206] Effects on the tumor stroma or local endothelium by cancer cells secreting factors that dissolve the tumor stroma or activate the local endothelium for T cell infiltration can be assessed *in vitro* or *in vivo*. It is contemplated that expression of genes targeting the tumor stroma and/or the local endothelium will increase T cell infiltration and reduce the size
 20 of the tumor.

Example 6: Multi Gene Payload in mutICP4mutICP47 for Targeting the Tumor Stroma

- [00207] This example describes the design and assessment of a mutICP4mutICP47 HSV-1 vector with a multi gene payload for targeting the tumor stroma and activating the
 25 local endothelium to favor T cell infiltration of the tumor to reduce the size of the tumor using a salivary gland model (see, *e.g.*, (Barone *et al.* PNAS (2015) 112 (35) 11024-11029).

- [00208] Briefly, both submandibular salivary glands of wild-type BALB/c mice (8-10 weeks old) were cannulated under anesthesia via the excretory duct with either 3x10⁷ PFU of mutICP4mutICP47 HSV-1 vector expressing GFP or a combination of mutICP4mutICP47
 30 HSV-1 vectors which expressed one of each murine LTB, CXCL13, CCL19, CCL21, or IL-7 (6x10⁶ PFU of each vector, total dose 3x10⁷ PFU, "Payloads", prepared as described in **Example 2**), and then salivary glands were harvested 15 days later for flow cytometry analysis using antibodies against CD31, PDGFR α and PDPN (markers of stromal cell activation). The number of positive cells was quantified.

5 [00209] The results show that payload-encoding mutICP4mutICP47 viruses increased the number of CD31+ endothelial cells and PDGFRa+PDPN+ stromal cells in salivary glands (FIG. 5A-5B). These data demonstrate that stromal and endothelial activation can be achieved using a combination of mutICP4mutICP47 viruses each carrying a payload of one of the murine LTB, CXCL13, CCL19, CCL21, and IL-7 genes.

10 **Example 7: Assessment of expression TGFβ pathway components after mutICP4 mutICP47 infection**

[00210] This example describes the assessment TGFβ pathway component expression after infection with a mutICP4mutICP47-GFP HSV-1 vector.

[00211] The TGFβ pathway is a multifunctional cytokine that regulates stromal, 15 endothelial, and immune cells, and therapeutic targeting of this pathway has been described in cancer.

[00212] To determine expression of the TGFβ pathway components with mutICP4mutICP47 virus after infection of cancer cells, Hs578T cells were infected with 10 PFU/cell of mutICP4mutICP47-GFP (with GFP encoded as a marker, no payload) 20 (uninfected cells as control) for 24 hours, and RNA was extracted from the cells for RNAseq analysis. TGFβ1 mRNA expression was quantified.

[00213] The results show that TGFβ1 expression was decreased ~50% 24 h post infection after infection with mutICP4mutICP47 vector (FIG. 5C). Thus, infection with the mutICP4mutICP47 viral backbone itself decreases expression of TGFβ1.

25 **Example 8: Multi Gene Payload for T cell Survival in the Tumor Microenvironment**

[00214] This example describes the design and assessment of viral vectors with a multi gene payload for supporting T cell survival in the tumor microenvironment (TME).

[00215] T cells in the tumor microenvironment can be supported by different mechanisms. For example, the recruitment of T cells to the tumor site can be improved by 30 the presence of T cell recruitment factors such as the chemokine ligands CCL19 or CCL21 in the tumor microenvironment.

[00216] The local T cells can be supported to elicit a strong and durable anti-tumor response. For example, the local expression of T cell trophic factors such as IL-7, IL-12, IL-15, IL-18, and IFNγ can elicit a strong and durable anti-tumor response from the local or 35 recruited T cells. Additionally, TGFβ signaling in cells within the tumor can be suppressed

- 5 by soluble TGFβRII expression. Further, local expression of a transgene encoding a co-stimulatory molecule such as CD40L or OX40L depending on the target TME and a combination with other vectors or therapeutics can stimulate local or recruited T cells.

[00217] T cell support or T cell recruitment can be modulated together or separately by these factors.

- 10 [00218] One or more of CCL19, CCL21, IL-7, IL-12, IL-15, IL-18, IFNγ, soluble TGFβRII, CD40L, or OX40L can be cloned into a non-replicating McKrae strain HSV-1 vector as described in **Example 2**, and expressed and purified as described in **Example 1**. Cancer cells can be transduced with one or more GOI containing McKrae strain HSV-1 virus at different MOIs as described in **Example 3**, and assessed for oncolysis, payload expression
15 and/or secretion, and immunogenicity as described in **Example 3** and **Example 4**.

[00219] Effects on the T cell recruitment or T cell function by cancer cells secreting these factors can be assessed *in vitro* or *in vivo*. It is contemplated that expression of genes for T cell recruitment or T cell function will result in the recruitment of T cells to the tumor site and support the function of local T cells, thereby reducing the size of the tumor.

- 20 **Example 9: Multi Gene Payload in mutICP4mutICP47 for T cell and NK cell Survival in the Tumor Microenvironment**

[00220] This example describes the design and assessment of a mutICP4mutICP47 HSV-1 vector with a multi gene payload for supporting T cell and NK cell survival in the tumor microenvironment (TME).

- 25 [00221] Immune-mediated tumor cell killing can be assessed in *ex vivo* coculture models which include peripheral blood mononuclear cells (PBMC) and target tumor cells. The cytotoxic cells in these models include CD8+ T and NK cells present in PBMCs.

- [00222] To determine cytotoxic activity after treatment with mutICP4mutICP47 HSV-1 vector with or without a multi gene payload in an *in vitro* breast cancer model, CellTracker
30 Red labelled Hs578T cells (human breast cancer cell line) were infected with mutICP4mutICP47 vectors encoding GFP, human IFNγ, human IL-12, or combinations thereof at a total viral dose of 0.5 PFU/cell. After 2 h, CellTracker Violet labelled human PBMCs were added to the culture at a ratio of approximately 10 PBMCs per 1 Hs578T cell, and co-incubated for 72 hours. Recombinant human IL-15 (rh IL-15) was added to
35 mutICP4mutICP47-GFP infected cells to mimic viral-mediated delivery of human IL-15. A

5 PBMC activator cocktail (ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator + rh IL-2) served as a positive control for maximal tumor cell killing under these conditions. After 72 hours, samples were collected, and live Hs578T cells were quantified by flow cytometry.

[00223] The results show that viral-mediated expression of IFN γ or IL-12 in Hs578T:PBMC cocultures decreased the number of live Hs578T cells to a greater extent than
 10 viral backbone treatment alone (mutICP4mutICP47-GFP) (**FIG. 6A**). Similarly, the addition of rh IL-15 to mutICP4mutICP47-GFP infected cells also increased Hs578T tumor cell killing. The combination of IFN γ - and IL-12-expressing mutICP4mutICP47 vectors further enhanced the immune-mediated tumor cell killing in this assay. These data support the use of human IFN γ , IL-12, IL-15, and combinations thereof encoded in a vector designed to support
 15 T or NK cell function and immune-mediated tumor cell killing in the tumor microenvironment.

Example 10: IFN γ Payload in mutICP4mutICP47 for T cell and NK cell Survival in the Tumor Microenvironment

[00224] This example describes the assessment of a mutICP4mutICP47 HSV-1 vector
 20 with a IFN γ payload for supporting T cell and NK cell survival in the tumor microenvironment (TME).

[00225] To determine T cell and NK cell activity after treatment with mutICP4mutICP47 HSV-1 vector with or without a IFN γ payload, Hs578T cells were infected with 1 or 3 PFU/cell of mutICP4mutICP47 encoding GFP or mutICP4mutICP47
 25 encoding human IFN γ for 2 hours in OPTI-MEM + 5% FBS, then cocultured with PBMC for 24 or 72 hours. Cells were collected after 24 or 72 hours, stained with fluorescent conjugated antibodies, and analyzed by flow cytometry.

[00226] The results show that mutICP4mutICP47 infection increased the number of Ki67⁺Granzyme B⁺ CD8⁺ T cells after 24 h, an effect that was further increased after 72
 30 hours of coculture (**FIGs. 6B and 6C**). MutICP4mutICP47-IFN γ had a stronger effect in T cell activation than mutICP4mutICP47-GFP.

[00227] The results show that mutICP4mutICP47-IFN γ induced a greater number of Granzyme B⁺ Ki67⁻ NK cells and antigen-presenting cells (CD11c⁺CD16⁺CD14⁻ Ki67⁺MHCII⁺⁺) after 24 h than either uninfected control or mutICP4mutICP47-GFP (**FIGs. 6D and 6E**).

