SUMMARY

[4a] In one aspect, the present disclosure refers to a non-natural herpes simplex virus ("HSV"), wherein the virus comprises an alanine-to-threonine mutation at position 151 of the gE ~~one or more mutation(s) in a virulence gene that is from the group of:~~

~~(a) a glycoprotein E ("gE") encoding gene,~~

~~(b) an Infected Cell Protein 0 ("ICP0") encoding gene,~~

~~(c) a DNA packaging terminase subunit 1 encoding gene,~~

~~(d) an ICP8 encoding gene, or~~

~~(e) an ICP34.5 encoding gene; and optionally further comprising a gene encoding a dysfunctional ICP34.5 protein and/or a gene encoding a dysfunctional ICP6 protein~~.

[4b] In another aspect, the present disclosure refers to a composition comprising the non-natural HSV as described herein and a carrier and/or further comprising a cryopreservative that facilitates the freezing and thawing of the non-natural HSV without loss of significant virulence, and optionally wherein the carrier is a pharmaceutically available carrier.

[4c] In another aspect, the present disclosure refers to a cell infected with the non-natural HSV as described herein, optionally a lymphocyte.

[4d] In another aspect, the present disclosure refers to a method to infect a cell, comprising contacting the cell with the non-natural HSV as described herein or the composition as described herein, optionally wherein the cell is a lymphocyte; and/or wherein the cell has been infected with an Epstein-Barr virus ("EBV").

[4e] In another aspect, the present disclosure refers to a method to prepare a viral vector comprising introducing a transgene into the non-natural HSV as described herein.

[4f] In another aspect, the present disclosure refers to an *in vitro* method for inhibiting the growth or metastasis of cancer cell, comprising contacting the cell with an effective amount of the non-natural HSV ~~vector~~ as described herein or the composition as described herein.

[4g] In yet another aspect, the present disclosure refers to a use of the non-natural HSV ~~vector~~ as described herein or the composition as described herein in the manufacture of a

medicament for treating cancer in a subject, wherein an effective amount of the non-natural HSV ~~vector~~ as described herein or the composition as described herein is to be administered to the subject, and optionally wherein the cancer is of the type, or the cancer cell is selected from a cell of the type: pancreatic cancer, renal cancer, small cell lung cancer, brain cancer, neural cancer, bone cancer, lymphoma, myeloma, colon cancer, uterine cancer, breast cancer, leukemia, liver cancer, prostate cancer, skin cancer, or melanoma; and/or wherein the HSV ~~vector~~ or the pharmaceutical composition is to be administered by injection, infusion, instillation, and/or inhalation; and/or wherein the administration comprises a therapy of the group of: a first line therapy, a second line therapy, a third line therapy, a fourth line therapy, or a fifth line therapy; and/or an effective amount of an anticancer therapy is to be further administered to the subject.

~~**[4h]** In yet another aspect, the present disclosure refers to a method of producing the HSV vector as described herein, comprising~~

~~(a) introducing to a host cell a 17TermA HSV vector and an rRp450 HSV vector;~~

~~(b) growing the host cell for at least 3 passages; and~~

~~(c) isolating an HSV particle produced by the host cell.~~

[4hi] In yet another aspect, the present disclosure refers to an *in vitro* method for inducing cell lysis, comprising contacting the cell with the non-natural HSV as described herein or the composition as described herein.

[4ij] In yet another aspect, the present disclosure refers to a kit comprising the HSV ~~vector~~ as described herein or the composition as described herein, and instructions for use.

[4ijk] In yet another aspect, the present disclosure refers to a method of producing the HSV ~~vector~~ as described herein, comprising

(a) introducing to a host cell a polynucleotide encoding a viral genome of the HSV ~~vector~~;

(b) growing the host cell; and

(c) isolating an HSV particle produced by the host cell.

[4k] In yet another aspect, the present disclosure refers to the non-natural HSV ~~vector~~ as described herein or the composition as described herein for use in therapy.

[5] The present disclosure provides a non-natural herpes simplex virus ("HSV"), wherein the virus comprises, alternatively consists essentially of, or yet further consists of a mutation in one or more of: (a) a glycoprotein E ("gE")-encoding gene, (b) an Infected Cell Protein 0 ("ICP0")-encoding gene, (c) a DNA packaging terminase subunit 1-encoding gene, (d) an ICP8-encoding gene, or (e) an ICP34.5-encoding gene.

[6] In another example, the present disclosure provides a composition or a pharmaceutical composition that comprises, alternatively consists essentially of, or yet further consists of the non-natural HSV of this disclosure. In another example, provided in this disclosure is a method to infect a cell, comprising, or alternatively consisting essentially of, or yet further consisting of contacting the cell with the non-natural HSV or a composition or pharmaceutical composition containing the non-natural HSV.

