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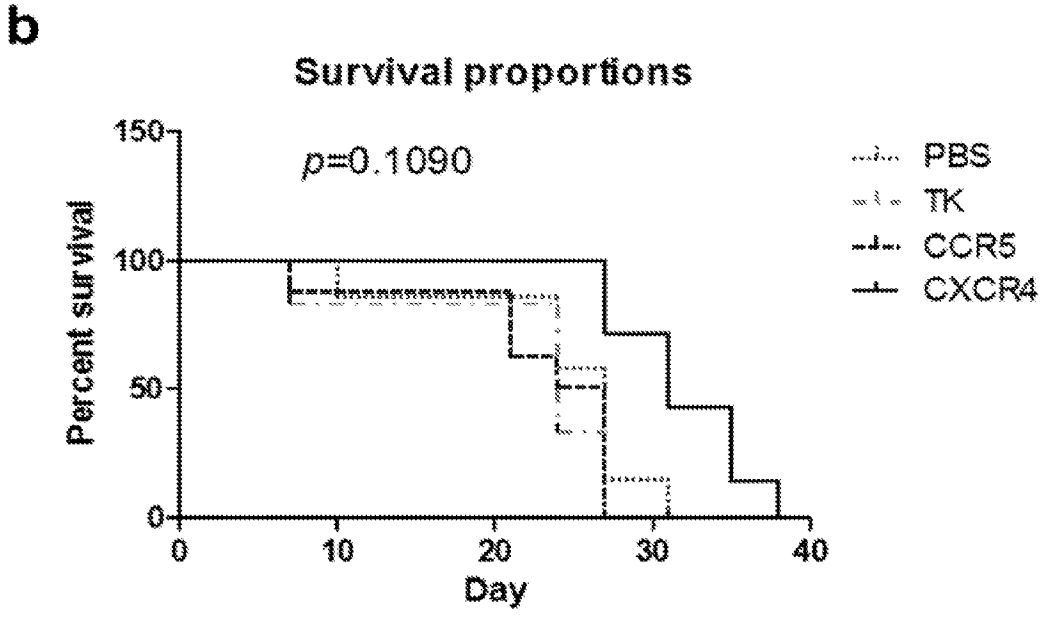
(54) Title: PLATFORM ONCOLYTIC VECTOR FOR SYSTEMIC DELIVERY

FIG. 5B



(57) Abrégé/Abstract:
This disclosure provides a modified oncolytic virus that can contain modifications in the viral genome and exogenous nucleic acids coding for proteins. The modified oncolytic virus can be utilized as a platform vector for systemic delivery.

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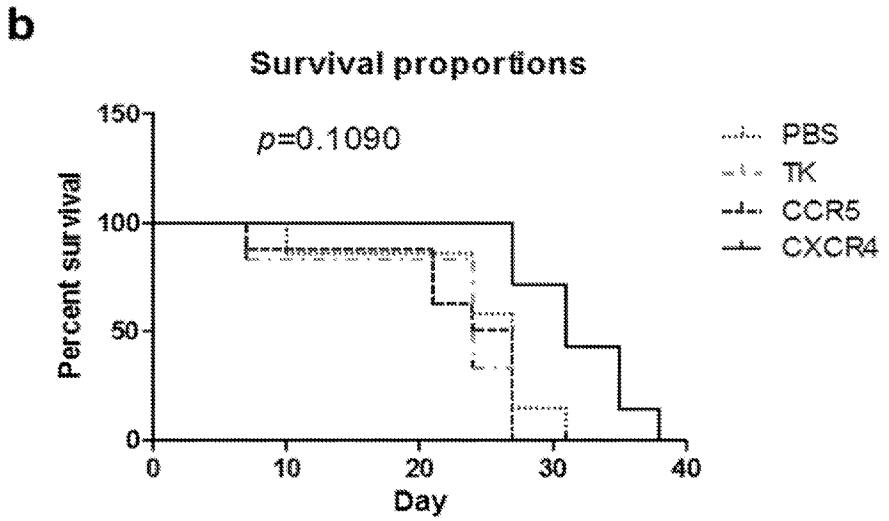
(54) Title: PLATFORM ONCOLYTIC VECTOR FOR SYSTEMIC DELIVERY

FIG. 5B



(57) Abstract: This disclosure provides a modified oncolytic virus that can contain modifications in the viral genome and exogenous nucleic acids coding for proteins. The modified oncolytic virus can be utilized as a platform vector for systemic delivery.

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PLATFORM ONCOLYTIC VECTOR FOR SYSTEMIC DELIVERY

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/579,517 filed October 31, 2017 which is incorporated by reference herein in its entirety.

INCORPORATION BY REFERENCE

[0002] All publications, patents, patent applications, and NCBI accession numbers mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference, and as if set forth in their entireties. In the event of a conflict between a term as used herein and the term as defined in the incorporated reference, the definition of this disclosure controls.

SUMMARY

[0003] One aspect of the present disclosure provides a modified oncolytic virus, comprising an exogenous nucleic acid that codes for a chemokine receptor, a membrane associated protein that can be capable of degrading hyaluronan, a microbial protein that is capable of degrading hyaluronan, or any combinations thereof.

[0004] In some embodiments, the chemokine receptor can comprise at least one of CXCR4 and CCR2.

[0005] In some embodiments, the modified oncolytic virus can comprise the exogenous nucleic acid that can code for the membrane associated protein. In some embodiments, the exogenous nucleic acid that codes for the membrane associated protein can code for a hyaluronidase. In some embodiments, the hyaluronidase can be PH-20. In some embodiments, the PH-20 can be GPI-anchored. In some embodiments, the modified oncolytic virus can comprise the exogenous nucleic acid that can code for the microbial protein. In some embodiments, the microbial protein comprises a secreted hyaluronidase. In some embodiments, the secreted hyaluronidase comprises at least one of HysA, lin, sko, rv, or any combinations thereof. In some embodiments, the microbial protein comprises HysA.

[0006] In some embodiments, the modification can enhance production of enveloped extracellular form (EEV) of the virus.

[0007] In some embodiments, the modified oncolytic virus can comprise the modification in the genome of the virus. In some embodiments, the modification can comprise a mutation or a deletion of the B5R gene, wherein said deletion is a partial deletion. In some embodiments, the modification can comprise a mutation or a deletion in a SCR region of the B5R gene, wherein said SCR region comprises SCR1, SCR3, SCR4, or any combinations thereof, and wherein the SCR region does not comprise SCR2.

[0008] In some embodiments, the modified oncolytic virus can comprise the deletion of the B5R gene, and the deletion can be a partial deletion of the B5R gene.

[0009] In some embodiments, the modified oncolytic virus can comprise a modification in the genome of the virus, wherein the modification can comprise a mutation or a deletion of the A52R gene. In some embodiments, the modification can comprise the deletion of the A52R gene.

[0010] In some embodiments, the modified oncolytic virus can further comprise at least one additional modification in the genome of the virus, wherein the additional modification can comprise a mutation or a deletion of a further viral gene.

[0011] In some embodiments, the further viral gene can comprise at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, and N1L, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0012] In some embodiments, the modified oncolytic virus can further comprise at least one additional exogenous nucleic acid.

[0013] In some embodiments, the at least one additional exogenous nucleic acid can comprise a nucleic acid sequence that codes for a protein or a fragment thereof that: modulates NFκB (NF-kappaB) signaling, promotes reduction of interstitial fluid pressure (IFP) in a tumor, modulates STAT3-mediated gene activation, promotes T cell activation, promotes attraction of NK cells to virus-infected cells, modulates metabolic program of virus-infected cells, modulates fatty acid uptake by virus-infected cells, promotes therapeutic targeting of MDSCs, or any combinations thereof.

[0014] In some embodiments, the at least one additional exogenous nucleic acid can comprise a nucleic acid coding for at least one ofHMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, and a functional domain or fragment or variant thereof, or any combinations thereof .

[0015] In some embodiments, the modified oncolytic virus can comprise: (a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; and (b) the exogenous nucleic acid that codes for a hyaluronidase.

[0016] In some embodiments, the modified oncolytic virus can comprise: (a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; and (b) the mutation or the deletion of B5R gene.

[0017] In some embodiments, the modified oncolytic virus can comprise: (a) the exogenous nucleic acid that codes for a hyaluronidase; and (b) the mutation or the deletion of B5R gene.

[0018] In some embodiments, the modified oncolytic virus can comprise: (a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; (b) the exogenous nucleic acid that codes for a hyaluronidase; and (c) the mutation or the deletion of B5R gene.

[0019] In some embodiments, the modified oncolytic virus can comprise: (a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; and (b) the mutation or the deletion of the further viral gene can comprise at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0020] In some embodiments, the modified oncolytic virus can comprise: (a) the exogenous nucleic acid that codes for a hyaluronidase; and (b) the mutation or the deletion of the further viral gene can comprise at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0021] In some embodiments, the modified oncolytic virus can comprise: (a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; (b) the exogenous nucleic acid that codes for a hyaluronidase; and (c) the mutation or the deletion of the further viral gene can comprise at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0022] In some embodiments, the modified oncolytic virus can comprise: (a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; (b) the mutation or the deletion of B5R gene; and (c) the mutation or the deletion of the further viral gene can comprise at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0023] In some embodiments, the modified oncolytic virus can comprise: (a) the exogenous nucleic acid that codes for a hyaluronidase; (b) the mutation or the deletion of B5R gene; and (c) the mutation or the deletion of the further viral gene can comprise at least one of F13L,

A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0024] In some embodiments, the modified oncolytic virus can comprise: (a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2 ; (b) the exogenous nucleic acid that codes for a hyaluronidase ; (c) the mutation or the deletion of B5R gene; and (d) the mutation or the deletion of the further viral gene can comprise at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0025] In some embodiments, the modified oncolytic virus can comprise: (a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; and (b) the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0026] In some embodiments, the modified oncolytic virus can comprise: (a) the exogenous nucleic acid that codes for a hyaluronidase; and (b) the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0027] In some embodiments, the modified oncolytic virus can comprise: (a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; (b) the exogenous nucleic acid that codes for a hyaluronidase; and (c) the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0028] In some embodiments, the modified oncolytic virus can comprise: (a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; (b) the mutation or the deletion of B5R gene; and (c) the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0029] In some embodiments, the modified oncolytic virus can comprise: (a) the exogenous nucleic acid that codes for ahyaluronidase; (b) the mutation or the deletion of B5R gene; and (c) the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0030] In some embodiments, the modified oncolytic virus can comprise: (a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; (b) the exogenous nucleic acid that codes for a hyaluronidase; (c) the mutation or the deletion of B5R gene; and (d) the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0031] In some embodiments, the modified oncolytic virus can comprise: (a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; (b) the exogenous nucleic acid that codes for a hyaluronidase; (c) the mutation or the deletion of B5R gene; (d) the mutation or the deletion of the further viral gene can comprise at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, and a functional domain or fragment or variant thereof, or any combinations thereof; and (e) the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0032] In some embodiments, the hyaluronidase comprises PH-20. In some embodiments, the hyaluronidase comprises HysA. In some embodiments, the modified oncolytic virus can further comprise an exogenous nucleic acid that codes for a viral VH1 protein.

[0033] In some embodiments, the modified oncolytic virus can comprise the exogenous nucleic acid coding for the viral VH1 protein, wherein the exogenous nucleic acid can be from a genome of a poxvirus, wherein the poxvirus can be not vaccinia virus. In some embodiments, the poxvirus can comprise a measles virus, a poliovirus, a poxvirus, a vaccinia virus, an adenovirus, an adeno associated virus, a herpes simplex virus, a vesicular stomatitis virus, a reovirus, a Newcastle disease virus, a senecavirus, a lentivirus, a mengovirus, or a myxomavir.

[0034] In some embodiments, the viral genome can comprise a thymidine kinase gene. In some embodiments, a thymidine kinase gene can be deleted from the viral genome.

[0035] In some embodiments, the modified oncolytic virus can further comprise a thymidine kinase gene from a herpes simplex virus.

[0036] In some embodiments, the modified oncolytic virus exhibits enhanced intratumoral and intertumoral spreading, enhanced immune evasion, enhanced tumor-specific replication, enhanced tumor-targeted delivery, compared to an otherwise identical oncolytic virus that does not comprise the modifications as disclosed herein.

[0037] In some embodiments, the modified oncolytic virus can comprise a vaccinia virus, an adeno associated virus, an adenovirus, a reovirus, a lentivirus, a herpes simplex virus, a vesicular stomatitis virus, a mengovirus, or a myxomavir. In some embodiments, the modified oncolytic virus can be a vaccinia virus.

[0038] Another aspect of the present disclosure provides an oncolytic vaccinia virus that can comprise at least two of the following: (a) a modification that enhances intratumoral and intertumoral spreading of the virus; (b) a modification that enhances systemic delivery of the virus; (c) a modification that enhances tumor-specific replication of the virus; and (d) a modification that enhances immune evasion of the virus.

[0039] Yet another aspect of the present disclosure provides an oncolytic vaccinia virus that can comprise an exogenous nucleic acid that codes for a protein, or a fragment thereof, that enhances degradation of an extracellular matrix (ECM) of a tumor.

[0040] In some embodiments of the oncolytic vaccinia virus, the protein or the fragment thereof that enhances degradation of the ECM can be a hyaluronidase. In some embodiments, the hyaluronidase can be a membrane associated hyaluronidase, such as PH-20. In some embodiments, the PH-20 can be GPI-anchored. In some embodiments, the hyaluronidase can be a microbial hyaluronidase, such as HysA.

[0041] In some embodiments, the oncolytic vaccinia virus can comprise at least one of the following: (a) a modification that enhances intratumoral and intertumoral spreading of the virus; (b) a modification that enhances systemic delivery of the virus; (c) a modification that enhances tumor-specific replication of the virus; and (d) a modification that enhances immune evasion of the virus.

[0042] Yet another aspect of the present disclosure provides an oncolytic vaccinia virus that can comprise an exogenous nucleic acid that codes for a chemokine receptor, wherein expression of the chemokine receptor from the virus enhances systemic delivery of the virus.

[0043] In some embodiments, the chemokine receptor can comprise at least one of CXCR4 and CCR2.

[0044] In some embodiments, the oncolytic vaccinia virus can comprise the exogenous nucleic acid that codes for the chemokine receptor, and can further comprise at least one of the following: (a) a modification that enhances degradation of an ECM of a tumor; and (b) a modification that enhances production of EEV form of the virus;

[0045] Yet another aspect of the present disclosure provides an oncolytic vaccinia virus that can comprise a first modification in the genome of the virus that enhances production of EEV form of the virus, and at least one of the following further modifications: (a) a modification

that enhances intratumoral and intertumoral spreading of the virus; (b) a modification that enhances systemic delivery of the virus; (c) a modification that enhances tumor-specific replication of the virus; and (d) a modification that enhances immune evasion of the virus.

[0046] In some embodiments, the first modification can comprise a mutation or a deletion of the B5R gene.

[0047] In some embodiments, the oncolytic vaccinia virus can comprise a mutation or a deletion of a further viral gene, wherein the further viral gene can comprise at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, and A52R, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0048] In some embodiments, the oncolytic vaccinia virus can further comprise a nucleic acid sequence that codes for a protein or a fragment thereof that: modulates NFκB signaling, promotes reduction of interstitial fluid pressure (IFP), modulates STAT3-mediated gene activation, promotes T cell activation, promotes attraction of NK cells to virus-infected cells, modulates metabolic program of virus-infected cells, modulates fatty acid uptake by virus-infected cells, promotes therapeutic targeting of MDSCs, or any combinations thereof.

[0049] In some embodiments, the at least one additional exogenous nucleic acid can comprise a nucleic acid coding for at least one of HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0050] In some embodiments, the oncolytic vaccinia virus can comprise: (a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; and (b) the exogenous nucleic acid that codes for a hyaluronidase.

[0051] In some embodiments, the oncolytic vaccinia virus can comprise: (a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; and (b) the mutation or the deletion of B5R gene.

[0052] In some embodiments, the oncolytic vaccinia virus can comprise: (a) the exogenous nucleic acid that codes for a hyaluronidase; and (b) the mutation or the deletion of B5R gene.

[0053] In some embodiments, the oncolytic vaccinia virus can comprise: (a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; (b) the exogenous nucleic acid that codes for a hyaluronidase; and (c) the mutation or the deletion of B5R gene.

[0054] In some embodiments, the oncolytic vaccinia virus can comprise: (a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; and (b) the mutation or the deletion of the further viral gene can comprise at least one of F13L, A36R, A34R, A33R,

B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, A52R, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0055] In some embodiments, the oncolytic vaccinia virus can comprise: (a) the exogenous nucleic acid that codes for a hyaluronidase; and (b) the mutation or the deletion of the further viral gene can comprise at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, A52R, and a functional domain or fragment or variant thereof, or any combinations thereof a functional domain thereof, or any combinations thereof.