- 5 [00228] Infection with mutICP4mutICP47-GFP or mutICP4mutICP47-IFN γ decreased live tumor cell number and PBMC-mediated target cell killing (**FIG. 6F**). The effects of mutICP4mutICP47 viruses on T cell activation were dose-dependent. These data demonstrate that the infection of cancer cells with a mutICP4mutICP47 encoding human IFN γ vector regulates T and NK cell function, increases antigen presentation capability, and
10 activates immune-mediated tumor cell killing.

Example 11: Assessment of Chemokine Expression in Cancer Cells after Infection with mutICP4mutICP47-IFN γ

- [00229] This example describes the assessment the effect of a mutICP4mutICP47 HSV-1 vector with a IFN γ payload on chemokine expression in cancer cells.
- 15 [00230] Chemokines in the tumor microenvironment are important factors which regulate the recruitment of T and NK cells into tumors.
- [00231] To determine expression of chemokines after infection of cancer cells with mutICP4mutICP47-GFP or mutICP4mutICP47-IFN γ , Hs578T cells were infected with 10 PFU/cell of mutICP4mutICP47 (with GFP encoded as a marker) or mutICP4mutICP47
20 vector encoding human IFN γ for 24 h and RNA was extracted from the cells for RNaseq analysis. Number of gene counts are reported for each gene of interest (****p<0.0001).
- [00232] The results show that infection with mutICP4mutICP47 vector upregulated the expression of multiple T and NK cell chemokines including CCL2, CXCL9, CXCL10, CXCL11, and CX3CL1, and this effect could be further enhanced by encoding human IFN γ
25 in the vector (**FIG. 6G**). The data show that the response to the mutICP4mutICP47 viral backbone itself supports T and NK cells recruitment to tumors.

Example 12: Multi Gene Payload for CAR T Cell Survival in the TME

- [00233] This example describes the design and assessment of viral vectors with a multi gene payload for supporting CAR T cell survival in the TME.
- 30 [00234] T cells in the tumor microenvironment can be supported by different mechanisms. For example, the recruitment of CAR T cells the tumor site can be improved by the presence of T cell recruitment factors such as the chemokine ligands CCL19 or CCL21 in the tumor microenvironment.
- [00235] The local CAR T cells can be supported to elicit a strong and durable anti-
35 tumor response. For example, the local expression of CAR T cell trophic factors such as IL-

5 7, IL-15, and IL-18 can elicit a strong and durable anti-tumor response from the local or recruited CAR T cells. Additionally, TGF β signaling in cells within the tumor can be suppressed by soluble TGF β RII expression. Further, local expression of a transgene encoding a co-stimulatory molecule such as CD40L or OX40L depending on the target TME and a combination with other vectors or therapeutics can stimulate local or recruited CAR T
10 cells.

[00236] The ectopic or overexpression of a cognate CAR antigen in the tumor cells such as mesothelin can re-direct CAR T cells to target tumor cells which express the antigen.

[00237] CAR T cell support or CAR T cell recruitment can be modulated together or separately by these factors.

15 [00238] One or more of CCL19, CCL21, IL-7, IL-15, IL-18, soluble TGF β RII, CD40L, or OX40L can be cloned into a non-replicating McKrae strain HSV-1 vector as described in **Example 2**, and expressed and purified as described in **Example 1**. Cancer cells can be transduced with one or more GOI containing McKrae strain HSV-1 virus at different MOIs as described in **Example 3**, and assessed for oncolysis, payload expression and/or
20 secretion, and immunogenicity as described in **Example 3** and **Example 4**.

[00239] Effects on the CAR T cell recruitment or CAR T cell function by cancer cells secreting these factors can be assessed *in vitro* or *in vivo*. It is contemplated that expression of genes for CAR T cell recruitment or CAR T cell function will recruit CAR T cells to the tumor site and support the function of local CAR T cells and reduce the size of the tumor.

25 Additionally, it is expected that expression of ectopic tumor antigens in the tumor will recruit CAR T cells with a cognate CAR to the tumor and reduce the size of the tumor.

Example 13: Multi Gene Payload in mutICP4mutICP47 HSV-1 vectors for Inducing Tertiary Lymphoid Structures in a Tumor Bed

[00240] This example describes the design and assessment of mutICP4mutICP47
30 HSV-1 vectors with a multi gene payload for inducing tertiary lymphoid structures (TLS) within the tumor bed.

[00241] Tertiary lymphoid structures (TLS) act as a functional niche for the maturation of the T and B cell response against locally displayed tumor antigens and, whilst originally described in the context of chronic inflammation, have been more recently recognized as
35 elements able to support the organization of a robust immune response against solid tumors.

- 5 TLS formation appears to associate with improved efficacy of immunotherapy in mice and humans, providing ICI-activated TILs with recruitment and survival signals within the tumor itself.

- [00242] To determine the induction of TLS in the tumor bed by a mutICP4mutICP47 HSV-1 vector, a model of intraglandular delivery of vectors in the salivary glands was used.
- 10 This model is an environment that has been demonstrated to be highly permissive to TLS formation. Briefly, both submandibular glands of wild-type BALB/c mice (8-10 weeks old) were cannulated under anesthesia via the excretory duct with either 3×10^7 PFU of mutICP4mutICP47 HSV-1 vector expressing GFP or a combination of mutICP4mutICP47 HSV-1 vectors which express murine TNF, CCL19, IL-17a, and IL-7 (6×10^6 PFU of each
- 15 vector including a GFP encoding vector, total dose 3×10^7 PFU), and then salivary glands were harvested 15 days later for flow cytometry and immunofluorescent staining (**FIG. 7A**). Dendritic cells (CD11c), CD4⁺ T cells (CD4), B cells (B220), and nuclei (DAPI) were visualized (**FIGs. 7B and 7C**). Two areas of interest (A1, A2) are shown at both low magnification (10X) and high magnification (40X) (**FIG. 7C**).
- 20 [00243] The results show that the mutICP4mutICP47 viral backbone alone enhanced immune infiltration into the salivary gland (measured as CD45⁺ cells by flow cytometry) (**FIG. 7A**) and formed small immune cell aggregates characterized by dendritic cells and CD4⁺ T cells (**FIG. 7B and 7C**). Administration of a combination of mutICP4mutICP47 vectors expressing TNF, CCL19, IL-17a, and IL-7, which have reported roles in TLS
- 25 formation, increased the number, size, and organization of the TLS structures observed as demonstrated by spatially defined B and T cell zones infiltrated with dendritic cells in immunofluorescent images. The data shows that the response to the mutICP4mutICP47 viral backbone itself supports underlying lymphoid neogenesis processes, and this can be enhanced by viral-mediated expression of a combination of factors known to regulate TLS formation,
- 30 such as TNF, CCL19, IL-17a, and IL-7. It is believed that lymphotoxin β and CXCL13, which are thought to play a role in TLS establishment and maintenance, can also be used to support TLS formation.

5 **Example 14: Multi Gene Payload for Promoting Phagocytic Innate Immune Surveillance**

[00244] This example describes the design and assessment of viral vectors with a multi gene payload for promotion of the phagocytic innate immune surveillance and elimination of tumor cells.

10 [00245] The binding of SIRP α of tumor cells to CD47 of macrophages results in the inhibition of phagocytosis. This “don’t eat me” signal allows tumor cells to evade immune destruction by macrophages. To block this pathway, a mutICP4mutICP47 vector encoding a SIRP α -IgG fusion protein was constructed. To determine the activity of this vector in an *ex vivo* phagocytosis model, macrophages were co-cultured with mutICP4mutICP47 SIRP α -IgG
15 medium. Briefly, a STEMCELL EasySep™ Human Monocyte Enrichment Kit was used to enrich CD14⁺ cells from human PBMCs. The enriched cells were plated in a 24 well plate and cultured for 8 days supplied with STEMCELL ImmunoCult™-SF Macrophage Differentiation Medium and 50ng/ml m-CSF. On day 4, fresh medium was added, and on day 6, 50ng/ml IFN γ was added to stimulate the cells to polarize to M1 macrophages. On
20 day 8, CFSE labeled Raji cells were pretreated (30 min) with IgG control or anti-CD47 antibody (positive control) or with conditioned medium from Hs578T cells infected with 10 PFU/cell mutICP4mutICP47-GFP or 3 or 10 PFU/cell mutICP4mutICP47-SIRP α -IgG. Macrophages were then added to the CFSE-labelled Raji cells and cocultured for 3 hours. Samples were collected, and flow cytometry was used to measure phagocytosis. %
25 phagocytosis is defined as the percentage of macrophages (CD14⁺) that are positive for CFSE.

[00246] The results show that the conditioned medium from mutICP4mutICP47-SIRP α -IgG infected Hs578T cells induced a higher percentage of phagocytosis than the IgG control, the anti-CD47 antibody, or mutICP4mutICP47-GFP, and this effect was dose-
30 dependent (**FIG. 8**). These data demonstrate that the infection of cells with mutICP4mutICP47 viral vector encoding a SIRP α -IgG fusion protein increases macrophage phagocytosis.