[7] In one example, the present disclosure provides a method of preparing the non-natural HSV of this disclosure comprising, or alternatively consisting essentially of, or yet further consisting of mutating a gene in a non-natural HSV viral particle or introducing into the non-natural HSV a transgene. In another example, the method of producing the non-natural HSV vector, comprises, or alternatively consists essentially of, or yet further consists of: (a) introducing to a host cell a 17TermA HSV vector and an rRp450 HSV vector; (b) growing the host cell for at least 3 passages; and (c) isolating a HSV particle produced by the host cell.

[8] Also provided is a method for inhibiting the growth or metastasis of a cancer cell or a metastatic cancer cell, the method comprising, or consisting essentially of, or yet further consisting of, contacting the cell with an effective amount of the non-natural HSV vector or a composition or a pharmaceutical composition containing the non-natural HSV vector as described herein. The contacting is *in vitro* or *in vivo*. In one example, the contacting is *in vivo* by administration of the non-natural HSV or a composition or a pharmaceutical composition to a subject. *In vitro*, the method is practiced by placing the non-natural HSV in contact with the cell. The *in vitro* method can be used to test for new therapies or as a personalized assay to determine if the therapy is suitable for the cancer to be treated.
Additional cancer therapies

WHAT IS CLAIMED IS:

1. A non-natural herpes simplex virus ("HSV"), wherein the virus comprises an alanine-to-threonine mutation at position 151 of the gE~~one or more mutation(s) in a virulence gene that is from the group of:~~

~~(a) a glycoprotein E ("gE") encoding gene,~~

~~(b) an Infected Cell Protein 0 ("ICP0") encoding gene,~~

~~(c) a DNA packaging terminase subunit 1 encoding gene,~~

~~(d) an ICP8 encoding gene, or~~

~~(e) an ICP34.5 encoding gene; and optionally further comprising a gene encoding a dysfunctional ICP34.5 protein and/or a gene encoding a dysfunctional ICP6 protein~~.

~~2. The non-natural HSV of claim 2, wherein the gene encoding the dysfunctional ICP34.5 protein comprises a polynucleotide having a sequence selected from SEQ ID NO. 5, SEQ ID NO. 7 and a sequence at least 95% identical to SEQ ID No. 5, or 7 and maintaining the mutation at the nucleotide in any one of SEQ ID No. 5, or 7.~~

~~3. The non-natural HSV of claim 1 or 2, wherein the gene encoding the dysfunctional ICP6 protein comprises a polynucleotide having a sequence selected from SEQ ID NO. 45, SEQ ID NO. 47, and a sequence at least 95% identical to SEQ ID No. 45, or 47 and maintaining the mutation at the nucleotide in any one of SEQ ID No. 45, or 47.~~

~~4. The non-natural HSV of any one of claims 1-3, wherein the HSV lacks a gene encoding a functional ICP34.5 protein and/or a functional ICP6 protein.~~

~~5. The non-natural HSV of any one of claims 1-4 or an equivalent thereof, wherein the mutation or equivalent encodes:~~

~~(a) an alanine-to-threonine mutation at position 151 of the gE protein,~~

~~(b) an arginine-to-histidine mutation at position 258 of the ICP0 protein,~~

~~(c) an alanine-to-threonine mutation at position 376 of the DNA packaging terminase subunit 1 protein,~~

~~(d) a threonine-to-methionine mutation at position 1155 of the ICP8 protein, or~~

~~(e) a proline-to-histidine mutation at position 119 of the ICP34.5 protein.~~

~~6~~2. The non-natural HSV of ~~any one of~~ claim~~s~~ 1~~-5~~, comprising ~~one or more of the following:~~

~~(a) a polynucleotide encoding an amino acid sequence selected from SEQ ID NOs. 2, 6, 8, 10, and 52, and/or a polynucleotide having a sequence selected from SEQ ID NOs. 1, 5, 7, 9, and 51;~~

~~(b) a polypeptide having an amino acid sequence selected from SEQ ID NOs. 2, 6, 8, 10, and 52;~~

~~(c)~~ a polynucleotide encoding an amino acid sequence as shown by SEQ ID NO:~~selected from SEQ ID NOs.~~ 13, ~~15, 17 and 19,~~ and/or a polynucleotide having a sequence as shown by SEQ ID NO:~~selected from SEQ ID NOs.~~ 12~~, 14, 16 and 18;~~

~~(d) a polypeptide having an amino acid sequence selected from SEQ ID NOs. 13, 15, 17 and 19;~~

~~(e) a polynucleotide encoding an amino acid sequence selected from SEQ ID NOs. 21, 23 and 26, and/or a polynucleotide having a sequence selected from SEQ ID NOs. 20, 22, 24, 25, and 53, or a sequence thereof free of one or two or more introns;~~

~~(f) a polypeptide having an amino acid sequence selected from SEQ ID NOs. 21, 23 and 26;~~