[0056] In some embodiments, the oncolytic vaccinia virus can comprise: (a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; (b) the exogenous nucleic acid that codes for a hyaluronidase; and (c) the mutation or the deletion of the further viral gene can comprise at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, A52R, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0057] In some embodiments, the oncolytic vaccinia virus can comprise: (a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; (b) the mutation or the deletion of B5R gene; and (c) the mutation or the deletion of the further viral gene can comprise at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, A52R, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0058] In some embodiments, the oncolytic vaccinia virus can comprise: (a) the exogenous nucleic acid that codes for a hyaluronidase; (b) the mutation or the deletion of B5R gene; and (c) the mutation or the deletion of the further viral gene can comprise at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, A52R, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0059] In some embodiments, the oncolytic vaccinia virus can comprise: (a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2 ; (b) the exogenous nucleic acid that codes for a hyaluronidase ; (c) the mutation or the deletion of B5R gene; and (d) the mutation or the deletion of the further viral gene can comprise at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, A52R, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0060] In some embodiments, the oncolytic vaccinia virus can comprise: (a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; and (b) the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-

R, LIGHT, ITAC, fractalkine, CCL5, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0061] In some embodiments, the oncolytic vaccinia virus can comprise: (a) the exogenous nucleic acid that codes for the hyaluronidase; and (b) the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-R, LIGHT, ITAC, fractalkine, CCL5, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0062] In some embodiments, the oncolytic vaccinia virus can comprise: (a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; (b) the exogenous nucleic acid that codes for the hyaluronidase; and (c) the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-R, LIGHT, ITAC, fractalkine, CCL5, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0063] In some embodiments, the oncolytic vaccinia virus can comprise: (a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; (b) the mutation or the deletion of B5R gene; and (c) the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-R, LIGHT, ITAC, fractalkine, CCL5, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0064] In some embodiments, the oncolytic vaccinia virus can comprise: (a) the exogenous nucleic acid that codes for the hyaluronidase; (b) the mutation or the deletion of B5R gene; and (c) the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-R, LIGHT, ITAC, fractalkine, CCL5, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0065] In some embodiments, the oncolytic vaccinia virus can comprise: (a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; (b) the exogenous nucleic acid that codes for the hyaluronidase; (c) the mutation or the deletion of B5R gene; and (d) the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-R, LIGHT, ITAC, fractalkine, CCL5, and a functional domain or fragment or variant thereof, or any combinations thereof.

[0066] In some embodiments, the oncolytic vaccinia virus can comprise: (a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; (b) the exogenous nucleic acid that codes for the hyaluronidase; (c) the mutation or the deletion of B5R gene; (d) the mutation or the deletion of the further viral gene can comprise at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, A52R, and a functional domain or fragment or variant thereof, or any combinations thereof; and (e)

the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, and a functional fragment or domain or variant thereof, or any combinations thereof.

[0067] One aspect provides an oncolytic vaccinia virus, comprising an exogenous nucleic acid that can code for a chemokine receptor, a protein that is capable of degrading hyaluronan, or any combinations thereof. In some embodiments, the protein that is capable of degrading hyaluronan, wherein the protein comprises PH-20 or HysA.

[0068] One aspect provides a modified oncolytic virus that can comprise a mutation or a deletion of A52R gene. In some embodiments, the modified oncolytic virus can comprise the deletion of A52R gene.

[0069] In some embodiments, the modified oncolytic virus can comprise an exogenous nucleic acid that can code for a chemokine receptor, a protein that is capable of degrading hyaluronan, or any combinations thereof. In some embodiments, the modified oncolytic virus can comprise the exogenous nucleic acid that can code for the chemokine receptor, wherein the chemokine receptor can comprise CXCR4 or CCR2. In some embodiments, the modified oncolytic virus can comprise the exogenous nucleic acid that codes for the protein that is capable of degrading hyaluronan, wherein the protein can comprise a membrane associated hyaluronidase or a secreted hyaluronidase. In some embodiments, the modified oncolytic virus can comprise the exogenous nucleic acid that can code for the membrane associated hyaluronidase, wherein the membrane associated hyaluronidase can be PH-20. In some embodiments, the PH-20 can be GPI-anchored. In some embodiments, the modified oncolytic virus can comprise the exogenous nucleic acid that can code for the secreted hyaluronidase, wherein the secreted hyaluronidase can be HysA.

[0070] One aspect provides a modified oncolytic virus that can comprise a mutation or a deletion of K7R gene. In some embodiments, the modified oncolytic virus can comprise the deletion of the K7R gene. In some embodiments, the modified oncolytic virus can further comprise at least one of an exogenous nucleic acid that can code for a cytokine, an exogenous nucleic acid that can code for a cytokine receptor, and an exogenous nucleic acid that can code for a chemokine. In some embodiments, the modified oncolytic virus comprises the exogenous nucleic acid that can code for the cytokine, wherein the cytokine can comprise IL15. In some embodiments, the modified oncolytic virus can comprise the exogenous nucleic acid that can code for the cytokine receptor, wherein the cytokine receptor can comprise IL15-Rα. In some embodiments, the modified oncolytic virus can comprise exogenous nucleic acid that can code for the chemokine, wherein the chemokine can

comprise CCL5. In some embodiments, the modified oncolytic virus can further comprise an exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, LIGHT, ITAC, a fractalkine, and a functional domain or fragment or variant thereof, or any combinations thereof. In some embodiments, the modified oncolytic virus can further comprise a mutation or a deletion of a further viral gene comprising at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, N1L, A52R, and a functional domain or fragment or a variant thereof, or any combinations thereof.

[0071] One aspect provides a modified oncolytic virus that can comprise exogenous nucleic acids that can code for IL15 and IL15-Rα. One aspect provides a modified oncolytic virus that can comprise exogenous nucleic acids that can code for IL15 and CCL5. One aspect provides a modified oncolytic virus that can comprise an exogenous nucleic acid that can code for at least one of: IL15, IL15-Rα, ITAC, fractalkine, and a functional domain or fragment or variant thereof, or any combinations thereof. In some embodiments, the virus can exhibit enhanced activation and attraction of natural killer cells.

[0072] In some embodiments, the modified oncolytic virus can exhibit enhanced intratumoral and intertumoral spreading, enhanced immune evasion, enhanced tumor-specific replication, enhanced tumor-targeted delivery, compared to an otherwise identical oncolytic virus that does not comprise the modifications as disclosed herein. In some embodiments, the modified oncolytic virus can comprise a poxvirus, an adeno associated virus, an adenovirus, a reovirus, a lentivirus, a herpes simplex virus, a vesicular stomatitis virus, a mengovirus, or a myxomavir. In some embodiments, the modified oncolytic virus can comprise the poxvirus. In some embodiments, the poxvirus can be a vaccinia virus. In some embodiments, the modified oncolytic virus can be suitable for systemic delivery.

[0073] In some embodiments, the oncolytic vaccinia virus can exhibit enhanced intratumoral and intertumoral spreading, enhanced immune evasion, enhanced tumor-specific replication, enhanced tumor-targeted delivery, compared to an otherwise identical vaccinia virus that does not comprise the modifications as disclosed herein.

[0074] In some embodiments, the oncolytic vaccinia virus can be suitable for systemic delivery.

[0075] In some embodiments, the oncolytic vaccinia virus may be capable of immune evasion.

[0076] In some embodiments, the systemic delivery can comprise oral administration, parenteral administration, intranasal administration, sublingual administration, rectal administration, transdermal administration, or any combinations thereof.

[0077] In some embodiments, the parenteral delivery can comprise an intravenous injection.

[0078] In some embodiments, the oncolytic vaccinia virus can be suitable for intratumoral delivery.

[0079] In some embodiments, the oncolytic vaccinia virus can comprise an exogenous nucleic acid that codes for a viral VH1 protein.

[0080] In some embodiments, the exogenous nucleic acid can be from a genome of a poxvirus, wherein the poxvirus can be not vaccinia virus. In some embodiments, the poxvirus can comprise a betaentomopoxvirus, a yatapoxvirus, a cervidpoxvirus, a gammaentomopoxvirus, a leporipoxvirus, a suipoxvirus, a molluscipoxvirus, a crocodylidpoxvirus, a alphaentomopoxvirus, a capripoxvirus, a avipoxvirus, a parapoxvirus.

[0081] In some embodiments, the viral genome can comprise a thymidine kinase gene. In some embodiments, a thymidine kinase gene can be deleted from the viral genome.

[0082] In some embodiments, the oncolytic vaccinia virus can comprise a thymidine kinase gene from a herpes simplex virus.

[0083] Yet another aspect of the present disclosure provides a pharmaceutical composition can comprise a modified oncolytic virus as disclosed herein or an oncolytic vaccinia virus as disclosed herein. In some embodiments, the pharmaceutical composition can comprise a solubilizing agent and an excipient. In some embodiments, the excipient can comprise one or more of a buffering agent, a stabilizer, an antioxidant, a binder, a diluent, a dispersing agent, a rate controlling agent, a lubricant, a glidant, a disintegrant, a plasticizer, a preservative, or any combinations thereof. In some embodiments, the excipient can comprise di-sodium hydrogen phosphate dihydrate, sodium dihydrogen phosphate dihydrate, sodium chloride, myo-inositol, sorbitol, or any combinations thereof. In some embodiments, the pharmaceutical composition does not comprise a preservative. In some embodiments, the pharmaceutical composition can comprise one or more of a preservative, a diluent, and a carrier. In some embodiments, the pharmaceutical composition can comprise an additional active ingredient or a salt thereof. In some embodiments, the solubilizing agent can be sterile water. In some embodiments, the pharmaceutical composition can comprise an additional active ingredient, wherein the additional active ingredient can be a further oncolytic virus. Yet another aspect of the present disclosure provides a method of enhancing therapeutic effect of an oncolytic virus upon systemic delivery of the virus to a subject, comprising a systemic administration of a modified oncolytic virus as disclosed herein, an oncolytic vaccinia virus as disclosed herein, or a pharmaceutical composition as disclosed herein.

[0084] In some embodiments, the systemic administration can comprise oral administration, parenteral administration, intranasal administration, sublingual administration, rectal administration, transdermal administration, or any combinations thereof. In some embodiments, the parenteral administration can comprise intravenous injection.

[0085] Yet another aspect of the present disclosure provides a process for engineering a modified oncolytic virus can comprise: (i) obtaining a modified oncolytic virus DNA backbone vector, the modified oncolytic virus DNA backbone vector can comprise one or more modifications as disclosed herein; (ii) further modifying the modified oncolytic virus DNA vector to produce an engineered DNA vector; (iii) transfecting mammalian cells with the engineered DNA vector; (iv) culturing the mammalian cells under conditions suitable for viral replication; and (v) harvesting the viral particles.

[0086] Yet another aspect of the present disclosure provides a process for engineering an oncolytic vaccinia virus can comprise: (i) obtaining an oncolytic vaccinia virus DNA backbone vector, the oncolytic vaccinia virus DNA backbone vector can comprise one or more modifications as disclosed herein; (ii) further modifying the oncolytic vaccinia virus DNA vector to produce an engineered DNA vector; (iii) transfecting mammalian cells with the engineered DNA vector; (iv) culturing the mammalian cells under conditions suitable for viral replication; and (v) harvesting the viral particles.

[0087] Yet another aspect of the present disclosure provides a process for producing a modified oncolytic virus as disclosed herein, comprising: (i) generating a modified oncolytic virus DNA vector, the modified oncolytic virus DNA vector can comprise the modifications as disclosed herein; (ii) transfecting mammalian cells with the modified oncolytic virus DNA vector; (iii) culturing the mammalian cells under conditions suitable for viral replication; and (iv) harvesting the viral particles.

[0088] Yet another aspect of the present disclosure provides a process for producing an oncolytic vaccinia virus as disclosed herein, comprising: (i) generating an oncolytic vaccinia virus DNA vector, the oncolytic vaccinia virus DNA vector can comprise the modifications as disclosed herein; (ii) transfecting mammalian cells with the oncolytic vaccinia virus DNA vector; (iii) culturing the mammalian cells under conditions suitable for viral replication; and (iv) harvesting the viral particles. In some embodiments, the mammalian cells comprise HeLa cells, 293 cells, or Vero cells.

[0089] Yet another aspect of the present disclosure provides a kit that can comprise: a modified oncolytic virus as disclosed herein, an oncolytic vaccinia virus as disclosed herein, or a pharmaceutical composition as disclosed herein, and instruction for administering the

modified oncolytic virus, the oncolytic vaccinia virus, or a pharmaceutical composition; and instructions for administering said pharmaceutical composition to a subject to treat a disorder associated with pathological angiogenesis.

[0090] Yet another aspect of the present disclosure provides a kit, comprising: a modified oncolytic virus as disclosed herein, an oncolytic vaccinia virus as disclosed herein, or a pharmaceutical composition as disclosed herein, and instruction for administering the modified oncolytic virus, the oncolytic vaccinia virus, or a pharmaceutical composition; a container; and instructions for administering said pharmaceutical composition to a subject to treat a disorder associated with pathological angiogenesis.

[0091] Yet another aspect of the present disclosure provides a kit for treating a cancer, comprising: a modified oncolytic virus as disclosed herein, an oncolytic vaccinia virus as disclosed herein, or a pharmaceutical composition as disclosed herein, and instruction for administering the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition; and instructions for administering said pharmaceutical composition to a subject to treat a disorder associated with pathological angiogenesis.

[0092] Yet another aspect of the present disclosure provides a kit for treating a cancer, comprising: a modified oncolytic virus as disclosed herein, an oncolytic vaccinia virus as disclosed herein, or a pharmaceutical composition as disclosed herein, and instruction for administering the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition; a container; and instructions for administering said pharmaceutical composition to a subject to treat a disorder associated with pathological angiogenesis. In some embodiments, the cancer can be a solid tumor, a leukemia, or a lymphoma.

[0093] Yet another aspect of the present disclosure provides a kit for treating a tumor, comprising: a modified oncolytic virus as disclosed herein, an oncolytic vaccinia virus as disclosed herein, or a pharmaceutical composition as disclosed herein, and instruction for administering the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition; and instructions for administering said pharmaceutical composition to a subject to treat a disorder associated with pathological angiogenesis.

[0094] Yet another aspect of the present disclosure provides a kit for treating a tumor, comprising: a modified oncolytic virus as disclosed herein, an oncolytic vaccinia virus as disclosed herein, or a pharmaceutical composition as disclosed herein, and instruction for administering the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition; a container; and instructions for administering said

pharmaceutical composition to a subject to treat a disorder associated with pathological angiogenesis. In some embodiments, the tumor can be a solid tumor, a leukemia, or a lymphoma. In some embodiments, the subject can be in need of the treatment. In some embodiments, the subject can be human. In some embodiments, the instructions for administering can comprise instructions for a systemic administration. In some embodiments, the systemic administration can comprise oral administration, parenteral administration, intranasal administration, sublingual administration, rectal administration, transdermal administration, or any combinations thereof. In some embodiments, the parenteral administration can comprise intravenous injection.

[0095] Yet another aspect of the present disclosure provides a method of treating a tumor, comprising: administering to a subject a therapeutically effective amount of a modified oncolytic virus as disclosed herein, an oncolytic vaccinia virus as disclosed herein, or a pharmaceutical composition as disclosed herein.

[0096] In some embodiments, the method of treating a tumor can comprise administering to a subject a therapeutically effective amount of a modified oncolytic virus as disclosed herein, an oncolytic vaccinia virus as disclosed herein, or a pharmaceutical composition as disclosed herein, wherein the tumor can be a solid tumor, a leukemia, or a lymphoma.

[0097] Yet another aspect of the present disclosure provides a method of treating a cancer, comprising administering to a subject a therapeutically effective amount of a modified oncolytic virus as disclosed herein, an oncolytic vaccinia virus as disclosed herein, or a pharmaceutical composition as disclosed herein.

[0098] In some embodiments, the method of treating a cancer can comprise administering to a subject a therapeutically effective amount of a modified oncolytic virus as disclosed herein, an oncolytic vaccinia virus as disclosed herein, or a pharmaceutical composition as disclosed herein, wherein the cancer can be a solid tumor, a leukemia, or a lymphoma.

[0099] In some embodiments, the method of treating a cancer can comprise administering to a subject a therapeutically effective amount of a modified oncolytic virus as disclosed herein, an oncolytic vaccinia virus as disclosed herein, or a pharmaceutical composition as disclosed herein, wherein the method further can comprise administration of a further therapy.