Example 15: Multi Gene Payload for NK cell or CAR NK cell Survival in the TME

[00247] This example describes the design and assessment of viral vectors with a multi
35 gene payload for promotion of the NK cell and CAR NK cell survival in the TME.

- 5 **[00248]** NK cells and CAR NK cell in the tumor microenvironment can be supported by different mechanisms. For example, the recruitment of NK cells and CAR NK cell to the tumor site can be improved by the presence of NK cell and CAR NK cell recruitment factors such as the chemokine ligands CCL2, CX3CL1, CXCL16, CCL5, CXCL9, CXCL10, CXCL11, or CAR antigen in the tumor microenvironment in the tumor microenvironment.
- 10 **[00249]** The local NK cells and CAR NK cell can be supported to elicit a strong and durable anti-tumor response. For example, the local expression of NK cell and CAR NK cell trophic factors such as IL-2, IL-15, IL-18, and IFN α can elicit a strong and durable anti-tumor response from the local or recruited NK cells and CAR NK cell. Additionally, TGF β signaling in cells within the tumor can be suppressed by soluble TGF β RII expression.
- 15 **[00250]** NK cell and CAR NK cell support or NK cell and CAR NK cell recruitment can be modulated together or separately by these factors.
- [00251]** One or more of CCL2, CX3CL1, CXCL16, CCL5, CXCL9, CXCL10, CXCL11, as IL-2, IL-15, IL-18, IFN α , or soluble TGF β RII can be cloned into a non-replicating McKrae strain HSV-1 vector as described in **Example 2**, and expressed and
- 20 purified as described in **Example 1**. Cancer cells can be transduced with one or more GOI containing McKrae strain HSV-1 virus at different MOIs as described in **Example 3**, and assessed for oncolysis, payload expression and/or secretion, and immunogenicity as described in **Example 3** and **Example 4**.
- [00252]** Effects on the NK cell and CAR NK cell recruitment or NK cell and CAR NK
- 25 cell function by cancer cells secreting these factors can be assessed *in vitro* or *in vivo*. It is contemplated that expression of genes for NK cell and CAR NK cell recruitment or NK cell and CAR NK cell function will result in the recruitment of NK cells and CAR NK cell to the tumor site and support the function of local NK cells and CAR NK cell, thereby reducing the size of the tumor.

30 **INCORPORATION BY REFERENCE**

[00253] The entire disclosure of each of the patent and scientific documents referred to herein is incorporated by reference for all purposes.

5

EQUIVALENTS

[00254] The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the
10 foregoing description, and all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.

SEQUENCE LISTING

SEQ ID NO: 1 – GenBank Accession No.: JQ730035.1

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 149221 tgtcgcgcgc gcccgccgag gggcccgacc cectgggcgg ctggcggcgg cagccccggg
 149281 gggccagcca caggcgggcg cccgcggccg ccgccttga ggctactgc tccccgcgcg
 149341 ccgtggccga gctcacggac caccgcgtgt tccccgtccc ctggcgaccg gccctcatgt
 149401 ttgaccgcgc ggccctggcc tcgatcgccg cgcggtgcgc cgggcccgcc cccgcgcgcc
 149461 aggcgcgctg cggcgggcgc gacgacgaga acccccaccc ccacggggcc gccggggggc
 149521 gcctcttttg ccccttgcgc gcctcgggcc cgtgcgcgcg catggcgccc tggatgcgc
 149581 agatccccga ccccgaggac gtgcgcgtgg tgggtgctga ctgcgcgtg cggggcgagg
 149641 acctggccgg cggcggggcc tcgggggggc cgcggagtg gtccgccgag cgcggcgggc
 149701 tgtcctgcct gctggcggcc ctggccaacc ggctgtgcgg gccggacacg gccgcctggg
 149761 cgggcaactg gaccggcgcc ccgacgtgt cggcgctggg cgcgcagggc gtgctgtcgc
 149821 tgtccacgcg ggacctggcc ttgcgcgggg cgttgagtt tctggggctg ctgccagcg
 149881 ccggcgaccg cgggctcatc gtggtcaaca ccgtgcgcgc ctgcgactgg ccgcgcgacg
 149941 gggccgcggt gtgcgcgcag cagcctacc tggcgtgcga cctgctgcc gccgtgcagt
 150001 gcgcgctgcg ctggccggcg gcgcgggacc tgcgcgcac ggtgctggcc tcgggcgcgc
 150061 tgttcggccc ggggggtctt gcgcgcgtgg aggcgcgcga cgcgcgcctg taccgcgacg
 150121 cgcgcgcgct gcgcctgtgc cgcggcgcca acgtgcgcta ccgcgtgcgc acgccttcg
 150181 gcccgacac gccggtgccc atgtcccgc gcgagtaccg ccgggcctg ctgccgcgc
 150241 tggacggccg ggcggcggcc tcggggacca ccgacgccat ggcccccggc gcgcgcgact
 150301 tctcgagga ggaggccac tcgcaccgcg cctgcgcgcg ctggggcctg ggcgcgccgc
 150361 tgcggcccggt gtacgtggcg ctggggcgcg aggcggtgcg cgcggcccg gcccggtggc
 150421 gcgggcgcgc gagggacttt tgcgccgcg cctgctgga gccgcacgac gacgcccccc
 150481 cgctggtgct gcgcggcgac gacgacgacg gccggggggc cctgcgcgcg gcgcgcgcgc
 150541 ggattcgctg ggccctcgcc acgggcgcga gcggcacctg gctggcgcg gcggggggccg
 150601 tggagggtgct gggggcgag gcgggcttg ccacgcccc gcgacgggac gttgtggact
 150661 gggaaggcgc ctgggacgaa gacgacggcg gcgcgttcga gggggacggg gtgctgtaac
 150721 gggccgggac ggggcggggc gcttgtgaaa cccgaagacg caataaacg caacgacctg
 150781 attaaagttt gcagtagcgt tgtttattcg aggggcggga gggggcgagg ggcgggaggg
 150841 ggcgaggggc gggagggggc gaggggcggg agggggcgag gggcgggagg gggcgagggg
 150901 cgggaggggc gaggggcggg agggggcgag gggcgggagg ggcgaggggc gggagggggc
 150961 gaggggcggg aggggcgagg ggcgggaggg ggcgaggggc gggagggggc gaggggcggg
 151021 aggggcgagg ggcgggaggg ggcgaggggc gggaggggcg aggggcgggc gggagggggc
 151081 gaggggcggg agggggcgag ggcgggaggg gggcgagggg cgggaggggg cgaggggcg
 151141 tgggtggtgc cgggcgcccc cggagggttt ggatctctga cctgagattg gcggcactga
 151201 ggtagagatg cccgaacccc cccgagggag cgcgggacgc ggctggggag ggctggggct
 151261 ggggagggct ggggctgggg agggctgggg ctggggaggg ctggggctgg ggaggctgg
 151321 ggctggggag ggctggggct ggggagggct ggggctgggg agggctgggg ctggggaggg
 151381 gcggtggtgt gtagcaggag cgtgtgtttg cgcggggta cgtctggag agcgggaggt
 151441 gcgcggtgac gtgtggatga ggaacaggag ttgttgcgcg gtgagttgtc gctgtgagtt
 151501 gtgtgtttg gcaggtgtgg tggatgacgt gacgtgtgac gtgcggattg cgcctgtctt
 151561 tgttggtgtt ttacctgtgg cagcccgggc ccccgcgggg cgcgcgcgcg cgcaaaaaag
 151621 gcgggcggcg gtccggcgcg cgtgcgcgcg cgcggcgggc gtggggggcg gggccgcggg
 151681 agcgggggag gagcggggga ggagcggggg gaggagcggg gggagggagc ggggagggag
 151741 cggggggagg agcgggggga ggagcggggg gaggagcggg gggagggagc ggggagggag
 151801 cggggggagg agcgggggga ggagcggggg gaggagcggg gggagggagc ggggagggag
 151861 cggggggagg agcgggggga ggagcggggg aggagcggcc agaccccgga aacgggcccc
 151921 ccccaaaaaca caccctccgg gggtcgcgcg cggcccttta aaggcgggcg gcgg

SEQ ID NO: 3 – ICP4 protein (McKrae strain, AFK50409.1)

MASENKQRPGSPGPTDGPPTPSPDRDERGALGWAETEEGDDPDHDPDHPDLDDARRDGRAPAAGTDAGEDAGD
 AVSPRQLALLASMVEEAVRTIPTDPAASPRTPAFRADDDGDEYDDAADAAGDRAPARGRAREAPLRGAYDPDPTD
 RLSRPPPAQPPQRRRHGRRRPSASSTSSDSGSSSSSSASSSSSSDEDEDDDGNDAAHDHAREARAVGRGPSSAAPEA
 PGRTPPPPGPPPLSEAAPKPRAAARTPAASAGRIERRRARAAGRDATGRFTAGQPRVELDADAASGAIFYARYRD