~~(g) a polynucleotide encoding an amino acid sequence selected from SEQ ID NOs. 28, 30, 32 and 34, and/or a polynucleotide having a sequence selected from SEQ ID NOs. 27, 29, 31, and 33;~~

~~(h) a polypeptide having an amino acid sequence selected from SEQ ID NOs. 28, 30, 32 and 34;~~

~~(i) a polynucleotide encoding an amino acid sequence selected from SEQ ID NOs. 36, 38, 40, and 42, and/or a polynucleotide having a sequence selected from SEQ ID NOs. 35, 37, 39 and 41;~~

~~(j) a polypeptide having an amino acid sequence selected from SEQ ID NOs. 36, 38, 40, and 42;~~

~~(k) a polynucleotide encoding an amino acid sequence selected from SEQ ID NOs. 44, 46, 48 and 50, and/or a polynucleotide having a sequence selected from SEQ ID NOs. 43, 45, 47 and 49;~~

~~(l) a poly peptide having an amino acid sequence selected from SEQ ID NOs. 44, 46, 48 and 50~~.

~~7~~3. The non-natural HSV of claim 1 or claim 2~~any one of claims 1-6~~, further comprising a polynucleotide having sequence that is identical to at least a fragment of a virulence gene from a 17TermA HSV; and/or further comprising a polynucleotide having sequence that is identical to ~~at least a fragment of~~ a virulent gene from an rRp450 HSV; and/or further comprising a transgene.

~~8~~4. A composition comprising the non-natural HSV of any one of claims 1-~~7~~ 3 and a carrier and/or further comprising a cryopreservative that facilitates the freezing and thawing of the non-natural HSV without loss of significant virulence, and optionally wherein the carrier is a pharmaceutically available carrier.

~~9~~5. A cell infected with the non-natural HSV of any one of claims 1-~~8~~3, optionally a lymphocyte.

~~10~~6. A method to infect a cell, comprising contacting the cell with the non-natural HSV of any one of claims 1-~~14~~ 3 or the composition of ~~any one of claims 15-17~~claim 4, optionally wherein the cell is a lymphocyte; and/or wherein the cell has been infected with an Epstein-Barr virus (“EBV”).

~~11~~7. A method to prepare a viral vector comprising introducing a transgene into the non-natural HSV of any one of claims 1-~~7~~3.

~~12~~8. An *in vitro* method for inhibiting the growth or metastasis of cancer cell, comprising contacting the cell with an effective amount of the non-natural HSV ~~vector~~ of any one of claims 1-~~7~~3 or the composition of claim ~~4~~8.

~~13~~9. Use of the non-natural HSV ~~vector~~ of any one of claims 1-~~7~~ 3 or the composition of claim ~~4~~8 in the manufacture of a medicament for treating cancer in a subject, wherein an effective amount of the non-natural HSV ~~vector~~ of any one of claims 1-~~7~~ 3 or the composition of claim ~~8~~ 4 is to be administered to the subject, and optionally wherein the cancer is of the type, or the cancer cell is selected from a cell of the type: pancreatic cancer, renal cancer, small cell lung cancer, brain cancer, neural cancer, bone cancer, lymphoma, myeloma, colon cancer, uterine cancer, breast cancer, leukemia, liver cancer, prostate cancer, skin cancer, or melanoma; and/or wherein the HSV ~~vector~~ or the pharmaceutical composition is to be administered by injection, infusion, instillation, and/or inhalation; and/or wherein the administration comprises a therapy of the group of: a first line therapy, a second line therapy, a third line therapy, a fourth line therapy, or a fifth line therapy; and/or an effective amount of an anticancer therapy is to be further administered to the subject.

~~14. A method of producing the HSV vector of any one of claims 1-7, comprising~~

~~(a) introducing to a host cell a 17TermA HSV vector and an rRp450 HSV vector;~~

~~(b) growing the host cell for at least 3 passages; and~~

~~(c) isolating an HSV particle produced by the host cell.~~

~~15~~10. An *in vitro* method for inducing cell lysis, comprising contacting the cell with the non-natural HSV of any one of claims 1-~~7~~ 3 or the composition of claim ~~8~~4.

~~16~~11. A kit comprising the HSV ~~vector~~ of any one of claims 1-~~7~~ 3 or the composition of claim ~~4~~8, and instructions for use.

~~17~~12. A method of producing the HSV ~~vector~~ of any one of claims 1-~~7~~3, comprising

(a) introducing to a host cell a polynucleotide encoding a viral genome of the HSV ~~vector~~;

(b) growing the host cell; and

(c) isolating an HSV particle produced by the host cell.

~~18~~13. The non-natural HSV ~~vector~~ of any one of claims 1-~~7~~ 3 or the composition of claim ~~8~~ 4 for use in therapy.