[00100] In some embodiments, the further therapy can comprise chemotherapy, radiation, oncolytic viral therapy with an additional virus, treatment with immunomodulatory proteins, a CAR T cellular therapy (chimeric antigen receptor T cell therapy), an anti-cancer agent, or any combinations thereof.

[00101] In some embodiments, the further therapy can comprise administration of an immunomodulatory agent can comprise anti-CD33 antibody and variable region thereof, an anti-CD11b antibody and variable region thereof, a COX2 inhibitor, a cytokine, a chemokine, an anti-CTLA4 antibody or an antigen binding fragment thereof, an anti-PD-1 antibody or an antigen binding fragment thereof, an anti-PD-L1 antibody or an antigen binding fragment thereof, or a TLR agonist.

[00102] In some embodiments, the method of treating a cancer can comprise administration of the further therapy, wherein the further therapy can comprise administration of the anti-cancer agent, wherein the anti-cancer agent can be a chemotherapeutic agent. In some embodiments, the chemotherapeutic agent can be a prodrug. In some embodiments, upon administration of the prodrug in combination with the oncolytic vaccinia virus, the modified vaccinia virus, or a pharmaceutical composition can comprise the same, the prodrug can be converted to an active form. In some embodiments, the prodrug can comprise ganciclovir. In some embodiments, the method of treating a cancer can comprise administration of the further therapy, wherein the further therapy can be administered concurrently or sequentially. In some embodiments, the method of treating a cancer can comprise sequential administration of the further therapy, wherein the further therapy can be administered prior to administering the modified oncolytic virus as disclosed herein, the oncolytic vaccinia virus as disclosed herein, or the pharmaceutical composition as disclosed herein. In some embodiments, the method of treating a cancer can comprise sequential administration of the further therapy, wherein the further therapy can be administered after administering the modified oncolytic virus as disclosed herein, the oncolytic vaccinia virus as disclosed herein, or the pharmaceutical composition as disclosed herein.

[00103] Yet another aspect of the present disclosure provides a method of producing a toxic effect in a cancer cell, comprising: administering to a cancer cell a therapeutically effective amount of a modified oncolytic virus as disclosed herein, an oncolytic vaccinia virus as disclosed herein, or a pharmaceutical composition as disclosed herein. In some embodiments, the cancer cell can be present in a subject. In some embodiments, the subject can be in need of the method that producing the toxic effect in the cancer cell.

[00104] Yet another aspect of the present disclosure provides a method of treating a subject, comprising: producing a toxic effect in a cancer cell that can be present in the subject by administering to the cancer cell a therapeutically effective amount of a modified oncolytic virus as disclosed herein, an oncolytic vaccinia virus as disclosed herein, or a pharmaceutical

composition as disclosed herein. In some embodiments, the subject can be in need of the treatment.

[00105] Yet another aspect of the present disclosure provides a method of treating cancer in a subject, comprising, infecting a cancer cell of the subject by administration of a therapeutically effective amount of a modified oncolytic virus as disclosed herein, an oncolytic vaccinia virus as disclosed herein, or a pharmaceutical composition as disclosed herein, wherein the administration can be a systemic administration.

[00106] Yet another aspect of the present disclosure provides a method of treating cancer in a subject, comprising administering cells infected with a modified oncolytic virus as disclosed herein or an oncolytic vaccinia virus as disclosed herein.

[00107] In some embodiments, the cancer can comprise melanoma, hepatocellular carcinoma, breast cancer, lung cancer, peritoneal cancer, prostate cancer, bladder cancer, ovarian cancer, leukemia, lymphoma, renal carcinoma, pancreatic cancer, epithelial carcinoma, gastric cancer, colon carcinoma, duodenal cancer, pancreatic adenocarcinoma, mesotheliorna, glioblastoma multiforrne, astrocytoma, multiple myeloma, prostate carcinoma, hepatocellular carcinoma, cholangiosarcoma, pancreatic adenocarcinoma, head and neck squamous cell carcinoma, colorectal cancer, intestinal-type gastric adenocarcinoma, cervical squamous-cell carcinoma, osteosarcoma, epithelial ovarian carcinoma, acute lymphoblastic lymphoma, myeloproliferative neoplasms, or sarcoma.

[00108] In some embodiments, the cancer cell can be present in an organ of the subject selected from the group consisting of: the bladder, blood, bone, bone marrow, brain, breast, colon, esophagus, gastrointestine, gum, head, kidney, liver, lung, nasopharynx, neck, ovary, prostate, skin, stomach, testis, tongue, or uterus. In some embodiments, the cancer can be metastatic.

[00109] Yet another aspect of the present disclosure provides a method of treating a cancer in a subject, comprising: administering a modified oncolytic virus as disclosed herein or pharmaceutical composition can comprise the same in combination with a chemotherapeutic prodrug.

[00110] Yet another aspect of the present disclosure provides a method of treating a cancer in a subject, comprising: administering an oncolytic vaccinia virus as disclosed herein or a pharmaceutical composition can comprise the same in combination with a chemotherapeutic prodrug.

[00111] Yet another aspect of the present disclosure provides a method of treating a cancer in a subject, comprising: (i) administering a modified oncolytic virus as disclosed herein or a

pharmaceutical compositions can comprise the same; (ii) assaying a viral titer in a first and a second biological sample isolated from the subject, wherein the first biological sample can comprise a cancer cell and the second biological sample can comprise a non-cancer cell; and (iii) administering a chemotherapeutic prodrug if the viral titer can be equal to or higher in the second sample than the first sample, wherein administration of the chemotherapeutic prodrug results in inhibition of replication of the modified oncolytic virus in the subject.

[00112] Yet another aspect of the present disclosure provides a method of treating a cancer in a subject, comprising: (i) administering an oncolytic vaccinia virus as disclosed herein or a pharmaceutical compositions can comprise the same; (ii) assaying a viral titer in a first and a second biological sample isolated from the subject, wherein the first biological sample can comprise a cancer cell and the second biological sample can comprise a non-cancer cell; and (iii) administering a chemotherapeutic prodrug if the viral titer can be equal to or higher in the second sample than the first sample, wherein administration of the chemotherapeutic prodrug results in inhibition of replication of the oncolytic vaccinia virus in the subject.

[00113] In some embodiments, the method of treating a cancer in a subject can increase efficacy of oncolytic virus based cancer therapy. In some embodiments, the method of treating a cancer in a subject, can comprise the administration of the modified oncolytic virus as disclosed herein, the oncolytic vaccinia virus as disclosed herein, or the pharmaceutical composition as disclosed herein, wherein the modified oncolytic virus, the oncolytic vaccinia virus, or a pharmaceutical composition can be administered at a dosage that can comprise about 10^6 PFU/mL to about 10^{10} PFU/mL of the oncolytic vaccinia virus.

[00114] In some embodiments, the method of treating a cancer in a subject can comprise the administration of the modified oncolytic virus as disclosed herein, the oncolytic vaccinia virus as disclosed herein, or the pharmaceutical composition as disclosed herein, wherein the modified oncolytic virus, the oncolytic vaccinia virus, or a pharmaceutical composition can be administered at a dosage that can comprise about 5×10^9 PFU/mL of the oncolytic vaccinia virus.

[00115] In some embodiments, the method of treating a cancer in a subject can comprise the administration of the modified oncolytic virus as disclosed herein, the oncolytic vaccinia virus as disclosed herein, or the pharmaceutical composition as disclosed herein, wherein the modified oncolytic virus, the oncolytic vaccinia virus, or a pharmaceutical composition can be administered, independently, in an initial dose for a first period of time, an intermediate dose for a second period of time, and a high dose for a third period of time. In some embodiments, the method of treating a cancer in a subject can comprise administration of the

initial, the intermediate, and the high dose, independently, wherein the initial dose can be lower than the intermediate dose and the intermediate dose can be lower than the high dose.

[00116] In some embodiments, wherein the first, second, and third periods of time can be each from about 1 week to about 3 weeks. In some embodiments, the method of treating a cancer in a subject can comprise administering the modified oncolytic virus as disclosed herein, the oncolytic vaccinia virus as disclosed herein, or the pharmaceutical composition as disclosed herein, wherein the modified oncolytic virus, the oncolytic vaccinia virus, and a pharmaceutical composition independently can comprise a liquid dosage form that can be administered at a volume of about 1 mL to about 5 mL, about 5 mL to 10 mL, about 15 mL to about 20 mL, about 25 mL to about 30 mL, about 30 mL to about 50 mL, about 50 mL to about 100 mL, about 100 mL to 150 mL, about 150 mL to about 200 mL, about 200 mL to about 250 mL, about 250 mL to about 300 mL, about 300 mL to about 350 mL, about 350 mL to about 400 mL, about 400 mL to about 450 mL, about 450 mL to 500 mL, about 500 mL to 750 mL, or about 750 mL to 1000 mL.

[00117] In some embodiments, the method of treating a cancer in a subject can comprise administering the modified oncolytic virus as disclosed herein, the oncolytic vaccinia virus as disclosed herein, or the pharmaceutical composition as disclosed herein, wherein the modified oncolytic virus, the oncolytic vaccinia virus, or a pharmaceutical composition can be administered in a liquid dosage form, a solid dosage form, an inhalable dosage form, an intranasal dosage form, a liposomal formulation, a dosage form can comprise nanoparticles, a dosage form can comprise microparticles, a polymeric dosage form, or any combinations thereof.

[00118] In some embodiments, the method of treating a cancer in a subject can comprise administering the modified oncolytic virus as disclosed herein, the oncolytic vaccinia virus as disclosed herein, or the pharmaceutical composition as disclosed herein, wherein the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition can be administered for a duration of about 1 week, about 2 week, about 3 weeks, about 4 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 9 weeks, about 10, weeks, or about 12 weeks.

[00119] In some embodiments, the method of treating a cancer in a subject can comprise administering the modified oncolytic virus as disclosed herein, the oncolytic vaccinia virus as disclosed herein, or a pharmaceutical composition as disclosed herein, wherein the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition can be

administered once daily, twice daily, once every week, once every two weeks, or once every three weeks.

[00120] In some embodiments, the method of treating a cancer in a subject can comprise administering the modified oncolytic virus as disclosed herein, the oncolytic vaccinia virus as disclosed herein, or a pharmaceutical composition as disclosed herein, wherein the modified oncolytic virus, the oncolytic vaccinia virus, or a pharmaceutical composition can be administered intravenously, intraperitoneally, or by an intratumoral injection.

[00121] In some embodiments, the method of treating a cancer in a subject can comprise administering the modified oncolytic virus as disclosed herein, the oncolytic vaccinia virus as disclosed herein, or a pharmaceutical composition as disclosed herein, wherein the modified oncolytic virus, the oncolytic vaccinia virus, or a pharmaceutical composition can be administered as a bolus injection or a slow infusion.

[00122] In some embodiments, the method of treating a cancer in a subject can comprise administering the modified oncolytic virus as disclosed herein, the oncolytic vaccinia virus as disclosed herein, or a pharmaceutical composition as disclosed herein, wherein the administration of the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition results in a first peak viral load after about 1 hour to about 3 days and a second peak viral load after about 3 days to about 10 days from administration of a first dose.

[00123] In some embodiments, the method of treating a cancer in a subject can comprise administration of the further therapy, wherein the further therapy can be administered for a duration of about 1 week, about 2 week, about 3 weeks, about 4 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 9 weeks, about 10, weeks, or about 12 weeks. In some embodiments, the method of treating a cancer in a subject can comprise administration of the further therapy, wherein the further therapy can be administered once daily, once every week, once every two weeks, or once every three weeks. In some embodiments, the method of treating a cancer in a subject can comprise administration of the further therapy, wherein the further therapy can be administered in a liquid dosage form, a solid dosage form, an inhalable dosage form, an intranasal dosage form, a liposomal formulation, a dosage form can comprise nanoparticles, a dosage form can comprise microparticles, a polymeric dosage form, or any combinations thereof. In some embodiments, the method of treating a cancer in a subject can comprise administration of the further therapy, wherein the further therapy can be administered orally, intravenously, by an intratumoral injection, or by radiation. In some embodiments, the method of treating a cancer in a subject can comprise the administration of

the modified oncolytic virus as disclosed herein, the oncolytic vaccinia virus as disclosed herein, or the pharmaceutical composition as disclosed herein to a subject in need thereof, wherein the subject can be human. In some embodiments, the method of treating a cancer in a subject can comprise the administration of the modified oncolytic virus as disclosed herein, the oncolytic vaccinia virus as disclosed herein, or the pharmaceutical composition as disclosed herein to the subject in need thereof, wherein prior to administration of the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition the subject has been diagnosed with a cancer. In some embodiments, the method of treating a subject can comprise the administration of the modified oncolytic virus as disclosed herein, the oncolytic vaccinia virus as disclosed herein, or the pharmaceutical composition as disclosed herein to the subject in need thereof, wherein prior to administration of the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition the subject has been diagnosed with a tumor. In some embodiments, the method of treating a cancer in a subject can comprise the administration of the modified oncolytic virus as disclosed herein, the oncolytic vaccinia virus as disclosed herein, or a pharmaceutical composition as disclosed herein to the subject in need thereof in combination with the further therapy, wherein prior to administration of the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition or the further therapy the subject has been diagnosed with a cancer or a tumor. In some embodiments, the method of treating a subject can comprise the administration of the modified oncolytic virus as disclosed herein, the oncolytic vaccinia virus as disclosed herein, or a pharmaceutical composition as disclosed herein to the subject in need thereof in combination with the further therapy, wherein prior to administration of the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition or the further therapy the subject has been diagnosed with a tumor.

BRIEF DESCRIPTION OF THE DRAWINGS

[00124] The novel features of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of this disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of this disclosure are utilized, and the accompanying drawings of which.

[00125] **FIG. 1** shows the effect of an exemplary modified vaccinia virus of this disclosure, containing an exemplary mutation in B5R gene, on tumor growth. The graph quantifies the

relative tumor size over the days post implantation in BALB/c mice bearing subcutaneous RENCA tumors, which were treated with a single dose of a control vaccinia virus injected intravenously (Control (IV)), a modified WR.TK-GMCSF vaccinia virus, where TK gene was deleted and an exogenous nucleic acid encoding GMCSF was added, injected intratumorally (WR.TK-GMCSF (IT)), or the exemplary modified vaccinia virus (WR.B5Rmut.TK-) where TK gene was deleted and a mutation was introduced in B5R gene, injected intravenously (WR.B5Rmut.TK-(IV)) or intratumorally (WR.B5Rmut.TK-(IT)).

[00126] **FIGS. 2A-2B** show the effects of an exemplary mutation in B5R gene on the delivery and spreading of an exemplary modified vaccinia virus (WR.B5RmutTK- virus) (referred to as B5R in FIG. 2A and 2B), in tumors. **FIG. 2A** and **FIG. 2B** are graphs showing the quantification of the viral genomes per gram of tumor collected from non-immunized and immunized C57/BL6 mice bearing B16 tumors, respectively. The mice treated with a single injection of the WR.B5RmutTK- virus were compared with mice treated with another modified vaccinia virus WR.TK-A34R K151E, where TK was deleted and the viral A34R gene was mutated with an amino acid change, K151 to E (referred to as WI in **FIG. 2A and 2B**).

[00127] **FIGS. 3A-3B** show the effect of an exemplary mutation in B5R gene on the replication of an exemplary modified vaccinia virus (WR.B5RmutTK- virus) (referred to as B5R in FIG. 3A and 3B), in tumors. **FIG. 3A** and **FIG. 3B** are graphs quantifying the plaque forming capability of the viruses, WR.TK-A34R K151E, where TK was deleted and the viral A34R gene was mutated with an amino acid change, K151 to E (referred to as WI in **FIG. 3A and 3B**) and B5R, per gram of collected tumor (PFU/g) in an *in vitro* plaque assay.

[00128] **FIG. 4** shows the effect of exemplary modified vaccinia viruses having an exogenous nucleic acid that codes for CXCR4 or CCR5 on viral expression of luciferase in tumors. The graph quantifies the luciferase-mediated photon intensity in the tumors by bioluminescence imaging in mice bearing orthotopic (mammary fat pad) 4T1 tumors. The mice were treated with a single dose of a modified vaccinia virus where TK gene was deleted and an exogenous nucleic acid encoding mouse CCR5 was added (mCCR5/TK- virus), a modified vaccinia virus where TK gene was deleted and an exemplary exogenous nucleic acid encoding mouse CXCR4 was added (mCXCR4/TK- virus), or a TK- virus, where TK gene was deleted. All viruses were engineered to express luciferase.