GYVSGEPWPAGPPPPGRVLYGGLGDSRPGLWGAPEAEAEARRRFEASGAPAAVWAPELGDAAQQYALITRLLYTPDA
 EAMGWLQNPRVPGDVALDQACFRI SGAARNSSSFITGSVARAVPHLGYAMAAGRFGWGLAHAAAAMVMSRRYDRAQ
 KGFLLTSLRRAYAPLLARENAALTGAAGSPGAGADDEGVAAAVVAAAAAPGERAVPAGYGAAGILAAALGRLSAAPAS
 PAGGDDPDAARHADADDDAGRRAQAGRVAVECLAACRGILEALAEFGDGLAAVPGLAGARPASPPRPEGPAGPAS
 PPPHADAPRLRAWLRELRFVRDALVLMRLRGDLRVAGGSEAAVAAVRVSLVAGALGPALPRDPRLPSSAAAAAADL
 LFENQSLRPLLAAGPRRSSSSSGVAAAAAASAPREGRRKSPGPARP PGGGGPRPPKTKKSGADAPGSDARAPLPAPA
 PPSTPPGPEPAPAQPAAPRAAAQARPRVALSRRPAEGPDPLGGWRRQPPGPSHTAAPAAAALEAYCSPRAVAELT
 DHPLFPVPWRPALMFDPALASIAARCAGPAPAAQAACGGGDDDENPHPHGAAGGRLFGPLRASGPLRRMAAWMRQI
 PDPEDVRVVLYSLPLGEDLAGGGASGGPPEWSAERGGLSCLLAALANRLCGPDTAAGNWTGAPDVSAAGQGV
 LLSTRDLAFAGAVEFLGLLASAGDRLIVVNTVRACDWPADGPAVSRQHAYLACDLLPAVQCAVRWPAARDLRRTVL
 ASGRVFGPGVFARVEAAHARLYPDAPPLRLCRGGNVRYRVTRFPGDTPVPMSPREYRRAVLPAALDGRAAASGTTDA
 MAPGAPDFCEEEAHSHRACARWGLGAPLRFPVYVALGREAVRAGPARWRGPRRDFCARALLEPDDAPPLVLRGDDDG
 PGALPPAPPPIRWASATGRSGTVLAAAGAVEVLGAEGALATPPRRDVVDWEGAWDEDDGGAFEGDGV

SEQ ID NO: 4 – ICP4 protein (McKrae strain, AFK50423.1)

MASENKQRPSPGPTDGPPTPSPDRDERGALGWGAETEEGGDDPDHDPDHPHDLDDARRDGRAPAAAGTDAGEDAGD
 AVSPRQLALLASMVVEAVRTIPTPDPAASPPRTPAFRADDDDGDEYDDAADAAGDRAPARGRAREAPLRGAYPDPTD
 RLSPPPAQPPQRRRHGRRRPSASSTSSDSGSSSSSSASSSSSSSDEDEDDGNDAAADHAREARAVGRGPSAAPEA
 PGRTPPPPGPPPLSEAAPKPRAAARTPAASAGRIERRRARAAGVAGRDATGRFTAGQPRRVELDADAASGAFYARYD
 GYVSGEPWPAGPPPPGRVLYGGLGDSRPGLWGAPEAEAEARRRFEASGAPAAVWAPELGDAAQQYALITRLLYTPDA
 EAMGWLQNPRVPGDVALDQACFRI SGAARNSSSFITGSVARAVPHLGYAMAAGRFGWGLAHAAAAMVMSRRYDRAQ
 KGFLLTSLRRAYAPLLARENAALTGAAGSPGAGADDEGVAAAVVAAAAAPGERAVPAGYGAAGILAAALGRLSAAPAS
 PAGGDDPDAARHADADDDAGRRAQAGRVAVECLAACRGILEALAEFGDGLAAVPGLAGARPASPPRPEGPAGPAS
 PPPHADAPRLRAWLRELRFVRDALVLMRLRGDLRVAGGSEAAVAAVRVSLVAGALGPALPRDPRLPSSAAAAAADL
 LFENQSLRPLLAAGPRRSSSSSGVAAAAAASAPREGRRKSPGPARP PGGGGPRPPKTKKSGADAPGSDARAPLPAPA
 PPSTPPGPEPAPAQPAAPRAAAQARPRVALSRRPAEGPDPLGGWRRQPPGPSHTAAPAAAALEAYCSPRAVAELT
 DHPLFPVPWRPALMFDPALASIAARCAGPAPAAQAACGGGDDDENPHPHGAAGGRLFGPLRASGPLRRMAAWMRQI
 PDPEDVRVVLYSLPLGEDLAGGGASGGPPEWSAERGGLSCLLAALANRLCGPDTAAGNWTGAPDVSAAGQGV
 LLSTRDLAFAGAVEFLGLLASAGDRLIVVNTVRACDWPADGPAVSRQHAYLACDLLPAVQCAVRWPAARDLRRTVL
 ASGRVFGPGVFARVEAAHARLYPDAPPLRLCRGGNVRYRVTRFPGDTPVPMSPREYRRAVLPAALDGRAAASGTTDA
 MAPGAPDFCEEEAHSHRACARWGLGAPLRFPVYVALGREAVRAGPARWRGPRRDFCARALLEPDDAPPLVLRGDDDG
 PGALPPAPPPIRWASATGRSGTVLAAAGAVEVLGAEGALATPPRRDVVDWEGAWDEDDGGAFEGDGV

SEQ ID NO: 5 – ICP47 protein (McKrae strain)

MSWALEMADTFLDNMVRGPRTYADVRDEINKRGREDREAARTAVHDPERPLLRSPGLLPKIAPNASLGVAHRRRTGGT
 VTDSRPNFVTR

SEQ ID NO: 6 – Cell surface hyaluronidase

MYATDSRGHSPAFLQFPQNGNSRHPSGYVPGKVVLPRPPPPKQASAKFTSIRREDRATFAFSPEEQQAQRESQKQK
 RHKNTFCFAITSFSSFFIALAILGISSKYAPDENCQDQNPRLRNWDPGQDSAKQVVIKEGDMLRLTSDATVHSIVI
 QDGGLLVFGDNKDGSRNITLRTHYILIQDGGALHIGAEKCRYKSKATITLYGKSDEGESMPTFGKKFIGVEAGGTLE
 LHGARKASWTLARTLNSSGLPFGSYTFEKDFSRLGNVRVIDQDTAKILESERFDTHEYRNESRRLQEFRLFQDPGR
 IVAIAVGDSAAKSLQGTIQMIQERLGSELIQGLGYRQAWALVGVIDGGSTSCNESVRNYENHSSGGKALAQREFYT
 VDGQKFSVTAYSEWIEGVSLSGFRVEVDGVKLNLLDDVSSWKPGDQIVVASTDYSMYQAEFTLLPCSECSHFQVK
 VKETPQFLHMEIIDGVDMAEVGILTRNIVIQGEVEDSCYAENQCQFFDYDTFGGHIMIMKNFTSVHLSYVELKHM
 GQQQMGRYPVHFHLCGDVDYKGGYRHATFVDGLSIHHSFRCITVHGTNGLLIKDTIGFDTLGHCFLEDGIEQRNT
 LFHNLGLLTKPGTLLPTDRNNSMCTTMRDKVFGNYIPVPATDCMAVSTFWIAHPNNNLINNAAGSQDAGIYLFHK
 EPTGESSGLQLLAKPELTPLGIFYNNRVHSNFKAGLFIDKGVKTTNSSAADPREYLCLDNSARFRPHQDANPEKPRV
 AALIDRLIAFKNNDNGAWVRGGDIIVQNSAFADNIGILTASDGSFSPSDEGSSQEVSESLFVGESRNYGFGQGGNKY
 VGTGGIDQKPRITLPNRNRTFPIRGFQIYDGPILHTRSTFKKYVPTPDYSSAIGFLMKNWSQITPRNNISLVKFGPHV
 SLNVFFGKPGPWFEDCEMDGDKNSIFHDIDGSVTGYKDAYVGRMDNYLIRHPSCVNVSKWNAVICSGTYAQVYVQTW

STQNLSMTITRDEYPSNPMVLRGINQKAAPFQYQPVVMLEKGYTIHWNGPAPRTTFLYLNVFNKNDWIRVGLCYPSN
TSFQVTFGYLQRQNGSLSKIIEYEPVHSLEELQRKQSERKFYFDSSTGLFLYLKAKSHRHGHSYCSSQGCERVKIQ
AATDSKDISNCMAKAYPQYYRKPSVVKRMPAMLTGLCQCGCTRQVVFTSDPHKSYLPVQFQSPDKAETQRGDPSVIS
VNGTDFTRFSAVLLLVDPSCSVFFRLTEKTVFPLADVSRIEYLTGIPPRSIVLLSTRGEIKQLNISHLLVPLGL
AKPAHLVDKGSTIFLGFSGNFKPSWTKLFTSPAGQGLGVLEQFIPLQLDEYGCPRATTVRRRDLELLKQASKAH

SEQ ID NO: 7 – human Matrix metalloproteinase-9 (MMP-9)

MSLWQPLVLVLLVVGCCFAAPRQRQSTLVLPFGDLRTNLTDRQLAEYLYRYGYTRVAEMRGESKSLGPALLLLQKQ
LSLPETGELDSATLKAMRTPRCGVDPDLGRFQTFEGDLKWHHHNITYWIQNYSEDLPRAVIDDAFARAFALWSAVTPL
TFTRVYSRDADIVIQFGVAEHGDGYFPDGDGLLAHAFPPGPGIQGDAHFDDELWSLGKGVVPTFRGNADGAACH
FPFIFEGRSYSACTTDGRSDGLPWCSTTANYDTDDRFGFCPSERLYTQDGNADGKPCQFPFIQGGQSYSACTTDGRS
DGYRWCATTANYDRDKLFGFCPTRADSTVMGGNSAGELCVFPFTFLGKEYSTCTSEGRGDGRLWCATTSNFSDSKK
GFCPDQGYSLFLVAHEFGHALGLDHSSVPEALMYPMYRFTGEPPLHKDDVNGIRHLYGPRPEPEPRPPTTTPTQPT
APPTVCPTGPPTVHPSERPTAGPTGPPSAGPTGPPTAGPSTATTVPLSPVDDACNVNIFDAIAEIGNQLYLFKDGKY
WRFSEGRGRSRPQGFPLIADKWPALPRKLDVSFEERLSKKLFFFSGRQVWVYTGASVLGPRRLDKLGLGADVAQVTGA
LRSGRGKMLLFSGRRLWRFDVKAQMVDP RSASEVDRMFPGVPLDTHDVFQYREKAYFCQDRFYWRVSRSELNQVDQ
VGYVTYDILQCPED

SEQ ID NO: 8 – human Interleukin-1 beta

MAEVPPELASEMMAYYSGNEDDLFFEADGPKQMKCSFQDLDLCPDGGIQLRISDHHSYKGFQQAASVVVAMDKLRKM
LVPCPQTFQENDLSTFFPFIFEEEPFFDFTWDNEAYVHDAPVRSINCTLRDSQQKSLVMSGPYELKALHLQGDMEQ
QVVFMSFVQGEESNDKI PVALGLKEKNLYLSCVLKDDKPTLQLESVDPKNYPKKMEKRFVFNKIEINNKLFEFESA
QFPNWIYSTSQAENMPVFLGGTKGGQDITDFTMQFVSS

SEQ ID NO: 9 – human Interleukin-6

MNSFSTSAFGPVAFSLGLLLVLPAAFPAPVPPGEDSKDVAAPHRQPLTSSERIDKQIRYILDGISALRKETCNKSNM
CESSKEALAENNLNLPKMAEKDGCQSGFNEETCLVKIITGLLEFEVYLEYLQNRFEESSEEQARAVQMSTKVLIQFL
QKKAKNLDAITTPDPTNASLLTKLQAQNLQWLQDMTHLILRSFKEFLQSSLRALRQM

SEQ ID NO: 10 – human Interleukin-18

MAAEPVEDNCINFVAMKFIDNTLYFIAEDDENLESDFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTSDSCRDN
APRTIFIIISMYKDSQPRGMAVTISVKCEKISTLSCENKIIISFKEMNPPDNIKDTKSDIIFQRSVPGHDKMQFESS
SYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNE

SEQ ID NO: 11 – TNFA_HUMAN Tumor necrosis factor

MSTESMIRDVELAEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFCLLHFGVIGPQREEFPRDLSLISPLAQAV
RSSSRTPSDKPVAVHVANPQAEGLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGGCPSTHVLLTH
TISRIVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPYILGGVFQLEKGDRLSAEINRPDYLDFAESGQVYFGII
AL

SEQ ID NO: 12 – CCL19_HUMAN C-C motif chemokine 19

MALLLALSLLVLTSPAPTLSGTND AEDCCLSVTQKPIPGYIVRNHFYLLIKDGCRVPAVVFTTLRGRQLCAPPDQP
WVERIIQRLQRTSAKMKRRSS

SEQ ID NO: 13 – CCL21_HUMAN C-C motif chemokine 21

MAQSLALSLLILVLAFGIPRTQGSDDGAQDCCLKYSQRKIPAKVVRSYRKQEPSLGCSSIPAILFLPRKRSQAEKAD
PKELWVQQLMQHLDKTPSPQKPAQGCGRKDRGASKTGKKGKSGCKRTERSQT PKGP

SEQ ID NO: 14 – IL7_HUMAN Interleukin-7

MFHVSFRYIFGLPPLILVLLPVASSDCDIEGKDGKQYESVLMVSIQQLDSMKEIGSNCLNNEFNFFKRHICDANKE
GMFLFRAARKLRQFLKMNSTGDFDLHLKLVSEGTIILLNCTGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLC
FLKRLQEIKTCKWNKILMGTKHEH

SEQ ID NO: 15 - IL12A_HUMAN Interleukin-12 subunit alpha (p35)

MCPARSLLLVATLVLLDHLSLARNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPTCTSEEDHEDITKD
KTSTVEACLPLELTKNESCLNSRETSFTNGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIF
LDQNMALVIDELMQALNFNSETVPQKSSLEEDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS

SEQ ID NO: 16 - IL12B_HUMAN Interleukin-12 subunit beta (p40)

MCHQQLVISWFSVLFLASPLVAIWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLTI
QVKEFGDAGQYTKHGGEVLSSHLLLLHKKEDGIWSTDI LKDQKEPKNKTFLRCEAKNYSGRFTCWWTITSTDLTF
SVKSSRGSSDPQGVTCGAATLSAERVVRGDNKEYEYSVEQCEDSACPAAEESLPIEVMDAVHKLKYENYTSFFIRD
I IKPDPKPNLQ LKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSATVICRKNASISV
RAQDRYSSSWSEWASVPCS

SEQ ID NO: 17 - IL15_HUMAN Interleukin-15

MRISKPHLRISISIQCYLCLLNSHFLTEAGIHVFILGCFSSAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTES
DVHPSCKVTAMKCFLELQVISLES GDASIHDTVENLII LANNLS SNGNVTESGCKECEELEEKNIKEFLQSFVHI
VQMFINTS

SEQ ID NO: 18 - IL18_HUMAN Interleukin-18

MAAEPVEDNCINFVAMKFIDNTLYFIAEDDENLESDFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDN
APRTIFII SMYKDSQPRGMAVTISVKCEKISTLSCENKII SFKEMNPPDNIKDTSKDIIFFQRSVPGHDKMQFESS
SYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNEH

SEQ ID NO: 19 - IFNG_HUMAN Interferon gamma

MKYTSYIILAFQLCIVLGS LGCYCQDPYVKEAENLKKYFNAGHSDVADNGTLFLGILKNWKEESDRKIMQSQIVSFYF
KLKFNKFDQSIQKSVETIKEDMNVKFFNSNKKRDDFEKLTNYSVTDLNVQRKAIHELIVMAELSPA AKTGKRKR
SQMLFRGRASQ

SEQ ID NO: 20 - TGFR2_HUMAN TGF-beta receptor type-2

MGRGLLRGLWPLHIVLWTRIASTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDFRSTCDNQKSCMSNCSITSIC
EKPQEVCAVVRKNDENITLETVCHDPKLPYHDFILEDAAAPKIMKEKKKPGETFFMCSCSSDECNDNII FSEEYN
TSNPDLVVIFQVTGISLLPPLGVAISVII FYCYRVNRQQLSSTWETGKTRKLMEFSEHCAIILED RSDISSTC
ANNINHNTELLPIELDTLVGKGRFAEVYKAKLKQNTSEQFETVAVKIFPYEYASWKTEKDIFSDINLKHENILQFL
TAEERKTELKQYWLITAFHAKGNLQEYLTRHVISWEDLRKLGS SLARGIAHLHSDHTPCGRPKMPIVHRDLKSSNI
LVKNDLTCCLCDFGLSLRLDPTLSVDDLANSQVGTARYMAPEVLES RMNLENVESFKQTDVYSMALVLWEMTSRCN
AVGEVKDYEPFPGSKVREHPCVESMKDNVLRDRGRPEI PSFWLNHQGIQMV CETLTECWDHDPPEARLTAQCVARFS
ELEHLDRLSGRSCSEKIPEDGSLNTTK