[00129] **FIGS. 5A-5B** show the effects of exemplary modified vaccinia viruses containing an exogenous nucleic acid that codes for CXCR4 or CCR5 on tumor growth and animal survival. **FIG. 5A** includes a graph showing the relative tumor size over the days post

implantation in mice bearing orthotopic (mammary fat pad) 4T1 tumors, which were treated with a single dose a modified vaccinia virus where TK gene was deleted and an exogenous nucleic acid encoding mouse CCR5 was added (CCR5 - virus), a modified vaccinia virus where TK gene was deleted and an exemplary exogenous nucleic acid encoding mouse CXCR4 was added (CXCR4 - virus), or a TK- virus, where TK gene was deleted. **FIG. 5A** also includes a chart listing statistic difference for each pair of comparison. **FIG. 5B** shows the survival curves of these mice.

[00130] **FIGS. 6A-6B** show the effects of expression of an exemplary exogenous nucleic acid coding for CXCR4 or CCR5 on viral expression of luciferase in tumors. **FIG. 6A** is a graph quantifying the luciferase-mediated photon intensity in the tumors during bioluminescence imaging in C57/BL6 mice bearing B16 tumors. The mice were treated with a single dose of a modified vaccinia virus where TK gene was deleted and an exogenous nucleic acid encoding mouse CCR5 was added (mCCR5/TK- virus), a modified vaccinia virus where TK gene was deleted and an exemplary exogenous nucleic acid encoding mouse CXCR4 was added (mCXCR4/TK- virus), or a TK- virus, where TK gene was deleted. **FIG. 6B** is a representative picture showing the luminescence signal from the bioluminescence imaging of the mice treated with the viruses on day 1 post injection. All viruses were engineered to express luciferase.

[00131] **FIG. 7** shows the effects of expression of an exemplary exogenous nucleic acid coding for CXCR4 or CCR5 on tumor growth. The graph quantifies the tumor volume in C57/BL6 mice bearing B16 tumors subcutaneously over the days post vaccinia virus treatment. The mice were treated with a single dose of a modified vaccinia virus where TK gene was deleted and an exogenous nucleic acid encoding mouse CCR5 was added (mCCR5/TK- virus), a modified vaccinia virus where TK gene was deleted and an exemplary exogenous nucleic acid encoding mouse CXCR4 was added (mCXCR4/TK- virus), or a TK- virus, where TK gene was deleted, or PBS.

[00132] **FIGS. 8A-8D** show the effects of expression of an exemplary exogenous nucleic acid coding for CCR5 on viral replication in tumors. **FIG. 8A** and **FIG. 8B** are graphs showing quantification of plaque-forming capability of three different viruses (mCCR5/TK- virus, mCXCR4/TK- virus, and TK- virus) in tumor cell line 4T1, starting at multiplicity of infection of 0.1 (4T1-MOI0.1) and 1 (4T1-MOI1), respectively. **FIG. 8C** and **FIG. 8D** are similar graphs showing quantification of plaque-forming capability of three different viruses (mCCR5/TK- virus, mCXCR4/TK- virus, and TK- virus) in tumor cell line B16, starting at MOI of 0.1 (B16-MOI0.1) and 1 (B16-MOI1), respectively.

[00133] **FIGS. 9A-9C** show the effects of B cells depletion on the increased viral expression of luciferase in tumors treated with an exemplary modified vaccinia virus having an exemplary exogenous nucleic acid that codes for CXCR4. **FIG. 9A**, **FIG. 9B**, and **FIG. 9C** are graphs quantifying the luciferase-mediated photon intensity in tumors measured at 24 h, 48h, and 72 hours post viral injection, respectively. Balb/C mice are wild type mice having B cells, while JH mice are B cell-depleted mice. The mice were injected with a single dose of a modified vaccinia virus where TK gene was deleted and an exogenous nucleic acid encoding mouse CCR5 was added (mCCR5/TK- virus), a modified vaccinia virus where TK gene was deleted and an exemplary exogenous nucleic acid encoding mouse CXCR4 was added (mCXCR4/TK- virus), or a TK- virus, where TK gene was deleted.

[00134] **FIGS. 10A-10C** show the effects of an exemplary modified vaccinia virus having an exemplary exogenous nucleic acid that codes for CXCR4 on B cell accumulation in tumors. **FIG. 10A**, **FIG. 10B**, and **FIG. 10C**are graphs quantifying the percentage of B cells in the lymphocytes collected from spleen, lymph node (LN), and tumor, respectively, in BALB/c mice bearing subcutaneous 4T1 tumor. The mice were treated with a single dose of a modified vaccinia virus where TK gene was deleted and an exemplary exogenous nucleic acid encoding mouse CXCR4 was added (mCXCR4/TK- virus), a TK- virus, where TK gene was deleted, or PBS.

[00135] **FIGS. 11A-11B** show the effects of an exemplary modified vaccinia virus having an exemplary exogenous nucleic acid coding for CXCR4 on T cell activation. **FIG. 11A** and **FIG. 11B** are graphs quantifying IFNgamma release by T cells in response to tumor cell 4T1 lysate and inactivated vaccinia virus (VV) mixed with tumor cell 4T1 lysate, respectively. The T cells were collected from mice bearing 4T1 tumors treated with a single dose of a modified vaccinia virus where TK gene was deleted and an exemplary exogenous nucleic acid encoding mouse CXCR4 was added (mCXCR4/TK- virus), or a TK- virus, where TK gene was deleted, or PBS. T cells were recovered from spleens after 14 days.

[00136] **FIGS. 12A-12B** show the effects of exemplary modified vaccinia viruses having an exemplary exogenous nucleic acid that encodes hyaluronidase PH-20 or matrixmetalloprotease MMP8, on viral expression of luciferase in tumors. **FIG. 12A** is a representative picture showing the luminescence signal in three BALB/c mice bearing RENCA tumors, which were treated with a single dose of an unmodified vaccinia virus (WR), a modified vaccinia virus having an exemplary exogenous nucleic acid that codes for MMP8 (WR MMP8), or a modified vaccinia virus having an exemplary exogenous nucleic

acid that codes for PH-20 (WR PH20 virus), respectively. **FIG. 12B** is a graph quantifying the photon intensity in the mice on day 2 after viral injection.

[00137] **FIGS. 13A-13C** show the therapeutic effects of an exemplary modified vaccinia virus having an exemplary exogenous nucleic acid that encodes hyaluronidase PH-20. **FIG. 13A** is a graph quantifying the luciferase-mediated photon intensity over the days after the viral injection in BALB/c mice bearing 4T1 tumors treated with either a modified vaccinia virus where the TK gene was deleted (TK-), or a modified vaccinia virus where the TK gene was deleted and which has an exemplary exogenous nucleic acid that codes for PH-20 TK-PH20DCK virus. **FIG. 13B** is a graph quantifying the relative tumor volume over the days after the viral injection in mice treated with a PBS, gemcitabine alone, TK- PH20 DCK virus alone, TK- virus alone, TK- virus combined with gemcitabine, or TK-PH20 DCK virus combined with gemcitabine, and **FIG. 13C** shows the survival curves for these mice.

[00138] **FIG. 14** shows the therapeutic effects 45 days after the viral injection of an exemplary modified vaccinia virus having an exemplary exogenous nucleic acid that encodes, in various combinations or individually, hyaluronidase PH-20, and GMCSF. The graph quantifies the tumor volume in the mice treated with a control sham, a modified vaccinia virus where the TK gene was deleted and which has an exemplary exogenous nucleic acid that codes for PH-20 (WR. TK-PH20 virus), where the PH-20 was with or without a GPI-anchor, a modified vaccinia virus where the TK gene was deleted and which has an exemplary exogenous nucleic acid that codes for GMCSF (WR.TK-GMCSF), either intratumorally (IT) or intravenously (IV), 45 days after the viral injection.

[00139] **FIG. 15** shows the effects of an exemplary modified vaccinia virus having a modified VH1 gene on tumor growth. The graph quantifies the relative tumor volume in the mice treated with a control sham intravenously (IV), a modified vaccinia virus where the TK gene was deleted and which has an exemplary exogenous nucleic acid that codes for GMCSF (WR.TK-GMCSF) intratumorally (IT), or a modified vaccinia virus where the TK gene was deleted and which has a modified VH1 gene (WR.TK-.VH1mut) intratumorally, over the days after the tumor implantation.

[00140] **FIG. 16** shows the effect of deletion of A52R and insertion of an exogenous nucleic acid encoding mouse CXCR4 on the delivery of modified vaccinia virus to tumors. BALB/c mice bearing RENCA tumors subcutaneously were treated with a single intravenous injection (1×10^7 PFU) of one of the four modified viruses. Tumors were harvested 24 hours later, and the number of viral genomes per milligram of tissue quantified by qPCR.

[00141] **FIG. 17** shows the effect of a modified vaccinia virus having a deletion of A52R and an insertion of an exogenous nucleic acid encoding mouse CXCR4 on tumor growth. BALB/c mice bearing RENCA tumors subcutaneously were treated with intravenous injections (1×10^7 PFU) of one of the same four modified viruses on day 1 and 4, and tumor volume was monitored.

[00142] **FIGS. 18A-18D** show that modified vaccinia viruses having a deletion of TK and an insertion of an exogenous nucleic acid encoding either HysA or MMP8 infect cultures of MC38 cancer cells, replicate, spread, and prevent expansion of the cancer cells. MC38 cells were seeded in a 96-well plate at a density of 5×10^3 cells per well. The following day, cells were infected with different GFP-expressing viruses at a MOI of 0.002 and imaged using IncuCyte to measure phase confluence (**FIG. 18A**), GFP area (**FIG. 18B**), GFP intensity (**FIG. 18C**), and virus infectivity (**FIG. 18D**).

[00143] **FIGS. 19A-19D** show that modified vaccinia viruses having a deletion of TK and an insertion of an exogenous nucleic acid encoding either HysA or MMP8 infect cultures of HCT116 cancer cells, replicate, spread, and prevent expansion of the cancer cells. HCT116 cells were seeded in a 96-well plate at a density of 5×10^3 cells per well. The following day, cells were infected with different GFP-expressing viruses at a MOI of 0.002 and imaged using IncuCyte to measure phase confluence (**FIG. 19A**), GFP area (**FIG. 19B**), GFP intensity (**FIG. 19C**), and virus infectivity (**FIG. 19D**).

[00144] **FIG. 20** shows the effect of expression of HysA on delivery of modified vaccina virus to tumors. The viruses used were: (i) the TK-HysA modified virus (ii) the TK-MMP8 modified virus, (iii) the TK-PH20-expressing modified virus, (iv) the TK- modified virus without an exogenous enzyme added, and (v) vehicle formulated buffer (VFB). BALB/c mice bearing RENCA tumors subcutaneously were treated with a single intravenous injection (1×10^7 PFU) of one of the viruses. Tumors were harvested 24 hours later, and the number of viral genomes per milligram of tissue quantified by qPCR.

[00145] **FIG. 21** shows the effect of a modified vaccinia virus having a deletion of TK- and an insertion of an exogenous nucleic acid encoding HysA, MMP8, or PH20 (labeled in **FIG. 21** as SPAM1) on tumor growth. BALB/c mice bearing RENCA tumors subcutaneously were treated with one intratumoral injection (1×10^7 PFU) of the TK-HysA, TK-MMP8, TK-PH20, or TK- modified virus, and tumor volume was monitored.

[00146] **FIGS. 22A-22D** shows that modified vaccinia viruses having a deletion of TK or A52R and an insertion of an exogenous nucleic acid encoding HysA infect cultures of MC38 and LLC cancer cells, replicate, spread, and prevent expansion of the cancer cells. LLC or

MC38 cells were seeded in a 96-well plate at a density of 5 x 10^3 cells per well. The following day, cells were infected with different GFP-expressing viruses at a MOI of 1 and imaged using IncuCyte to measure phase confluence (**FIG. 22A** and **FIG. 22C**) and GFP area (**FIG. 22B** and **FIG. 22D**).

[00147] **FIG. 23** shows that exemplary vaccinia viruses with exemplary mutations to promote NK cell activity show enhanced therapeutic effects in murine tumor models. BALB/c mice bearing RENCA tumors subcutaneously were treated with a single intratumoral injection (1 x 10^8 PFU) of K7R- IL15+ CCL5+, TK-LIGHT+, or TK-GMCSF+. Tumor volume was quantified on day 34 after treatment.

[00148] **FIG. 24** shows that exemplary vaccinia viruses with exemplary mutations to promote NK cell activity show enhanced therapeutic effects in murine tumor models. In another experiment, BALB/c mice bearing RENCA tumors subcutaneously were treated with a single intratumoral injection (1 x 10^7 PFU) of TK-LIGHT+, TK-GMCSF+, TK-ITAC+ fractalkine (CX3CL1)+, A52R- IL15+ IL15A+, TK- ITAC+ LIGHT+ A52R- IL15+IL15A+, or a vehicle formulated buffer, termed VFB, and tumor volume was monitored over time.

[00149] **FIGS. 25A-25B** shows that an exemplary vaccinia virus with exemplary exogenous nucleic acids encoding a chemokine receptor and an extracellular-matrix-degrading enzyme show spread between and killing of cancer cells. LLC cells were seeded in a 96-well plate at a density of 5 x 10^3 cells per well. The next day, cells were infected with different viruses at a MOI of 1 and imaged using IncuCyte to measure phase confluence (**FIG. 25A**) and GFP area (**FIG. 25B**).

[00150] **FIGS. 26A-26D** show that an exemplary modified high EEV-producing vaccinia virus having an exemplary deletion of SCR1 shows spread between and killing of cancer cells. A modified IHDJ virus was developed wherein the neutralizing antibody binding site on the surface of the EEV (B5R SCR1) was deleted (IHDJ-B5RcoΔSCR1). MC38 or HCT116 cells were seeded in a 96-well plate at a density of 5 x 10^3 cells per well. The following day, cells were infected with different viruses at a MOI of 0.002 and imaged using IncuCyte to measure phase confluence, as shown in **FIG. 26A** (MC38 cells) and **FIG. 26B** (HCT116 cells). Results of a plaque assay demonstrating the ability of the IHDJ and IHDJ-B5RcoΔSCR1 viruses to spread between and kill cancer cells is shown in **FIG. 26C**. Results of a comet tail assay demonstrating the ability of the WR IHDJ and IHDJ-B5RcoΔSCR1 viruses to spread between and kill cancer cells is shown in **FIG. 26D**.

DETAILED DESCRIPTION

[00151] While preferred embodiments of this disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from this disclosure. It should be understood that various alternatives to the embodiments of this disclosure described herein may be employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

Certain Definitions

[00152] The terminology used herein is for the purpose of describing particular cases only and is not intended to be limiting. As used herein, the singular forms "a", "an" and "the" can include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms "contains," "containing," "including", "includes," "having," "has", "with", or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term "comprising."

[00153] The term "about" or "approximately" can mean within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *e.g.*, the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the given value. Where particular values are described in the application and claims, unless otherwise stated the term "about" should be assumed to mean an acceptable error range for the particular value, such as ±10% of the value modified by the term "about".

[00154] The terms "individual," "patient," or "subject" can be used interchangeably. None of the terms require or are limited to situation characterized by the supervision (*e.g.* constant or intermittent) of a health care worker (*e.g.* a doctor, a registered nurse, a nurse practitioner, a physician's assistant, an orderly, or a hospice worker). In some embodiments, patients, subjects, or individuals can be under the supervision of a health care worker.

[00155] The terms "heterologous nucleic acid sequence," or "exogenous nucleic acid sequence," or "transgenes," as used herein, in relation to a specific virus can refer to a nucleic acid sequence that originates from a source other than the specified virus.

[00156] The term "mutation," as used herein, can refer to a deletion, an insertion of a heterologous nucleic acid, an inversion or a substitution, including an open reading frame ablating mutations as commonly understood in the art.

[00157] The term "gene," as used herein, can refer to a segment of nucleic acid that encodes an individual protein or RNA (also referred to as a "coding sequence" or "coding region"), optionally together with associated regulatory regions such as promoters, operators, terminators and the like, which may be located upstream or downstream of the coding sequence.

[00158] The terms "mutant virus" and "modified virus," as used interchangeably herein, can refer to a virus comprising one or more mutations in its genome, including but not limited to deletions, insertions of heterologous nucleic acids, inversions, substitutions or combinations thereof.

[00159] The term "naturally-occurring," as used herein with reference to a virus, can indicate that the virus can be found in nature, *i.e.*, it can be isolated from a source in nature and has not been intentionally modified.

[00160] The terms "inhibiting," "reducing" or "prevention," or any variation of these terms, referred to herein, can include any measurable decrease or complete inhibition to achieve a desired result.

[00161] A "promoter," as used herein, can be a control sequence that is a region of a nucleic acid sequence at which initiation and rate of transcription are controlled. In certain embodiments, a promoter may contain genetic elements at which regulatory proteins and molecules may bind such as RNA polymerase and other transcription factors. The terms "operatively positioned," "operatively linked," "under control" and "under transcriptional control" can mean that a promoter is in a correct functional location and/or orientation in relation to a nucleic acid sequence to control transcriptional initiation and/or expression of that sequence. In certain embodiments, a promoter may or may not be used in conjunction with an "enhancer," which refers to a cis-acting regulatory sequence involved in the transcriptional activation of a nucleic acid sequence.