SEQ ID NO: 21 - TGFR2_HUMAN Isoform 3 of TGF-beta receptor type-2

MGRGLLRGLWPLHIVLWTRIASTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDFRSTCDNQKSCFSKVHYEGKK
KAW

SEQ ID NO: 22 - CD40L_HUMAN CD40 ligand

MIETYNQTSRPSAATGLPISMKIFMYLLTVFLITQMIGSALFAVYLHRRLDKIEDERNLHEDFVFMKTIQRCNTGER
 SLSSLNCEEIKSQFEGFVKDIMLNKEETKKENSFEMQKGDQNPQIAAHVISEASSKTTSVLQWAEKGYTMSNNLVT
 LENGKQLTVKRQGLYYIYAQVTFCSNREASSQAPFIASLCLKSPGRFERILLRAANTHSSAKPCGQQSIHLGGVFEL
 QPGASVFNVTDPQSQVSHGTGFTSFGLLKL

SEQ ID NO: 23 - TNFL4_HUMAN Tumor necrosis factor ligand superfamily member 4

MERVQPLEENVGNAARPRFRERNKLLLVASVIQGLGLLLCFTYICLHFSALQVSHRYPRIQSIVQFTEYKKEKGFI
 L TSQKEDEIMKVQNNSVIINCDFYLI SLKGYFSQEVNISLHYQKDEEPLFQLKKVRSVNSLMVASLTYKDKVYLNVT
 TDNTSLDDFHVNGGELILIHQNPGEFCVL

SEQ ID NO: 24 - CCL2_HUMAN C-C motif chemokine 2

MKVSAALLCLLLIAATFIPQGLAQPDAINAPVTCCYNFTNRKISVQRLASYRRITSSKCPKEAVIFKTIVAKEICAD
 PKQKWVQDSMDHLDKQTQTPKT

SEQ ID NO: 25 - CX3CL1_HUMAN Fractalkine

MAPISLSWLLRLATFCHLTVLLAGQHHGVTKCNITCSKMTSKI PVALLIHYQQNQASCGKRAII LETRQHRLFCADP
 KEQWVKDAMQHLDRAAALTRNGGT FEKQIGEVKPRTPAAGGMDES VVLEPEATGESSSLEPTSSQEAQRALGTS
 PELPTGVTGSSGTRLPPTPKAQDGGPVGTFLFRVPVSTAATWQSSAPHQPGPSLWAEAKTSEAPSTQDPSTQASTA
 SSPAPEENAPSEGRVWVGQGS PRPENSLEREEMGPVPAHTDAFQDWGPGSMAHVSVPVPSSEGTSPSREPVASGSWT
 PKAEEPIHATMDPQRLGVLTITPVPDAQAATRRQAVGLLAFLGLLFCLGVAMFTYQSLQGCPRKMAGEMAEGRLRYI PR
 SCGSNSYVLVPV

SEQ ID NO: 26 - CXL16_HUMAN C-X-C motif chemokine 16

MGRDLRPGSRVLLLLLLLLLVYLTQPGNGNEGSVTGSCYCGKRISSDSPPSVQFMNRLRKHLRAYHRCLYYTRFQLL
 SWSVCGGNKDPWVQELMSCLDLKECGHAYSGI VAHQHLLPTSPPI SQASEGASSDIHTPAQMLLSTLQSTQRPTLP
 VGSLSSDKELTRPNETTIHTAGHSLAAGPEAGENQKQPEKNAGPTARTSATVPVLCCLLAI I FILTAALSYVLCKRRR
 GQSPQSSPDLPVHYIPVAPDSNT

SEQ ID NO: 27 - CCL5_HUMAN C-C motif chemokine 5

MKVSAALAVILIATALCAPASASPYSSDTPCCFAYIARPLPRAHIKEYFYTSGKCSNPAVVVTRKNRQVCANPE
 KKWVREYINSLEMS

SEQ ID NO: 28 - CXCL9_HUMAN C-X-C motif chemokine 9

MKKSGVFLFLGII LLVLIGVQTPVVRKGRCSISTNQGTIHLQSLKDLKQFAPSPSCEKIEIIATLKNGVQTCLNP
 DSADVKELIKKWEKQVSQKKKQKNGKKHQQKKVLRKSRQSRQKTT

SEQ ID NO: 29 - CXL10_HUMAN C-X-C motif chemokine 10

MNQTALICCLIFLTLSGIQGVPLSRTVRCTCISISNQPVNPRSLEKLEIIPASQFCPRVEIIATMKKKGEKRLNP
 ESKAIKNLLKAVSKERSKRSP

SEQ ID NO: 30 - CXL11_HUMAN C-X-C motif chemokine 11

MSVKGMAIALAVILCATVVQGFPMFKRGRCLCIGPGVKAVKVADIEKASIMYPNNCDKIEVITLKENKGQRCLNP
 KSKQARLI IKKVERKNF

SEQ ID NO: 31 - IL2_HUMAN Interleukin-2

MYRMQLLSICIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRMLTFKFYMPKKATELKHLO
CLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

SEQ ID NO: 32 - IFNA1_HUMAN Interferon alpha-1/13

MASPFALLMVLVLSCKSSCSLGCCLPETHSLDNRRITMLLAQMSRISPSSCLMDRHDGFGFPQEEFDGNQFQKAPAI
SVLHELIQQIFNLFTTKDSSAAWDEDLLDKFCTELYQQLNDLEACVMQEERVGETPLMNADSI LAVKKYFRITLYL
TEKKYSPCAWEVVRAEIMRSLSLSTNLQERLRKE

SEQ ID NO: 33 - TNFC_HUMAN Lymphotoxin-beta

MGALGLEGRGGRLLAVAGATSLVTLALLAVPITVLAVLALVPQDQGGGLVTETADPGAQAQGGGLGFQKLPEE
EPETDLSPGLPAAHILGAPLKGQGLGWETTKAQAFITSGTQFSDAEGALPQDGLYYLYCLVGYRGRAPPGGGDPQG
RSVTLRSSLYRAGGAYGPGTPELLEGAETVTPVLDPARRQGYGPLWYTSVGFGGLVQLRRGERVYVNI SHPDMVDF
ARGKTFFGAVMVG

SEQ ID NO: 34 - CXL13_HUMAN C-X-C motif chemokine 13

MKFISTSLMLLVSSLSVPVQGVLEVYYTSLRCRCVQESSVFI PRFIDRIQILPRGNGCPRKEIIVWKKNKSI VCV
DPQAEWITQRMMEVLRKRSSSTLPVPVFKRKIP

SEQ ID NO: 35 - IL1A_HUMAN Interleukin-1 alpha

MAKVPDMFEDLKNCYSENEEDSSSIDHLSLNQKSFYHVSYGPLHEGCMQSVLSISSETSKTSLKTFKESMVVATN
GKVLKKRRLSLSQSIDDDLEAIANDSEEEIIKPRSAPFSFLSNVKYNFMRIIKYEFILNDALNQSIIRANDQYLTA
AALHNLDEAVKFDMGAYKSSKDDAKITVILRISKTLQYVTAQDEDQPVLLKEMPEIPKTIITGSETNLLFFWETHGTK
NYFTSVAHPNLFIA TKQDYWVCLAGGPPSITDFQILENQA

SEQ ID NO: 36 - IL17_HUMAN Interleukin-17

AMTPGKTSLSVLLLLLSLEAIVKAGITIPRNP GCPNSEDKNFPRTVMVNLNIHNRNTNTNPKRSSDYNNRSTSPWNL
HRNEDPERYPSVIWEAKRHLGCINADGNVDYHMNSVPIQQEILVLRREPPHCPNSFRLEKILVSVGCTCVTPIVHH
VA

SEQ ID NO: 37 - SHPS1_HUMAN Tyrosine-protein phosphatase non-receptor type 1 (SIRPalpha)

MEPAGPAPGRLGPLLCLLLAASCAWSGVAGEEELQVIQPDKSVLVAAGETATLRCTATSLIPVGPIQWFRGAGPGRE
LIYNQKEGHFPRVTTVSDLTKRNNMDFSIRIGNITPADAGTYCYVKFRKGSFDDVEFKSGAGTELSVRAKPSAPVVS
GPAARATPQHTVSFTCESHGFSPRDITLKWFKNGNELSDFQTNVDPVGESVSYSIHSTAKVVLTRREDVHSQVICEVA
HVTLLQGDPLRG TANLSETIRVPPTLEVTTQPVRAENQVNVTCQVRKFYPQRLQLTWLENGNVSRTEASTVTENKDG
TYNWMWLLVNVSAHRDDVKLTCCQVEHMQPAVSKSHDLKVS AHPKEQGSNTAAENTGSNERNIYIVVGVCVCTLLVA
LLMAALYLVRIRQKKAQGSTSSTRLHEPEKNAREITQDTNDITYADLNLPKGKKPAPQAAEPNNHTEYASIQTSQP
ASEDTLTYADLDMVHLNRTPKQPAKPEPSFSEYASVQVPRK