[00162] The term "homology," as used herein, may be to calculations of "homology" or "percent homology" between two or more nucleotide or amino acid sequences that can be determined by aligning the sequences for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first sequence). The nucleotides at corresponding positions may then be compared, and the percent identity between the two sequences may be a function of the number of identical positions shared by the sequences (*i.e.*, % homology = # of

identical positions/total # of positions x 100). For example, a position in the first sequence may be occupied by the same nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent homology between the two sequences may be a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. In some embodiments, the length of a sequence aligned for comparison purposes may be at least about: 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 95%, of the length of the reference sequence. A BLAST® search may determine homology between two sequences. The homology can be between the entire lengths of two sequences or between fractions of the entire lengths of two sequences. The two sequences can be genes, nucleotides sequences, protein sequences, peptide sequences, amino acid sequences, or fragments thereof. The actual comparison of the two sequences can be accomplished by well-known methods, for example, using a mathematical algorithm. A non-limiting example of such a mathematical algorithm may be described in Karlin, S. and Altschul, S., Proc. Natl. Acad. Sci. USA, 90-5873-5877 (1993). Such an algorithm may be incorporated into the NBLAST and XBLAST programs (version 2.0), as described in Altschul, S. et al., Nucleic Acids Res., 25:3389-3402 (1997). When utilizing BLAST and Gapped BLAST programs, any relevant parameters of the respective programs (*e.g.*, NBLAST) can be used. For example, parameters for sequence comparison can be set at score= 100, word length= 12, or can be varied (*e.g.* , W=5 or W=20). Other examples include the algorithm of Myers and Miller, CABIOS (1989), ADVANCE, ADAM, BLAT, and FASTA. In another embodiment, the percent identity between two amino acid sequences can be accomplished using, for example, the GAP program in the GCG software package (Accelrys, Cambridge, UK).

[00163] The term “subject” can refer to an animal, including, but not limited to, a primate (*e.g.*, human), cow, sheep, goat, horse, dog, cat, rabbit, rat, or mouse. The terms “subject” and “patient” are used interchangeably herein in reference, for example, to a mammalian subject, such as a human subject.

[00164] The terms “treat,” “treating,” and “treatment” can be meant to include alleviating or abrogating a disorder, disease, or condition; or one or more of the symptoms associated with the disorder, disease, or condition; or alleviating or eradicating the cause(s) of the disorder, disease, or condition itself. Desirable effects of treatment can include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishing any direct or indirect pathological consequences of the disease, preventing metastasis,

decreasing the rate of disease progression, amelioration or palliation of the disease state and remission or improved prognosis.

[00165] The term "therapeutically effective amount" can refer to the amount of a compound that, when administered, can be sufficient to prevent development of, or alleviate to some extent, one or more of the symptoms of the disorder, disease, or condition being treated. The term "therapeutically effective amount" can also refer to the amount of a compound that is sufficient to elicit the biological or medical response of a cell, tissue, system, animal, or human that is being sought by a researcher, veterinarian, medical doctor, or clinician.

[00166] The term "pharmaceutically acceptable carrier," "pharmaceutically acceptable excipient," "physiologically acceptable carrier," or "physiologically acceptable excipient" can refer to a pharmaceutically-acceptable material, composition, or vehicle, such as a liquid or solid filler, diluent, excipient, solvent, or encapsulating material. A component can be "pharmaceutically acceptable" in the sense of being compatible with the other ingredients of a pharmaceutical formulation. It can also be suitable for use in contact with the tissue or organ of humans and animals without excessive toxicity, irritation, allergic response, immunogenicity, or other problems or complications, commensurate with a reasonable benefit/risk ratio. S*ee, Remington: The Science and Practice of Pharmacy*, 21st Edition; Lippincott Williams & Wilkins: Philadelphia, PA, 2005; *Handbook of Pharmaceutical Excipients*, 5th Edition; Rowe et al., Eds., The Pharmaceutical Press and the American Pharmaceutical Association: 2005; and *Handbook of Pharmaceutical Additives*, 3rd Edition; Ash and Ash Eds., Gower Publishing Company: 2007; *Pharmaceutical Preformulation and Formulation*, Gibson Ed., CRC Press LLC: Boca Raton, FL, 2004).

[00167] The term "pharmaceutical composition" can refer to a mixture of a compound disclosed herein with other chemical components, such as diluents or carriers. The pharmaceutical composition can facilitate administration of the compound to an organism. Multiple techniques of administering a compound exist in the art including, but not limited to, oral, injection, aerosol, parenteral, and topical administration. Pharmaceutical compositions can also be obtained by reacting compounds with inorganic or organic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like.

[00168] An "anti-cancer agent," as used herein, can refer to an agent or therapy that is capable of negatively affecting cancer in a subject, for example, by killing cancer cells, inducing apoptosis in cancer cells, reducing the growth rate of cancer cells, reducing the incidence or number of metastases, reducing tumor size, inhibiting tumor growth,

reducing the blood supply to a tumor or cancer cells, promoting an immune response against cancer cells or a tumor, preventing or inhibiting the progression of cancer, or increasing the lifespan of a subject with cancer. Non-limiting examples of anti-cancer agents can include biological agents (biotherapy), chemotherapy agents, and radiotherapy agents.

[00169] The term "oncolytic," as used herein, can refer to killing of cancer or tumor cells by an agent, such as an oncolytic pox virus, such as an oncolytic vaccinia virus, *e.g.*, through the direct lysis of said cells, by stimulating immune response towards said cells, apoptosis, expression of toxic proteins, autophagy and shut-down of protein synthesis, induction of anti-tumoral immunity, or any combinations thereof. The direct lysis of the cancer or tumor cells infected by the agent, such as an oncolytic vaccinia virus, can be a result of replication of the virus within said cells. In certain examples, the term "oncolytic," can refer to killing of cancer or tumor cells without lysis of said cells.

[00170] The term "oncolytic virus" as used herein can refer to a virus that preferentially infects and kills tumor cells. Under certain non-limiting circumstances, it is understood that oncolytic viruses can promote anti-tumor responses through dual mechanisms dependent on not only the selective killing of tumor cells, but also the stimulation of host anti-tumor immune responses.

[00171] In some embodiments, the oncolytic viruses can include, but are not limited to, (i) viruses that naturally replicate preferentially in cancer cells and are non-pathogenic in humans often due to elevated sensitivity to innate antiviral signaling or dependence on oncogenic signaling pathways; and (ii) viruses that are genetically-manipulated for use. In some embodiments, the oncolytic virus can be a measles virus, a poliovirus, a poxvirus, a vaccinia virus, an adenovirus, an adeno associated virus, a herpes simplex virus, a vesicular stomatitis virus, a reovirus, a Newcastle disease virus, a senecavirus, a lentivirus, a mengovirus, or a myxomavir. In certain embodiments, the oncolytic virus can be a pox virus. In certain embodiments, the oncolytic virus can be a vaccinia virus.

[00172] The term "modified oncolytic virus" as used herein can refer to an oncolytic virus that comprises a modification to its constituent, such as, but not limited to, a modification in the native genome ("backbone") of the virus like a mutation or a deletion of a viral gene, introduction of an exogenous nucleic acid, a chemical modification of a viral nucleic acid or a viral protein, and introduction of a exogenous protein or modified viral protein to the viral capsid. In general, oncolytic viruses may be modified (also known as "engineered") in order to gain improved therapeutic effects against tumor cells. In certain embodiments, the

modified oncolytic virus can be a modified pox virus. In certain embodiments, the modified oncolytic virus can be a modified pox virus.

[00173] The terms "systemic delivery," and "systemic administration," used interchangeably herein, in some cases can refer to a route of administration of medication, oncolytic virus or other substances into the circulatory system. The systemic administration may comprise oral administration, parenteral administration, intranasal administration, sublingual administration, rectal administration, transdermal administration, or any combinations thereof.

Modified Oncolytic Viruses

[00174] Provided herein, in some embodiments, is a modified oncolytic virus that can have one or more modifications that can result in a greater therapeutic effect against tumor cells, as compared to an otherwise identical virus that does not comprises the modifications. Some non-limiting examples of the greater therapeutic effect may include each or any combinations of: enhanced immune evasion of the virus, enhanced tumor-targeted systemic delivery of the virus, enhanced intratumoral and intertumoral spreading of the virus, and enhanced tumor-specific replication of the virus. The modified oncolytic virus of this disclosure, in some instances, can be utilized as a platform vector for systemic delivery.

[00175] Provided herein is a modified oncolytic virus having one or more modifications that can, in some embodiments, result in enhanced immune evasion of the virus, enhanced tumor-targeted systemic delivery of the virus, enhanced intratumoral and intertumoral spreading of the virus, and enhanced tumor-specific replication of the virus.

[00176] In some embodiments of this disclosure, provided is a modified oncolytic virus comprising a modification that can enhance immune evasion of the virus. Virus infection very often can induce immune responses from the host body against the viral invasion, which may consequently, deplete the inoculated viruses or diminish the toxic effect the virus is expected to produce against the tumor cells in therapeutic settings. Appropriate immune evasion therefore may significantly increase the efficacy of the therapeutic application of oncolytic viruses.

[00177] In some embodiments of the present disclosure, provided is a modified oncolytic virus comprising a modification that can enhance tumor-targeted systemic delivery of the virus. Typically oncolytic viruses can be either be (a) administered systemically or (b) inoculated topically over the tumor or, in many cases, injected directly into the tumor ("intratumoral delivery"). It is believed that systemic delivery of the oncolytic virus can

afford the opportunity to treat both the primary tumor and any overt or undiagnosed metastatic deposits simultaneously. As a result, this method of delivery can be a very attractive option for the treatment of patients with advanced/metastatic disease or patients with inaccessible disease such as those with pancreatic cancer or brain cancer, where access is difficult for example due to physiological barriers, such as blood-brain barrier. However, barriers can exist for successful systemic delivery of many oncolytic viruses. For instance, in some cases, as described above, host defense limits most oncolytic viruses' ability to infect tumors after systemic administration. Blood cells, complement, antibodies, and antiviral cytokines, as well as nonspecific uptake by other tissues such as the lung, liver and spleen, tissue-resident macrophages, and additionally poor virus escape from the vascular compartment are among the main barriers to systemic delivery of oncolytic viruses. In order to have effective systemic administration, in many cases, the oncolytic virus may need to persist in the circulation without depletion or degradation while selectively infecting tumor cells. In some embodiments of the present disclosure, disclosed oncolytic viruses can comprise a modification that can promote the persistent existence of the virus in the circulation system, at least through, as abovementioned, enhancement of immune evasion. On the other hand, enhanced tumor-targeted delivery of the virus can also be desirable under certain circumstances, as it may not only increase therapeutic efficacy against cancer, but may also alleviate the safety concerns around virus-mediated oncotherapy as the non-tumor infection can be limited, avoiding the undesired side effects of viral infection. Certain embodiments herein relate to an oncolytic virus comprising a modification that can promote the tumor-targeted delivery of the virus.

[00178] In some embodiments of the present disclosure, provided is a modified oncolytic virus comprising a modification that can enhance intratumoral and intertumoral spreading of the virus. Enhanced spreading of the oncolytic virus within and between tumors may be an effective manner to boost the therapeutic efficacy by increasing the number of the cancer cells that are infected by the virus.

[00179] In some embodiments of the present disclosure, provided is a modified oncolytic virus comprising a modification that can enhance tumor-specific replication of the virus. By enhancing the selective replication of the oncolytic viruses in tumor cells, both the safety and efficacy of the therapeutic application of the oncolytic viruses could be improved.

[00180] Provided herein, in some embodiments, is a modified oncolytic virus that can comprise an exogenous nucleic acid. Provided herein, in some embodiments, is a modified oncolytic virus that can comprise a modification to in the genome of the virus. Provided

herein, in some embodiments, is a modified oncolytic virus that can comprise an exogenous nucleic acid as well as a modification in the genome of the virus.

Exogenous Nucleic Acids

[00181] In some embodiments, provided herein is a modified oncolytic virus comprising an exogenous nucleic acid, also referred to herein as a transgene, that can code for a chemokine receptor. In some cases, the exogenous nucleic acid can be a therapeutic transgene. In some embodiments, provided herein is a modified oncolytic virus comprising an exogenous nucleic acid that can code for a membrane associated protein that can degrade hyaluronan, such as a hyaluronidase. In some embodiments, provided herein is a modified oncolytic virus comprising exogenous nucleic acids that can code for both a chemokine receptor and a hyaluronidase.

[00182] Chemokines are chemotactic cytokines that regulate the trafficking and positioning of cells by activating the seven-transmembrane spanning chemokine receptors. In some cases, chemokines can be divided into four subfamilies based on the position of the first two N-terminal cysteine residues, including the CC, CXC, CX3C and XC subfamilies. Differential expression of chemokine receptors on leukocytes may result in selective recruitment of specific cell types under particular conditions, providing appropriate and efficient immune responses tailored to the infecting pathogen or foreign insult. Beyond their pivotal role in the coordinated migration of immune cells to the site of inflammation, in many cases, chemokines may also play important roles in the development of lymphoid tissues, in the maturation of immune cells, and in the generation and delivery of adaptive immune responses.

[00183] Tumors are increasingly recognized as a complex microenvironment made up of many different cell types that cohabit and communicate with each other in a complicated signaling network. Chemokines are essential coordinators of cellular migration and cell-cell interactions and therefore have great impact on tumor development. In the tumor microenvironment, tumor-associated host cells and cancer cells release an array of different chemokines, resulting in the recruitment and activation of different cell types that mediate the balance between antitumor and pro-tumor responses. In addition to their primary role as chemoattractants, chemokines, in many cases, are also involved in other tumor-related processes, including tumor cell growth, angiogenesis and metastasis.

[00184] Tumor cells have been shown to acquire the ability to produce growth-promoting chemokines. For instance, melanoma has been found to express a number of chemokines, including CXCL1, CXCL2, CXCL3, CXCL8, CCL2 and CCL5, which have been implicated

in tumor growth and progression. CCL2 level can be found increased in neuroblastoma cell lines and primary tumor cells isolated from human patients. Immunostaining studies also suggest an elevated expression level of CXCL12 in a variety of cancers, including breast cancer, carcinoid, cervical cancer, colorectal cancer, endometrial cancer, liver cancer, lung cancer, lymphoma, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, stomach cancer.

[00185] Chemokine receptors are cytokine receptors found on the surface of certain cells that interact with chemokines. There have been 20 distinct chemokine receptors discovered in humans. Each has a 7-transmembrane structure and couples to G-protein for signal transduction within a cell, making them members of a large protein family of G protein-coupled receptors. Following interaction with their specific chemokine ligands, chemokine receptors may trigger a flux in intracellular calcium (Ca^{2+}) ions (calcium signaling). This may cause cell responses, including the onset of a process known as chemotaxis that traffics the cell to a desired location within the organism. In general, the term "chemokine receptor" as used herein can refer to a membrane associated protein that selectively binds to a chemokine ligand and induces the chemotaxis toward the chemokine ligand.

[00186] It is to be understood the chemokine receptor as disclosed herein in some cases can refer to not only the naturally occurring chemokine receptors identified in human bodies, but also include chemokine receptors from other sources, such as, but not limited to: (1) naturally occurring chemokine receptors identified in animals, like pigs, dogs, cows, sheep; (2) non-naturally occurring chemokine receptors, like mutant proteins, chimeric receptors, design proteins with binding affinity to a certain type(s) of chemokines. In some examples, a fragment of a naturally occurring chemokine receptor can be also considered a chemokine receptor, if the function of binding and responding to the corresponding chemokine and directing the chemotaxis of the cell is retained in the fragment. As provided herein, in some embodiments, the virus that comprises the exogenous nucleic acid coding for the chemokine receptor may force a virus-infected cell to express the chemokine receptor as the virus hijacks the host cell's gene expression machinery.

[00187] In some cases, the modified oncolytic viruses can comprise exogenous nucleic acid that can code for a cytokine receptor whose cognate cytokine can be expressed in tumor microenvironments (*e.g.*, IL15-R can have a cognate cytokine IL15 expressed in a tumor microenvironment). In some cases, the modified oncolytic viruses can express selected chemokine receptors whose cognate chemokines are likely to be expressed on tumors (*e.g.*, CXCR4 can have a cognate chemokine CXCL12 expressed on a tumor; CCR2 can have a

target CCL2 expressed on a tumor) and can be delivered systemically as a naked virus. Subsequent to entry of the modified oncolytic viruses into the blood stream, by systemic delivery, the viruses can infect lymphocytes, such as B-cells, and can re-direct the infected B-cells to the tumor, resulting in significantly increased viral load in the tumor. In certain embodiments, the increased viral load in the tumor can be achieved soon after the systemic delivery. Ability to deliver the modified oncolytic viruses disclosed herein, in a systemic manner, can provide an advantage over traditional intratumoral delivery methods for oncolytic viruses. While intratumoral delivery can be helpful in treating easily accessible tumors, in some instances, it is critical to treat inaccessible or metastatic cancer which is allegedly the predominant cause of death from the disease. In this context, it may be ineffective to rely on oncolytic viruses delivered intratumorally, as it will need systemic dissemination after administration to the distant sites. However, this dissemination often may be transient and ineffective, at least in part, due to the development of immune responses to the viral infection.

[00188] Chemokine receptors can be divided into different families. Non-limiting examples of chemokine receptors, as described herein can include CXC chemokine receptors, CC chemokine receptors, CX3C chemokine receptors and XC chemokine receptors that correspond to the 4 distinct subfamilies of chemokines they bind. Among the CXC chemokine receptors, CXCR1 and CXCR2 are closely related, while CXCR1 binds to CXCL8 and CXCL6, and CXCR2 binds to CXCL1 and CXCL7; CXCR3 binds to CXCL9, CXCL10, and CXCL11; CXCR4 binds to CXCL12 (or SDF-1); CXCR5 binds to CXCL13; CXCR6 binds to CXCL16. Among the CC chemokine receptors, CCR1's ligands include CCL4, CCL5, CCL6, CCL14, CCL15, CCL16, CCL23; CCR2's ligands include CCL2, CCL8, and CCL16; CCR3's ligands include CCL11, CCL26, CCL7, CCL13, CCL15, CCL24, CCL5, CCL28, and CCL18; CCR4's ligands include CCL3, CCL5, CCL17, and CCL22; CCR5's ligands include CCL3, CCL4, CCL5, CCL8, CCL11, CCL13, CCL14, and CCL16; CCR6's ligands include CCL20; CCR7's ligands include CCL19 and CCL21. CX3C chemokine receptor CX3CR1 has a ligand CXCL1. XC chemokine receptor XCR1 binds to both XCL1 and XCL2.

[00189] Non-limiting embodiments of the present disclosure provide a modified oncolytic virus that comprises an exogenous nucleic acid that can code for a chemokine receptor. In some embodiments, the chemokine receptor can be a CXC chemokine receptor, a CC chemokine receptor, a CX3C chemokine receptor, a XC chemokine receptor, or any combinations thereof. In some embodiments, the chemokine receptor can be CXCR1,

CXCR2, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, CXCR7, CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CX3CR1, XCR1, or any combinations thereof.

[00190] In certain embodiments, the modified oncolytic virus comprises an exogenous CXCR4-expressing nucleic acid. In certain embodiments, the modified oncolytic virus comprises an exogenous CCR2-expressing nucleic acid. Certain embodiments disclose a modified oncolytic virus comprising an exogenous nucleic acid that codes for both CXCR4 and CCR2, and both chemokines are expressed form the same virus. Under certain circumstances, CXCL12 and/or CCL2 typically expressed in the tumor microenvironment may attract the CXCR4 and/or CCR2-expressing lymphocytes or other migrating cells that are infected by the modified oncolytic virus, thereby enhancing the tumor-targeted delivery of the modified oncolytic virus.

[00191] In some embodiments, the modification of the oncolytic virus can result in at least about 1.1, 1.2, 1.5, 1.8, 2, 2.2, 2.5, 2.8, 3, 3.2, 3.5, 3.8, 4, 4.2, 4.5, 4.8, 5, 5.2, 5.5, 5.8, 6, 6.2, 6.5, 6.8, 7, 7.2, 7.5, 7.8, 8, 8.2, 8.5, 8.8, 9, 9.2, 9.5, 9.8, 10, 12, 14, 15, 16, 18, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 500, 800, 1000, 2500, 5000, 10^4, 2.5 x 10^4, 5 x 10^4, 7.5 x 10^4, 2.5 x 10^5, 5 x10^5, 7.5 x10^5, 10^6, 2.5 x10^6, 5 x10^6, 7.5 x10^6, 10^7, 2.5 x 10^7, 5 x 10^7, 7.5 x 10^7, 10^8, 2.5 x10^8, 5 x10^8, 7.5 x10^8, 10^9, 2.5 x 10^9, 5 x 10^9, 7.5 x 10^9, 10^{10} or even more folds increase in the efficacy of tumor-targeted systemic delivery of the virus, as compared to an otherwise identical oncolytic virus that does not comprise the modification. In certain embodiments, the efficacy of tumor-targeted systemic delivery of the virus can be measured by quantifying the viruses infecting the tumor cells, and optionally, in contrast with the viruses infecting non-tumor cells in the body. For instance, in some cases, the quantification of the virus can be performed by staining the viral particles in tissue sections, or blood smear in the cases of leukemia, lymphoma, or myeloma. In some cases, such quantification can be performed by reporter molecule(s) that is/are engineered to be expressed by the viruses, *e.g.*, luciferase, and fluorescent proteins. In some cases, such quantification can be performed by quantifying the viral genome in the tumor. Without being limited, it is also possible to measure the tumor-targeted systemic delivery of the virus by quantifying certain downstream effect(s) of viral infection in tumor cells, like cytokines in response to viral infection or lymphocyte accumulation. In some embodiments, the oncolytic virus comprises an exogenous nucleic acid that can code for CXCR4, CCR2, or both, and the presence of the exogenous nucleic acid can result in about 5 to 10 folds increase in the efficacy of tumor-target systemic delivery of the virus, as compared to an otherwise identical oncolytic virus that does not comprise the exogenous nucleic acid.

[00192] In some embodiments, provided herein is a modified oncolytic virus that comprises an exogenous nucleic acid that can code for a chemokine receptor, and the forced expression of chemokine receptor by the modified oncolytic virus can result in boosted immune responses against the infected tumor. Following infecting the tumor, the modified oncolytic viruses can replicate in the tumor cells and result in the expression of the chemokine receptors on the surface of the tumor cells. These membrane receptors may function as decoy receptors, binding and sequestering the immunosuppressive chemokines within the tumor (*e.g.*, CXCL12 and/or CCL2). Consequently, the immunosuppressive microenvironment in the tumor can be altered, leading to enhanced immunotherapeutic activity of the modified oncolytic virus, as compared to an otherwise identical virus that does comprise the nucleic acid coding for the chemokine receptor. In some embodiments, the increase in immunotherapeutic activity can be at least about 1.1, 1.1, 1.2, 1.5, 1.8, 2, 2.2, 2.5, 2.8, 3, 3.2, 3.5, 3.8, 4, 4.2, 4.5, 4.8, 5, 5.2, 5.5, 5.8, 6, 6.2, 6.5, 6.8, 7, 7.2, 7.5, 7.8, 8, 8.2, 8.5, 8.8, 9, 9.2, 9.5, 9.8, 10, 12, 14, 15, 16, 18, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 500, 800, 1000, 2500, 5000, 10^4, 2.5 x 10^4, 5 x 10^4, 7.5 x 10^4, 2.5 x 10^5, 5 x 10^5, 10^6 or even higher folds. Without being limited, the increased immunotherapeutic activity can be reflected by increased B cell accumulation in the tumor, increased T cell response to tumor-related immunogens, or both. B cell accumulation can be measured, for example, by quantifying the B cells in the tumor, and T cell immuno-activity may be measured by, for example, interferon-γ (interferon-gamma) secretion in ELISPOT assays.

[00193] In some embodiments, provided herein is a modified oncolytic virus that comprises an exogenous nucleic acid that can code for a chemokine receptor, and the forced expression of chemokine receptor by the modified oncolytic virus can result in increased replication of the virus in tumor cells, as compared to an otherwise identical virus that does not comprise the nucleic acid coding for the chemokine receptor. In certain embodiments, the modified oncolytic virus can comprise an exogenous CCR2-expressing nucleic acid, which can increase the tumor-specific replication of the virus. In some embodiments, the modified oncolytic virus can comprise an exogenous CCR5-expressing nucleic acid, which can increase the tumor-specific replication of the virus. In some embodiments, the increase in tumor-specific replication can be at least about 1.1, 1.1, 1.2, 1.5, 1.8, 2, 2.2, 2.5, 2.8, 3, 3.2, 3.5, 3.8, 4, 4.2, 4.5, 4.8, 5, 5.2, 5.5, 5.8, 6, 6.2, 6.5, 6.8, 7, 7.2, 7.5, 7.8, 8, 8.2, 8.5, 8.8, 9, 9.2, 9.5, 9.8, 10, 12, 14, 15, 16, 18, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 500, 800, 1000, 2500, 5000, 10^4, 2.5 x 10^4, 5 x 10^4, 7.5 x 10^4, 2.5 x 10^5, 5

x 10^5, 10^6 or even higher folds. Exemplary methods for measuring the increase in viral delivery and spread in tumors can include, but are not limited to, fluorescence or bioluminescence based imaging of expression of a reporter gene, quantitative PCR for detection of tumor concentrations of viral genomes or plaque determination of plaque forming units or immunohistochemistry of viral proteins.

[00194] In some embodiments, the modified oncolytic virus comprises an exogenous nucleic acid that can code for a chemokine receptor that is a chimeric protein. At least part of its extracellular domain can be from a chemokine receptor that promotes the tumor-targeted delivery of the virus, and at least part of its intracellular domain can be from a chemokine receptor that promotes the tumor-specific replication, inhibits immunosuppressive activity, or conveys some other beneficial effects, or vice versa. For instance, the modified oncolytic virus can comprise a nucleic acid that codes for a protein having an intracellular GTPase domain of CCR5, and an extracellular chemokine-binding domain of CXCR4 or CCR2. In some case, by combining domains with different functionalities one may achieve further improvement in therapeutic performance of the modified oncolytic virus. It is one embodiment of this disclosure that the modified oncolytic virus can comprise exogenous nucleic acids that can code for at least one chemokine receptor. In some cases, the modified oncolytic virus can comprise exogenous nucleic acids that can code for two or more different chemokine receptors, which may be expressed simultaneously by the virus. Exemplary chemokine receptors that can be expressed simultaneously from the modified oncolytic viruses described herein can include CXCR4 and CCR2. In modified oncolytic viruses expressing more than one chemokine receptors, a combinatorial or synergistic effect against tumor cells may be achieved as to the therapeutic application of the oncolytic virus.

[00195] Therapeutic effects of oncolytic viruses can often be limited by their ineffective spreading in and between the solid tumors, at least in part due to the high amounts of extracellular matrix (ECM) and high interstitial fluid pressure (IFP) that exist in tumor. Certain embodiments of the present disclosure relate to a modified oncolytic virus that comprises an exogenous nucleic acid that can code for a protein that degrades ECM of a tumor. Exemplary proteins that can degrade ECM can be a membrane associated protein. In some cases, the membrane associated protein can comprise a glycosylphosphatidylinisotol (GPI) anchor.

[00196] Hyaluronan (HA) is an important structural element of ECM and a high molecular weight linear glycosaminoglycan consisting of repeating disaccharide units. It can be distributed widely throughout connective, epithelial, and neural tissues, and its expression

level can be significantly elevated in many types of tumors. Hyaluronidases are a family of enzymes that catalyze the degradation of HA. There are at least five functional hyaluronidases identified so far in human: HYAL1, HYAL2, HYAL3, HYAL4 and HYAL5 (also known as PH-20 or SPAM1), among which PH-20 is the only one known so far to be functional at relatively neutral pH. In some embodiments of the present disclosure, combining hyaluronidase with other tumor-targeting therapeutic agents (such as transgenes, also referred to herein as exogenous nucleic acid) can promote the therapeutic effect of the modified oncolytic virus at least by diminishing the ECM and enhancing the transportation of the therapeutic agent inside and between the tumors.

[00197] Some embodiments herein disclose a modified oncolytic virus that can comprise an exogenous nucleic acid coding for a membrane associated protein that is capable of degrading hyaluronan, such as a hyaluronidase. It should be noted that the term "hyaluronidase" as used herein can refer to any enzyme or a fragment thereof that catalyzes the degradation of HA in a tumor, including, but not limited to, PH-20 and its homologs from other species, as well as other engineered/design proteins with similar enzymatic function. As used herein, hyaluronidase can refer to a class of hyaluronan degrading enzymes. Hyaluronidases can include bacterial hyaluronidases (EC 4.2.2.1 or EC 4.2.99.1), hyaluronidases from leeches, other parasites, and crustaceans (EC 3.2.1.36), and mammalian-type hyaluronidases (EC 3.2.1.35). Hyaluronidases can be of any non-human origin including, but not limited to, murine, canine, feline, leporine, avian, bovine, ovine, porcine, equine, piscine, ranine, bacterial, and any from leeches, other parasites, and crustaceans. Exemplary non-human hyaluronidases include, hyaluronidases from cows, yellow jacket wasp, honey bee, white-face hornet, paper wasp, mouse, pig, rat, rabbit, sheep, chimpanzee, *Rhesus monkey*, orangutan, cynomolgus monkey, guinea pig, *Arthrobacter sp.* (strain FB24), *Bdellovibrio bacteriovorus*, *Propionibacterium acnes*, *Streptococcus agalactiae*, *Staphylococcus aureus*; strain MRSA252, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus suis*, *Vibrio fischeri*, and the *Streptomyces hyaluronolyticus* hyaluronidase enzyme, which is specific for hyaluronic acid and does not cleave chondroitin or chondroitin sulfate.

[00198] In some embodiments, the modified oncolytic virus can comprise an exogenous nucleic acid that can code for PH-20. In some embodiments, the modified oncolytic virus can comprise an exogenous nucleic acid that can code for PH-20, and the PH-20 can comprise a GPI-anchor. In some embodiments, the modified oncolytic virus can comprise an exogenous nucleic acid that can code for PH-20, and the PH-20 may lack a GPI-anchor. GPI

can function as an anchor of the protein to a cell membrane, therefore, generally, GPI-containing PH-20 may be anchored to the cell membrane, while PH-20 that does not have GPI may be in secretory form.

[00199] In some embodiments, exemplary amino acid sequences for the PH-20 can be SEQ ID NO: 36, SEQ ID NO: 130, or sequences that can be about 70%, about 75%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identical to SEQ ID NO: 36 or SEQ ID NO: 130.

[00200] The present disclosure identifies that retention of the C-terminal GPI anchor, unexpectedly, improves the spread of oncolytic viruses expressing the GPI-anchor containing PH-20, within a tumor microenvironment. In some examples, the modified oncolytic virus comprising an exogenous nucleic that can code for a GPI-anchored PH-20 can unexpectedly degrade tumor ECM and promote viral spreading to a greater extent, as compared to an otherwise identical virus that can comprise an exogenous nucleic acid that can code for a PH-20 lacking a GPI-anchor. In some other embodiments, the modified oncolytic virus can comprise nucleic acids that can code for both a GPI-anchored PH-20 and secretory PH-20 without the GPI-anchor.

[00201] In some cases, expression of the GPI-anchored PH-20 from the modified oncolytic vaccinia virus can have at least one of the following effect: (i) the PH-20 can be incorporated into the EEV outer envelope, and thereby may allow the EEV to spread more effectively, (ii) the viral infection may result in movement of infected cells, which may be enhanced if they express PH20 on their surface. Some embodiments of this disclosure identify that the secreted form of the PH-20, without the GPI-anchor can be less active in promoting production of the EEV form of the virus.

[00202] In some embodiments, at least some of the hyaluronidase-encoding nucleic acid can be derived from other sources than human beings. Hyaluronidases in different species can hydrolyze tumor HA in different manners,with different efficiencies, combinations thereof. Some embodiments herein relate to a modified oncolytic virus that can comprise an exogenous nucleic acid coding for multiple hyaluronidases from different species. The combination may provide a greater ECM degrading capability and subsequently lead to enhanced therapeutic effects.