5 WHAT IS CLAIMED IS:

1. A vector comprising a herpes simplex virus (HSV) genome, wherein the vector comprises an alteration that prevents expression of one or more functional ICP4 and ICP47 proteins.
2. The vector of claim 1, wherein the functional ICP4 and ICP47 proteins are characterized by the amino acid sequences of SEQ ID NO: 3 and 4 (ICP4) and SEQ ID NO: 5 (ICP47).
- 10 3. The vector of claim 1 or claim 2, wherein the HSV genome is an HSV-1 genome.
4. The vector of claim 3, wherein the HSV genome is a McKrae strain genome.
5. The vector of claim 3, wherein the vector, when administered to a subject, results in one or more of:
 - (a) delayed oncolysis;
 - 15 (b) increased immunogenicity; and
 - (c) increased immune activation.
6. The vector of any one of claims 1-5, wherein the vector comprises a nucleic acid sequence encoding one or more therapeutic polypeptides.
7. The vector of claim 6, wherein the therapeutic polypeptide:
 - 20 (a) targets the stroma of a tumor;
 - (b) supports T cell survival in a tumor microenvironment;
 - (c) induces tertiary lymphoid structures (TLS) in a tumor bed; and/or
 - (d) promotes phagocytic innate immune surveillance.
8. The vector of claim 7, wherein the therapeutic polypeptide targets the stroma of a tumor
25 by degrading an extracellular matrix protein.
9. The vector of claim 8, wherein the therapeutic polypeptide comprises hyaluronidase, MMP-9, or an inhibitor of lysyl oxidase.

- 5 10. The vector of claim 7, wherein the therapeutic polypeptide targets the stroma of a tumor by activating local endothelium to increase T cell infiltration.
11. The vector of claim 10, wherein the therapeutic polypeptide comprises a proinflammatory cytokine.
12. The vector of claim 11, wherein the proinflammatory cytokine comprises TNF, IL-1 β ,
10 IL-6 or IL-18.
13. The vector of claim 7, wherein the therapeutic polypeptide supports T cell survival in the tumor microenvironment by enhancing recruitment of T cells to a site of a tumor.
14. The vector of claim 13, wherein the therapeutic polypeptide comprises CCL19 or CCL21.
- 15 15. The vector of claim 7, wherein the therapeutic polypeptide supports T cell survival in the tumor microenvironment by supporting T cell function.
16. The vector of claim 15, wherein the therapeutic polypeptide comprises a T cell trophic factor.
17. The vector of claim 16, wherein the T cell trophic factor is selected from IL-7, IL-12, IL-
20 15, IL-18, and IFN γ .
18. The vector of claim 7, wherein the T cell is a CAR T cell.
19. The vector of claim 18, wherein the therapeutic polypeptide comprises soluble TGF β RII.
20. The vector of claim 18, wherein the therapeutic polypeptide comprises an antigen recognized by the CAR T cell.
- 25 21. The vector of claim 20, wherein the antigen recognized by the CAR T cell comprises mesothelin.
22. The vector of claim 21, wherein the therapeutic polypeptide comprises a co-stimulatory molecule.

- 5 23. The vector of claim 22, wherein the co-stimulatory molecule is selected from CD40L and OX40L.
24. The vector of claim 7, wherein the therapeutic polypeptide induces tertiary lymphoid structures (TLS) in a tumor bed.
25. The vector of claim 24, wherein the therapeutic polypeptide comprises CCL19,
10 lymphotoxin β , CXCL13, or TNF.
26. The vector of claim 7, wherein the therapeutic polypeptide promotes phagocytic innate immune surveillance.
27. The vector of claim 26, wherein the therapeutic polypeptide disrupts the Sirp α /CD47 axis.
- 15 28. The vector of claim 27, wherein the therapeutic polypeptide comprises a Sirp α -IgG fusion transgene.
29. The vector of claim 7, wherein the therapeutic polypeptide supports anti-tumor macrophage polarization.
30. The vector of claim 29, wherein the therapeutic polypeptide that supports anti-tumor
20 macrophage polarization comprises TNF, IL-1, IL-12, IL-17, or IFN γ .
31. A method of expressing a polypeptide in a subject comprising administering to the subject a vector comprising a variant of a herpes simplex virus (HSV) strain whose genome contains an alteration such that the variant fails to express functional ICP4 and ICP47 proteins.
- 25 32. The method of claim 31, wherein the variant fails to express functional ICP4 and ICP47 proteins characterized by the amino acid sequences of SEQ ID NO: 3 and 4 (ICP4) and SEQ ID NO: 5 (ICP47).
33. The method of claim 31 or 32, wherein the HSV strain is an HSV-1 strain.
34. The method of claim 33, wherein the HSV strain is a McKrae strain.

- 5 35. The method of any of claims 31-34, wherein the vector, when administered to the subject, results in one or more of:
- (a) delayed oncolysis;
 - (b) increased immunogenicity; and
 - (c) increased immune activation.
- 10 36. The method of any one of claims 31-35, wherein the HSV strain comprises a nucleic acid encoding a therapeutic polypeptide.
37. The method of claim 36, wherein the therapeutic polypeptide:
- (a) targets the stroma of a tumor;
 - (b) supports T cell survival in a tumor microenvironment;
 - 15 (c) induces tertiary lymphoid structures (TLS) in a tumor bed; and/or
 - (d) promotes phagocytic innate immune surveillance.
38. The method of claim 37, wherein the therapeutic polypeptide targets the stroma of a tumor by degrading an extracellular matrix protein.
39. The method of claim 38, wherein the therapeutic polypeptide comprises hyaluronidase,
- 20 MMP-9, or an inhibitor of lysyl oxidase.
40. The method of claim 37, wherein the therapeutic polypeptide targets the stroma of a tumor by activating local endothelium to increase T cell infiltration.
41. The method of claim 40, wherein the therapeutic polypeptide comprises a proinflammatory cytokine.
- 25 42. The method of claim 41, wherein the proinflammatory cytokine comprises TNF, IL-1 β , IL-6 or IL-18.
43. The method of claim 37, wherein the therapeutic polypeptide supports T cell survival in the tumor microenvironment by enhancing recruitment of T cells to a site of a tumor.

- 5 44. The method of claim 43, wherein the therapeutic polypeptide comprises CCL19 or CCL21.
45. The method of claim 37, wherein the therapeutic polypeptide supports T cell survival in the tumor microenvironment by supporting T cell function.
46. The method of claim 45, wherein the therapeutic polypeptide comprises a T cell trophic
10 factor.
47. The method of claim 46, wherein the T cell trophic factor is selected from IL-7, IL-12, IL-15, IL-18, and IFN γ .
48. The method of claim 37, wherein the T cell is a CAR T cell.
49. The method of claim 48, wherein the therapeutic polypeptide comprises soluble
15 TGF β RII.
50. The method of claim 48, wherein the therapeutic polypeptide comprises an antigen recognized by the CAR T cell.
51. The method of claim 50, wherein the antigen recognized by the CAR T cell comprises mesothelin.
- 20 52. The method of claim 48, wherein the therapeutic polypeptide comprises a co-stimulatory molecule.
53. The method of claim 52, wherein the co-stimulatory molecule is selected from CD40L and OX40L.
54. The method of claim 37, wherein the therapeutic polypeptide induces tertiary lymphoid
25 structures (TLS) in a tumor bed.
55. The method of claim 54, wherein the therapeutic polypeptide comprises CCL19, lymphotoxin β , CXCL13, or TNF.
56. The method of claim 37, wherein the therapeutic polypeptide promotes phagocytic innate immune surveillance.

- 5 57. The method of claim 56, wherein the therapeutic polypeptide disrupts the Sirp α /CD47 axis.
58. The method of claim 57, wherein the therapeutic polypeptide comprises a Sirp α -IgG fusion transgene.
59. The method of claim 36, wherein the therapeutic polypeptide supports anti-tumor
10 macrophage polarization.
60. The method of claim 59, wherein the therapeutic polypeptide that supports anti-tumor macrophage polarization comprises TNF, IL-1, IL-12, IL-17, or IFN γ .
61. A method of preparing a vector comprising a variant herpes simplex virus (HSV) genome which contains an alteration such that the variant fails to express functional ICP4 and
15 ICP47 proteins, and wherein the vector expresses a therapeutic polypeptide, the method comprising incubating cells transfected with:
- (a) a first nucleic acid molecule:
 - (i) comprising a portion of HSV genome but does not encode functional ICP4 and ICP47 proteins; and
 - 20 (ii) comprising a sequence that encodes a marker element, wherein the sequence that encodes the marker element is flanked by a first homology region (HR1) and a second homology region (HR2); and
 - (b) a second nucleic acid molecule comprising a sequence that encodes a therapeutic polypeptide, wherein the sequence encoding the therapeutic polypeptide is flanked by
25 a first homology region (HR1_) and a second homology region (HR2_), wherein HR1 is homologous to HR1_ and HR2 is homologous to HR2_ such that the sequence encoding the therapeutic polypeptide is integrated into the first nucleic acid molecule via homologous recombination.
62. The method of claim 61, wherein the cells are ICP4 and/or ICP47 complementing cells.
63. The method of claim 61, further comprising a step of purifying viral plaques that do not
30 express the marker element.