[00203] In some embodiments, the oncolytic virus can comprises an exogenous nucleic acid that can code for a hyaluronidase of microbial origin. In some examples, the hyaluronidase

of microbial origin can be HysA from *Staphylococcus aureus,* lin from *Loxosceles intermedia,* sko from *Streptomyces koganeiensis,* rv from *Mycobacterium tuberculosis*. In some examples, the hyaluronidase of microbial origin can be a secreted hyaluronidase. In some examples, the exogenous nucleic acid hysA can comprise a sequence as set forth in GenBank: CP020020.1, (*Staphylococcus aureu*s subsp. aureus strain ATCC 6538 chromosome, complete genome) range of nucleotides: 2248602 to 2250899). In some cases, the exogenous nucleic acid hysA can code for a protein that comprises an amino acid sequence as set forth in SEQ ID NO: 122. In some cases, the exogenous nucleic acid hysA can code for a protein that comprises an amino acid sequence as set forth in UniProtKB Accession No. Q59801 (HYAS_STAA8) (SEQ ID NO: 123). In some embodiments, expression of a secreted hyaluronidase, such as HysA, enhances replication, spread, therapeutic activity (*e.g.*, cancer cell killing potential) of a modified oncolytic virus as described herein. In some cases, the exogenous nucleic acid lin can code for a protein that comprises an amino acid sequence as set forth in SEQ ID NO: 124 or SEQ ID NO: 127. In some cases, the exogenous nucleic acid rv can code for a protein that comprises an amino acid sequence as set forth in SEQ ID NO: 125 or SEQ ID NO: 128. In some cases, the exogenous nucleic acid sko can code for a protein that comprises an amino acid sequence as set forth in SEQ ID NO: 126 or SEQ ID NO: 129. In some embodiments, exemplary amino acid sequences for the microbial hyaluronidase can be SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, or SEQ ID NO: 129, or sequences that can be about 70%, about 75%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identical to S SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, or SEQ ID NO: 129.

[00204] Furthermore, in some embodiments, the modified oncolytic virus can comprise exogenous nucleic acids that can code for both a hyaluronidase and a matrix metalloprotease. Collectively, matrix metalloproteases are capable of degrading all kinds of ECM proteins. The addition of a matrix metalloprotease may further enhance the ECM degradation effect and promote virus spreading. One example of matrix metalloproteases can be MMP8. Other examples can include, but not limited to, MMP1, MMP2, MMP3, MMP7, MMP9, MMP10, MMP11, MMP12, MMP13, MMP14, MMP15, MMP17, MMP18, MMP19, MMP20, MMP21, MMP23A, MMP23B, MMP24, MMP25, MMP26, MMP27, and MMP28.

[00205] In some embodiments, the modified oncolytic virus that comprises the nucleic acid that can code for a hyaluronidase may have increased virus spreading in and between tumors as compared to an otherwise identical virus that does not comprise the hyaluronidase-encoding nucleic acid. In some embodiments, such increase can be at least about 1.1, 1.1, 1.2, 1.5, 1.8, 2, 2.2, 2.5, 2.8, 3, 3.2, 3.5, 3.8, 4, 4.2, 4.5, 4.8, 5, 5.2, 5.5, 5.8, 6, 6.2, 6.5, 6.8, 7, 7.2, 7.5, 7.8, 8, 8.2, 8.5, 8.8, 9, 9.2, 9.5, 9.8, 10, 12, 14, 15, 16, 18, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 500, 800, 1000, 2500, 5000, 10^4, 2.5 x 10^4, 5 x 10^4, 7.5 x 10^4, 2.5 x 10^5, 5 x 10^5, 10^6 or even higher folds. In certain embodiments, the modified oncolytic virus comprises an exogenous nucleic acid coding PH-20, and the viral spreading within tumor can be increased at least about 100 folds, as compared to an otherwise identical virus that does not comprise the PH-20-encoding nucleic acid. Exemplary methods for measuring the increase in viral spread can include, but are not limited to, fluorescence or bioluminescence based imaging of expression of a reporter gene, quantitative PCR for detection of tumor concentrations of viral genomes or plaque determination of plaque forming units or immunohistochemistry of viral proteins.

[00206] VH1 is a viral protein, identified as a dual specificity phosphatase against both phosphoserine- and phosphotyrosine-containing substrates. In particular, VH1 can inhibit phosphorylation and nuclear translocation of STAT1, a transcription factor responsive to a number of immune factors, such as interferon alpha and interferon gamma, suppressing immune response against virus-infected cells, at least in part through reduction in antigen presentation. More active VH1 expressed by the virus may have better immunosuppressive effect as to the virus-infected cells.

[00207] In some embodiments, the modified oncolytic virus can comprise an exogenous nucleic acid that can code for a viral VH1 protein. In some embodiments, the exogenous nucleic acid coding for the viral VH1 protein can be from a genome of poxvirus that is a different species than the modified oncolytic virus. In some embodiments, the poxvirus can comprise a betaentomopoxvirus, a yatapoxvirus, a cervidpoxvirus, a gammaentomopoxvirus, a leporipoxvirus, a suipoxvirus, a molluscipoxvirus, a crocodylidpoxvirus, an alphaentomopoxvirus, a capripoxvirus, an avipoxvirus, or a parapoxvirus. In some embodiments, the modified oncolytic virus can comprise a partial or a complete deletion of the native VH1 gene, and can comprise an exogenous VH1-encoding nucleic acid. The VH1 protein can also be a chimeric/fusion protein that has components from different species, artificially designed/engineered, or only a functional fragment of a naturally occurring VH1

protein that can retains the phosphatase activity. The VH1 protein encoded by the exogenous nucleic acid may be more active than the native VH1 protein of the backbone oncolytic virus.

[00208] In some embodiments, the modified oncolytic virus can comprise a vaccinia virus, wherein its native VH1 gene can be deleted from the genome, and a VH1 gene from other poxviruses can be inserted in its genome. In this case, a more active VH1 protein may lead to a lower toxicity of the virus. Alternatively or additionally, it may lead to a greater therapeutic benefit, at least partially due to the adaptive immune response more actively targeting the tumor antigens, and less potently targeting viral antigens.

[00209] In some embodiments, the modified oncolytic virus can comprise an exogenous nucleic acid that can code for a therapeutic protein. Examples of therapeutic proteins that can be coded by the exogenous nucleic acid contained with the modified oncolytic vaccinia virus described herein, can include, but are not limited to, antibodies or antigen binding fragments thereof, cytokines, growth factors, peptide hormones, cytokines, coagulation factors, plasma proteins, fusion proteins. In some embodiments, the modified oncolytic virus can comprise an exogenous nucleic acid that codes for proteins or fragment thereof that are immune checkpoint inhibitors, T-cell activators, therapeutic nanobodies, chemokines and immune activators, prodrug converting enzymes, directly cytotoxic compounds, or any combinations thereof. Each or any combinations of these proteins may contribute to a greater therapeutic benefit of the backbone oncolytic virus.

[00210] The exogenous nucleic acid, in some cases, can code for a transgene comprising a humanized anti-CD20 monoclonal antibody (*e.g.*, Gazyva), a VEGFR Fc-fusion (*e.g.*, Eylea), a CTLA-4 Fc-fusion (*e.g.*, Nulojix), a glucagon-like peptide-1 receptor agonist Fc-fusion (*e.g.*, Trulicity), VEGFR Fc-fusion (e.g., Zaltrap), a recombinant factor IX Fc fusion (e.g., Alprolix), a recombinant factor VIII Fc-fusion (*e.g.*, Eloctate), a GLP-1 receptor agonist-albumin fusion (*e.g.*, Tanzeum), a recombinant factor IX albumin fusion (*e.g.*, Idelvion), a PEGylated IFNβ-1a (*e.g.*, Plegridy), a recombinant factor VIII PEGylated (*e.g.*, Adynovate), a humanized anti-HER2/neu conjugated to emtansine (*e.g.*, Kadcyla), a mouse/human chimeric anti-CD30 (*e.g.*, Adcetris), an anti-human epidermal growth factor receptor 2 (HER2) (*e.g.*, Perjeta), Anti-IL-6 receptor (Actemra), an anti-CD20 (*e.g.*, obinutuzumab; Gazyva), an anti-integrin a4b7 (LPAM-1) (*e.g.*, Entyvio), an anti-PD-1 (e.g., Keytruda), an anti-dabigatran (e.g., Praxbind), an anti-IL-5 (*e.g.*, Nucala), an Anti-CD319 (SLAMF7) (*e.g.*, Empliciti), an anti-IL-17a (*e.g.*, Taltz), an anti-IL-5 (*e.g.*, Cinqair), an anti-PD-L1 (*e.g.*, Tecentriq), an anti-CD25 (*e.g.*, Zinbryta), an anti-CD30 (*e.g.*, Adcetris), an anti-IL-6 (*e.g.*, Sylvant), an anti-GD2 (*e.g.*, Unituxin), an anti- *Bacillus anthracis* (*e.g.*, Anthim), an anti-

TNFα (*e.g.*, Inflectra), a human anti-B-cell activating factor (BAFF) (*e.g.*, belimumab), a human anti-CTLA-4 (*e.g.*, ipilimumab), a CTLA-4 Fc-fusion (*e.g.*, belatacept), humanized anti-human epidermal growth factor receptor 2 (HER2) (*e.g.*, pertuzumab), a VEGFR Fc fusion (*e.g.*, ziv-afilbercept), a G-CSF (*e.g.*, tbo-filgrastim), human anti-VEGFR2 (KDR) (*e.g.*, ramucirumab), a mouse/human chimeric anti-IL-6 (*e.g.*, siltuximab), pembrolizumab, mouse bispecific anti-CD19/anti-CD3 (*e.g.*, blintumomab), nivolumab, a parathyroid hormone, a mouse/human chimeric anti-GD2 (*e.g.*, dinutuximab), a human anti-CD38 (*e.g.*, daratumumab), a human anti-epidermal growth factor receptor (EGFR)(*e.g.*, necitumumab), humanized anti-CD319(SLAMF7) (*e.g.*, elotuzumab), atezolizumab. In some embodiments, the modified oncolytic virus can comprise an exogenous nucleic acid coding for a protein or a fragment thereof that: can modulate NFκB signaling, can promote reduction of interstitial fluid pressure (IFP) in a tumor, can modulate STAT3-mediated gene activation, can promote T cell activation, can promote attraction of NK cells to virus-infected cells, can modulate metabolic program of virus-infected cells, can modulate fatty acid uptake by virus-infected cells, can promote therapeutic targeting of MDSCs, or any combinations thereof.

[00211] In some embodiments, the modified oncolytic virus can comprise an exogenous nucleic acid that can code for at least one of HMGB1, PIAS3, IL15, CCL5, a fragment thereof, or any combinations thereof. Each or any combinations of these proteins may contribute to a greater therapeutic benefit of the backbone oncolytic virus.

[00212] In some embodiments, the modified oncolytic virus can comprise an exogenous nucleic acid that codes for a fractalkine (such as CX3CL1), ITAC (such as CXCL11), LIGHT (such as tumor necrosis factor superfamily member 14, or TNFSF14), a fragment thereof, or any combinations thereof. Each or any combinations of these proteins may contribute to a greater therapeutic benefit of the backbone oncolytic virus.

[00213] In some embodiments, the immune checkpoint inhibitors can refer to inhibitors of immune checkpoint molecules such as, but not limited to, PD-1, PD-L1, PD-L2, CTLA4, TIM-3, LAG-3, CEACAM-1, CEACAM-5, VISTA, BTLA, TIGIT, LAIR1, CD 160, 2B4, TGFR-beta, and any combinations thereof.

[00214] In some embodiments, the T-cell activators can include, but not limited to, interleukin-1, interleukin-2, interleukin-4, interleukin-5, interleukin-6, interleukin-7, interleukin-11, interleukin-12, interleukin-13, interleukin-15 (IL15/IL-15), IL15-α (IL15-alpha), IL15-receptor (IL15-R), IL15-Rα (IL15-receptor alpha), interferon-alpha, interferon-gamma, tumor necrosis factors, anti-CD3 antibodies, anti-CD28 antibodies, anti-CTLA4 antibodies, anti-TGF-beta antibodies, anti-4-1BB antibodies, cell-based vaccines peptide

vaccines, DNA vaccines, growth factors, phytohemagglutinin, concanavalin-A, phorbol esters, and any combinations thereof.

[00215] In some embodiments, the therapeutic nanobody can refer to an antibody fragment consisting of a single monomeric variable antibody domain and having therapeutic effects against a tumor cell or a tumor.

[00216] In some embodiments, the other cytokines, chemokines, and immune activators can refer to any other proteins belonging to the corresponding category that may have therapeutic effects against a tumor cell or a tumor.

[00217] In some embodiments, the prodrug converting enzyme can refer to an enzyme that converts a molecule with less activity against a target into a molecule with more activity against a target. In some cases, the target can be a tumor cell or a tumor. The prodrug converting enzyme can include, but not limited to, cytosine deaminase, uracil phosphoribosyltransferase, thymidine kinase, and any combinations thereof.

[00218] In some embodiments, the directly cytotoxic compounds can refer to any molecules that are directly cytotoxic to the tumor cell without effecting through other cells or compounds. In some embodiments, the directly cytotoxic compound can include, but not limited to, proteins, peptides, mRNAs, or oligomers that may be expressed from the exogenous nucleic acids that are added to the modified oncolytic virus.

[00219] In various examples, the modified oncolytic virus can comprise one or more of exogenous nucleic acids described above, that can code for proteins, as described above, wherein the proteins can be the full-length proteins, truncated versions of the full-length proteins, functional domains of the full-length proteins, fragments of the full-length proteins, or variants of the full-length proteins, truncated versions, functional domains, or fragments. Variants can comprise, in some examples, amino acid substitutions (conservative or non-conservative), deletions, additions, modifications, or any combinations thereof.

Modifications of Viral Genome

[00220] Some embodiments of this disclosure can include a modified oncolytic virus that can comprise a modification in the genome of the virus. In some embodiments, the modified oncolytic virus can comprise at least one modification in the genome of the virus. In some embodiments, the modified oncolytic virus can comprise at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, or even more modifications in the genome of the virus. The modification of the viral genome can comprise a mutation, a deletion, or both of a viral gene. A deletion of a viral gene may include a partial or a complete deletion of the viral gene. It should be noted

that as used herein, "partial deletion" or "mutation" may refer to an *in situ* partial deletion or mutation of an endogenous viral gene, respectively. Alternatively, they may refer to replacing the endogenous viral gene with an otherwise identical exogenous nucleic acid that lacks a portion of the gene ("partial deletion") or has one or more nucleotide change in the gene ("mutation").

[00221] Replication of many viruses, including poxviruses, involves several stages with different morphologies in the various stages. One non-limiting example may be vaccinia virus, which is a large DNA virus that may replicate entirely within the cytoplasm of an infected cell. Generally vaccinia virus has a complex morphogenic pathway that culminates in the formation of two distinct infectious virions that are surrounded by different numbers of membranes. The first virion produced, which is called intracellular mature virus (IMV), is surrounded by a single membrane and remains within the cell until cell lysis. The other virion is surrounded by a second membrane and is exported from the cell before cell death. This virion is called cell-associated enveloped virus (CEV) if it is retained on the cell surface and extracellular enveloped virus (EEV) if it is released from the cell surface.

[00222] In the therapeutic applications of oncolytic vaccinia virus, the second membrane of the virion in its EEV form may significantly reduce the virus' sensitivity to neutralizing antibodies and complement that exist in the circulating system and other tissue environments, as compared to single membrane-wrapped IMV. Moreover, as EEV is in the released form, administration of EEV may make it easier for the virus to spread within and between tumors, thereby increasing the chance of infecting tumor cells. Therefore, increasing the production of EEV may result in greater therapeutic benefits when oncolytic viruses, such as poxviruses, *e.g.*, oncolytic vaccinia viruses, other oncolytic poxviruses, or other similar viruses are used at clinical settings. Some embodiments herein disclose a modified oncolytic virus that can comprise a modification in the viral genome that can enhance production of the EEV form of the virus and thereby the spreading of the virus within and between tumors. B5R, F13L, A36R, A34R, A33Rare examples of EEV-specific membrane proteins.

[00223] In some embodiments, the modified oncolytic virus can comprise a modification in the viral B5R gene, provided that said modification is not in the SCR2 domain. The B5R protein includes the following regions: (Signal peptide)-(Short Consensus Repeat (SCR) regions 1 to 4 (Transmembrane domain)-(Cytoplasmic Tail). SCR1 domain can contain a neutralizing antibody epitope found on the EEV. SCR3 can contain a P189S mutation site that can result in increased EEV release.

[00224] A modified oncolytic virus of this disclosure, in some examples, can comprise modifications in at least one of the SCR1, SCR3 and SCR4 domains of the B5R gene. For example, the modification can be a partial deletion of the B5R gene. In some cases, the modification of the B5R gene, *e.g.*, deletions in the SCR3 and SCR4 domains, can, at least partially, disrupt binding of neutralizing antibodies to B5R. Thus, a modified oncolytic virus according to this disclosure can be at least partially resistant to neutralization.

[00225] Additional modifications in the B5R SCR regions are also included in this disclosure, such that the modified oncolytic viruses may be optimized for EEV release, replication and antibody evasion. The B5R mutations, *e.g.*, removal of antibody binding site in B5R SCR1, can be combined with other EEV enhancing mutations, *e.g.*, random mutagenesis in A34R. In some cases, such combinations of modifications can result in increased EEV production.