- 5 64. The method of any of claims 61-64, wherein the HSV genome is an HSV-1 genome.
65. The method of claim 64, wherein the HSV genome is a McKrae strain genome.
66. The method of any one of claim 61-65, wherein the therapeutic polypeptide:
- (a) targets the stroma of a tumor;
- (b) supports T cell survival in a tumor microenvironment;
- 10 (c) induces tertiary lymphoid structures (TLS) in a tumor bed; and/or
- (d) promotes phagocytic innate immune surveillance.
67. The method of claim 66, wherein the therapeutic polypeptide targets the stroma of a tumor by degrading an extracellular matrix protein.
68. The method of claim 67, wherein the therapeutic polypeptide comprises hyaluronidase,
- 15 MMP-9, or an inhibitor of lysyl oxidase.
69. The method of claim 66, wherein the therapeutic polypeptide targets the stroma of a tumor by activating local endothelium to increase T cell infiltration.
70. The method of claim 69, wherein the therapeutic polypeptide comprises a proinflammatory cytokine.
- 20 71. The method of claim 70, wherein the proinflammatory cytokine comprises TNF, IL-1 β , IL-6 or IL-18.
72. The method of claim 66, wherein the therapeutic polypeptide supports T cell survival in the tumor microenvironment by enhancing recruitment of T cells to a site of a tumor.
73. The method of claim 72, wherein the therapeutic polypeptide comprises CCL19 or
- 25 CCL21.
74. The method of claim 73, wherein the therapeutic polypeptide supports T cell survival in the tumor microenvironment by supporting T cell function.

- 5 75. The method of claim 74, wherein the therapeutic polypeptide comprises a T cell trophic factor.
76. The method of claim 75, wherein the T cell trophic factor is selected from IL-7, IL-12, IL-15, IL-18, and IFN γ .
77. The method of claim 66, wherein the T cell is a CAR T cell.
- 10 78. The method of claim 77, wherein the therapeutic polypeptide comprises soluble TGF β RII.
79. The method of claim 77, wherein the therapeutic polypeptide comprises an antigen recognized by the CAR T cell.
80. The method of claim 79, wherein the antigen recognized by the CAR T cell comprises
- 15 mesothelin.
81. The method of claim 77, wherein the therapeutic polypeptide comprises a co-stimulatory molecule.
82. The method of claim 81, wherein the co-stimulatory molecule is selected from CD40L and OX40L.
- 20 83. The method of claim 66, wherein the therapeutic polypeptide induces tertiary lymphoid structures (TLS) in a tumor bed.
84. The method of claim 83, wherein the therapeutic polypeptide comprises CCL19, lymphotoxin β , CXCL13, or TNF.
85. The method of claim 66, wherein the therapeutic polypeptide promotes phagocytic innate
- 25 immune surveillance.
86. The method of claim 85, wherein the therapeutic polypeptide disrupts the Sirp α /CD47 axis.
87. The method of claim 86, wherein the therapeutic polypeptide comprises a Sirp α -IgG fusion transgene.

- 5 88. The method of claim 66, wherein the therapeutic polypeptide supports anti-tumor macrophage polarization.
89. The method of claim 88, wherein the therapeutic polypeptide that supports anti-tumor macrophage polarization comprises TNF, IL-1, IL-12, IL-17, or IFN γ .
90. A variant HSV strain comprising the vector of any of claims 1-30.
- 10 91. A cell transduced with a vector of any of claims 1-30.
92. A pharmaceutical composition comprising a vector of any of claims 1-30 and a pharmaceutically acceptable carrier.
93. A method of reducing the size of a tumor in a subject in need thereof, the method comprising administering to the subject a vector comprising a variant of a herpes simplex
- 15 virus (HSV) strain whose genome contains an alteration such that the variant fails to express functional ICP4 and ICP47 proteins, and wherein the vector comprises a nucleic acid encoding a therapeutic polypeptide that functions to reduce the size of the tumor.
94. The method of claim 93, wherein the variant fails to express functional ICP4 and ICP47 proteins characterized by the amino acid sequences of SEQ ID NO: 3 and 4 (ICP4) and SEQ
- 20 ID NO: 5 (ICP47), respectively.
95. The method of claim 93 or 94, wherein the HSV strain is an HSV-1 strain.
96. The method of claim 95, wherein the HSV strain is a McKrae strain.
97. The method of any one of claims 93-96, wherein the vector, when administered to the subject, results in one or more of:
- 25 (a) delayed oncolysis;
- (b) increased immunogenicity; and
- (c) increased immune activation.
98. The method of any one of claims 93-97, wherein the therapeutic polypeptide:
- (a) targets the stroma of a tumor;

- 5 (b) supports T cell survival in a tumor microenvironment;
- (c) induces tertiary lymphoid structures (TLS) in a tumor bed; and/or
- (d) promotes phagocytic innate immune surveillance.
99. The method of claim 98, wherein the therapeutic polypeptide targets the stroma of a tumor by degrading an extracellular matrix protein.
- 10 100. The method of claim 99, wherein the therapeutic polypeptide comprises hyaluronidase, MMP-9, or an inhibitor of lysyl oxidase.
101. The method of claim 98, wherein the therapeutic polypeptide targets the stroma of a tumor by activating local endothelium to increase T cell infiltration.
102. The method of claim 101, wherein the therapeutic polypeptide comprises a
- 15 proinflammatory cytokine.
103. The method of claim 102, wherein the proinflammatory cytokine comprises TNF, IL-1 β , IL-6 or IL-18.
104. The method of claim 98, wherein the therapeutic polypeptide supports T cell survival in the tumor microenvironment by enhancing recruitment of T cells to a site of a tumor.
- 20 105. The method of claim 100, wherein the therapeutic polypeptide comprises CCL19 or CCL21.
106. The method of claim 98, wherein the therapeutic polypeptide supports T cell survival in the tumor microenvironment by supporting T cell function.
107. The method of claim 102, wherein the therapeutic polypeptide comprises a T cell
- 25 trophic factor.
108. The method of claim 103, wherein the T cell trophic factor is selected from IL-7, IL-12, IL-15, IL-18, and IFN γ .
109. The method of claim 98, wherein the T cell is a CAR T cell.

- 5 110. The method of claim 105, wherein the therapeutic polypeptide comprises soluble TGF β RII.
111. The method of claim 105, wherein the therapeutic polypeptide comprises an antigen recognized by the CAR T cell.
112. The method of claim 107, wherein the antigen recognized by the CAR T cell comprises
10 mesothelin.
113. The method of claim 105, wherein the therapeutic polypeptide comprises a co-stimulatory molecule.
114. The method of claim 109, wherein the co-stimulatory molecule is selected from CD40L and OX40L.
- 15 115. The method of claim 98, wherein the therapeutic polypeptide induces tertiary lymphoid structures (TLS) in a tumor bed.
116. The method of claim 111, wherein the therapeutic polypeptide comprises CCL19, lymphotoxin β , CXCL13, or TNF.
117. The method of claim 98, wherein the therapeutic polypeptide promotes phagocytic
20 innate immune surveillance.
118. The method of claim 113, wherein the therapeutic polypeptide disrupts the Sirp α /CD47 axis.
119. The method of claim 114, wherein the therapeutic polypeptide comprises a Sirp α -IgG fusion transgene.
- 25 120. The method of claim 98, wherein the therapeutic polypeptide supports anti-tumor macrophage polarization.
121. The method of claim 120, wherein the therapeutic polypeptide that supports anti-tumor macrophage polarization comprises TNF, IL-1, IL-12, IL-17, or IFN γ .
122. A vector comprising a herpes simplex virus (HSV) genome, wherein the vector
30 comprises an alteration that prevents expression of one or more functional ICP4 and ICP47

- 5 proteins, and wherein the vector encodes one or more therapeutic polypeptides that support NK cell survival in the tumor microenvironment (TME).
123. The vector of claim 122, wherein the NK cell is a native NK cell or a CAR NK cell.
124. The vector of claim 122 or 123, wherein the one or more therapeutic polypeptides includes an NK cell recruitment factor.
- 10 125. The vector of claim 124, wherein the NK cell recruitment factor is a chemokine ligand.
126. The vector of claim 125, wherein the chemokine ligand is selected from CCL2, CX3CL1, CXCL16, CCL5, CXCL9, CXCL10, and CXCL11.
127. The vector of claim 122 or 123, wherein the one or more therapeutic polypeptides includes an NK cell trophic factor.
- 15 128. The vector of claim 127, wherein the NK cell trophic factor is selected from IL-2, IL-15, IL-18, and IFN α . 129. The vector of claim 122 or 123, wherein the one or more therapeutic polypeptides includes soluble TGF β RII.