[00226] In some embodiments, the modification comprises a deletion or mutation in B5R. The deletion may be a complete or partial deletion.

[00227] In some embodiments, the modified oncolytic virus can comprise a modification in the viral gene B5R, F13L, A36R, A34R, A33R, or any combinations thereof that increases production of EEV. In some embodiments, the modified oncolytic virus can comprise one or more of the following mutations: partial deletion of A33R, A34R Lys151 to Glu (K 151 E); complete or partial deletion of B5R; and/or mutation/deletion of A36R.

[00228] In some embodiments, the modified oncolytic virus can comprise a mutation or a deletion of a further viral gene. The further viral gene can comprise genes encoding certain secreted cytokine binding proteins, *e.g.*, B8R or B18R. Or it can comprise genes that may be responsible for immune suppressive activity, *e.g.*, N1L or A41L. It can also comprise genes coding for proteins with NFκB (NF-kappaB) inhibitory functions, *e.g.*, K7R, B15R, A52R. Alternatively, it is also possible that the modified oncolytic virus comprises a mutation or a deletion of any one or combination of the genes that can code for B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, or A52R. The protein B8R can, in some embodiments, refer to a secreted viral protein with homology to the IFN-gamma receptor, which in some cases can inhibit the interaction between host IFN-gamma and its receptor, thereby counteracting the antiviral effects of host IFNγ. The protein B18R can, in some embodiments, refer to a viral ankyrin-like protein that is secreted and, in some cases, can bind to type 1 interferon. The protein SPI-1 can, in some embodiments, refer to a viral serine proteinase inhibitor 1 and SPI-2 can refer to a viral serine proteinase inhibitor 2. The protein

B15R (also referred to as B14R in *Vaccinia virus* strain Copenhagen) can, in some embodiments, refer to a viral protein that, in some cases, can bind to the IKBKB subunit of the IKK complex, thereby preventing host NF-kappa-B activation in response to pro-inflammatory stimuli such as TNF-alpha or IL1B. The protein VGF can, in some embodiments, refer to pro-vaccinia growth factor that can stimulate cellular proliferation around infected cells. The protein E3L, in some embodiments, can refer to a viral protein that, in some cases, can bind to and sequester double-stranded RNA (dsRNA) synthesized during viral infection, thereby preventing recognition of the dsRNA by and subsequent activation of EIF2AK2/PKR. The protein K3L can, in some embodiments, refer to a viral protein that, in some cases, can act as a pseudosubstrate of EIF2AK2/PKR, thereby inhibiting eIF2α activation by PKR kinase and preventing translation shutoff in host cell. The protein A41L can, in some embodiments, refer to a secreted viral chemokine binding protein that, in some cases, can interact with certain cellular chemokines to interfere with chemokine-glycosaminoglycan (GAG) interactions at the cell surface to alter chemotaxis of nearby responsive cells. The protein K7R can, in some embodiments, refer to a viral Bcl-2-like protein that, in some cases, can bind to Toll-like receptor-adaptor proteins and the DEAD-box RNA helicase DDX3, thereby inhibiting the activation of NFκB and interferon regulatory factor 3. The protein N1L can, in some embodiments, refer to another viral Bcl-2-like protein that, in some cases, can bind to BH3 peptides of pro-apoptotic Bcl-2 family proteins. In some cases, N1L can inhibit NFκB activation and host cell apoptosis. The protein A52R can, in some embodiments, refer to another viral Bcl-2-like protein, which, in some cases can target host toll-like receptor signaling complexes to suppress innate immune response, in some cases can interact with host TRAF6 to activate p38 and subsequently induce the expression of several cytokines such as IL-10, and in some cases can interact with host IRAK2 to inhibit NF-kappa-B signaling. In some cases, a deletion in the N1L gene can, in some embodiments, result in increased memory T-cell response, upon administration of the modified oncolytic virus. Additional effects can, in some embodiments, include one or more of the following: deletion of A41L can, in some embodiments, result in increased CTL response; K7R deletion can, in some embodiments, lead to increased natural killer and T-cell response, modified B15R can, in some embodiments, reduce NFκB activation and a deletion of the B15R gene can, in some embodiments, enhance immune response, modified A52R can, in some embodiments, reduce NFκB activation and a deletion of the A52R gene can, in some embodiments, enhance immune response.

[00229] In some embodiments, the modified oncolytic virus can comprise an exogenous nucleic acid that can code for LIGHT. In some embodiments, the modified oncolytic virus can comprise an exogenous nucleic acid that can code for IL15. In some embodiments, the modified oncolytic virus can comprise an exogenous nucleic acid that can code for IL15, and exogenous nucleic acid that can code for CCL5. In some embodiments, the modified oncolytic virus can comprise an exogenous nucleic acid that can code for IL15, and exogenous nucleic acid that can code for IL15-Rα. In some embodiments, the modified oncolytic virus can comprise an exogenous nucleic acid that can code for ITAC (CXCL11), and an exogenous nucleic acid that can code for a fractalkine (CX3CL1). In some embodiments, the modified oncolytic virus can comprise an exogenous nucleic acid that can code for ITAC (CXCL11), an exogenous nucleic acid that can code for a fractalkine (CX3CL1), an exogenous nucleic acid that can code for IL15, and exogenous nucleic acid that can code for IL15-Rα.

[00230] In some embodiments, the modified oncolytic virus can comprise a mutation or deletion of the K7R gene, and can further comprise an exogenous nucleic acid that can code for a cytokine, *e.g.*, IL15. In some embodiments, the modified oncolytic virus can comprise a mutation or deletion of the K7R gene, and can further comprise an exogenous nucleic acid that can code for a chemokine, *e.g.*, IL15, and an exogenous nucleic acid that can code for a chemokine, *e.g.*, CCL5. In some embodiments, the modified oncolytic virus can comprise a mutation or deletion of the K7R gene, and can further comprise an exogenous nucleic acid that can code for a cytokine, *e.g.*, IL15, and an exogenous nucleic acid that can code for a receptor for the cytokine, *e.g.*, IL15Rα. In some embodiments, the modified oncolytic virus can comprise a mutation or deletion of the K7R gene, and can further comprise exogenous nucleic acid that can code for LIGHT. In some embodiments, the modified oncolytic virus can comprise a mutation or deletion of the K7R gene, and can further comprise an exogenous nucleic acid that can code for ITAC (CXCL11). In some embodiments, the modified oncolytic virus can comprise a mutation or deletion of the K7R gene, and comprise an exogenous nucleic acid that can code for a fractalkine (CX3CL1). In some embodiments, the modified oncolytic virus can comprise a mutation or deletion of the K7R gene, and can further comprise an exogenous nucleic acid that can code for ITAC (CXCL11), and an exogenous nucleic acid that can code for a fractalkine (CX3CL1).

[00231] In some embodiments, the modified oncolytic virus can comprise a mutation or deletion of the A52R gene, and can further comprise an exogenous nucleic acid that can code for a cytokine, *e.g.*, IL15. In some embodiments, the modified oncolytic virus can comprise a

mutation or deletion of the A52R gene, and can further comprise an exogenous nucleic acid that can code for a chemokine, *e.g.*, IL15, and an exogenous nucleic acid that can code for a chemokine, *e.g.*, CCL5. In some embodiments, the modified oncolytic virus can comprise a mutation or deletion of the A52R gene, and can further comprise an exogenous nucleic acid that can code for a cytokine, *e.g.*, IL15, and an exogenous nucleic acid that can code for a receptor for the cytokine, *e.g.*, IL15Rα. In some embodiments, the modified oncolytic virus can comprise a mutation or deletion of the A52R gene, and can further comprise exogenous nucleic acid that can code for LIGHT. In some embodiments, the modified oncolytic virus can comprise a mutation or deletion of the A52R gene, and can further comprise an exogenous nucleic acid that can code for ITAC (CXCL11). In some embodiments, the modified oncolytic virus can comprise a mutation or deletion of the A52R gene, and comprise an exogenous nucleic acid that can code for a fractalkine (CX3CL1). In some embodiments, the modified oncolytic virus can comprise a mutation or deletion of the A52R gene, and can further comprise an exogenous nucleic acid that can code for ITAC (CXCL11), and an exogenous nucleic acid that can code for a fractalkine (CX3CL1).

[00232] In some examples, the co-expression of a cytokine (*e.g.*, IL15) and its receptor (*e.g.*, IL15-Rα), from the modified oncolytic virus, can, in some cases, lead to enhanced immunomodulatory effects of the oncolytic virus, for example, due to improved ability of a complex formed by IL15 and IL15-Rα (IL15:IL15-R complex) to activate natural killer cells and promote T cell response. Without being bound by any specific theory, it is contemplated that the IL15 in the IL15:IL15-Rα complex can be presented to the IL15-Rβγ (IL15-receptor beta gamma complex) displayed on the surface of T cells and natural killer (NK) cells, thereby imparting potent immunomodulatory effects on the NK cells and the T cells.

[00233] In certain embodiments are provided modified oncolytic viruses wherein the A52R gene can be mutated or deleted, and further, wherein the modified oncolytic viruses can comprise an exogenous nucleic acid that can code for a secreted hyaluronidase, such as HysA. In certain embodiments are provided modified oncolytic viruses wherein the A52R gene can be mutated or deleted, and further, wherein the modified oncolytic viruses can comprise an exogenous nucleic acid that can code for a chemokine receptor, such as CXCR4. In certain embodiments are provided modified oncolytic viruses wherein the A52R gene can be mutated or deleted, and further, wherein the modified oncolytic viruses can comprise an exogenous nucleic acid that can code for a chemokine receptor, such as CXCR4, and an exogenous nucleic acid that can code for a secreted hyaluronidase, such as HysA.

[00234] In certain embodiments are provided modified oncolytic viruses wherein the A52R gene can be mutated or deleted, and further, wherein the modified oncolytic viruses can comprise an exogenous nucleic acid that can code for a membrane associated hyaluronidase, such as PH-20 (also known as SPAM-1). In certain embodiments are provided modified oncolytic viruses wherein the A52R gene can be mutated or deleted, and further, wherein the modified oncolytic viruses can comprise an exogenous nucleic acid that can code for a chemokine receptor, such as CXCR4. In certain embodiments are provided modified oncolytic viruses wherein the A52R gene can be mutated or deleted, and further, wherein the modified oncolytic viruses can comprise an exogenous nucleic acid that can code for a chemokine receptor, such as CXCR4, and an exogenous nucleic acid that can code for a membrane associated hyaluronidase, such as PH-20 (or SPAM-1).

[00235] In certain embodiments, the modified oncolytic virus can comprise a complete or a partial deletion of the viral thymidine kinase (TK) gene. According to certain embodiments, in the genome of the modified oncolytic virus disclosed herein, one or more of the exogenous nucleic acids are inserted in the loci of the deleted TK gene. Exemplary nucleic acid sequences for the viral genes disclosed herein, and amino acid sequences for the proteins coded by said genes, are provided in Table 3.

[00236] In some embodiments, in the modified oncolytic virus, such as in an oncolytic vaccinia virus, the viral TK gene may be replaced with a TK gene from a herpes simplex virus (HSV-TK). The HSV TK may function as a substitute for the deleted TK and may have multifaceted advantages. For instance, (i) HSV TK can be used as an additional therapeutic prodrug converting ganciclovir (GCV) into its cytotoxic metabolite in a tumor. In addition to the added therapeutic effect this modification can also serve as a suicide gene, e.g., vaccinia expressing cells can be killed efficiently through addition of GCV, thereby shutting down the virus in the case of an adverse event or uncontrolled replication. Thus, in some instances, the modified oncolytic virus of this disclosure can act as a safety switch). In additional examples, a mutated version of the HSV TK can be used to allow for PET imaging of labelled substrates with greatly increased sensitivity. Thus, in some cases, the modified oncolytic virus, comprising a HSV TK that can be used in PET imaging, can act as a reporter of viral replication *in vivo* to determine therapeutic activity early after treatment.

[00237] In some cases, the modified oncolytic virus can comprise a full-length viral backbone gene or viral backbone protein described above, or truncated versions thereof, or functional domains thereof, or fragments thereof, or variants thereof. In various examples,

the modified oncolytic virus can comprise mutation or deletion of one or more of viral backbones genes or viral backbone proteins, as described above. Mutations of the viral backbone genes and viral backbone proteins can comprise insertion, deletion, substitution, or modifications of nucleotides in nucleic acid sequences and amino acids in protein sequences. Deletion can comprise, in some examples, a complete or partial deletion of the viral backbone gene or protein.

Cancer Targets

[00238] In an embodiment of this disclosure, a method of treatment for a hyperproliferative disease, such as a cancer or a tumor, by the delivery of a modified oncolytic virus, such as an oncolytic vaccinia virus as described herein, is contemplated. Cancers that can be treated by a modified oncolytic virus, as described herein, can include, but are not limited to, melanoma, hepatocellular carcinoma, breast cancer, lung cancer, peritoneal cancer, prostate cancer, bladder cancer, ovarian cancer, leukemia, lymphoma, renal carcinoma, pancreatic cancer, epithelial carcinoma, gastric cancer, colon carcinoma, duodenal cancer, pancreatic adenocarcinoma, mesothelioma, glioblastoma multiforme, astrocytoma, multiple myeloma, prostate carcinoma, hepatocellular carcinoma, cholangiosarcoma, pancreatic adenocarcinoma, head and neck squamous cell carcinoma, colorectal cancer, intestinal-type gastric adenocarcinoma, cervical squamous-cell carcinoma, osteosarcoma, epithelial ovarian carcinoma, acute lymphoblastic lymphoma, myeloproliferative neoplasms, and sarcoma.

[00239] Cancer cells that can be treated by the methods of this disclosure include cells from the bladder, blood, bone, bone marrow, brain, breast, colon, esophagus, gastrointestine, gum, head, kidney, liver, lung, nasopharynx, neck, ovary, prostate, skin, stomach, testis, tongue, or uterus. In addition, the cancer may specifically be of the following histological type, though it is not limited to these: neoplasm, malignant; carcinoma; carcinoma, undifferentiated; giant and spindle cell carcinoma; small cell carcinoma; papillary carcinoma; squamous cell carcinoma; lymphoepithelial carcinoma; basal cell carcinoma; pilomatrix carcinoma; transitional cell carcinoma; papillary transitional cell carcinoma; adenocarcinoma; gastrinoma, malignant; cholangiocarcinoma; hepatocellular carcinoma; combined hepatocellular carcinoma and cholangiocarcinoma; trabecular adenocarcinoma; adenoid cystic carcinoma; adenocarcinoma in adenomatous polyp; adenocarcinoma, familial polyposis coli; solid carcinoma; carcinoid tumor, malignant; branchiolo-alveolar adenocarcinoma; papillary adenocarcinoma; chromophobe carcinoma; acidophil carcinoma; oxyphilic adenocarcinoma; basophil carcinoma; clear cell

adenocarcinoma; granular cell carcinoma; follicular adenocarcinoma; papillary and follicular adenocarcinoma; nonencapsulating sclerosing carcinoma; adrenal cortical carcinoma; endometroid carcinoma; skin appendage carcinoma; apocrine adenocarcinoma; sebaceous adenocarcinoma; ceruminous adenocarcinoma; mucoepidermoid carcinoma; cystadenocarcinoma; papillary cystadenocarcinoma; papillary serous cystadenocarcinoma; mucinous cystadenocarcinoma; mucinous adenocarcinoma; signet ring cell carcinoma; infiltrating duct carcinoma; medullary carcinoma; lobular carcinoma; inflammatory carcinoma; paget's disease, mammary; acinar cell carcinoma; adenosquamous carcinoma; adenocarcinoma w/squamous metaplasia; thymoma, malignant; ovarian stromal tumor, malignant; thecoma, malignant; granulosa cell tumor, malignant; androblastoma, malignant; sertoli cell carcinoma; leydig cell tumor, malignant; lipid cell tumor, malignant; paraganglioma, malignant; extra-mammary paraganglioma, malignant; pheochromocytoma; glomangiosarcoma; malignant melanoma; amelanotic melanoma; superficial spreading melanoma; malignant melanoma in giant pigmented nevus; epithelioid cell melanoma; blue nevus, malignant; sarcoma; fibrosarcoma; fibrous histiocytoma, malignant; myxosarcoma; liposarcoma; leiomyosarcoma; rhabdomyosarcoma; embryonal rhabdomyosarcoma; alveolar rhabdomyosarcoma; stromal sarcoma; mixed tumor, malignant; mullerian mixed tumor; nephroblastoma; hepatoblastoma; carcinosarcoma; mesenchymoma, malignant; brenner tumor, malignant; phyllodes tumor, malignant; synovial sarcoma; mesothelioma, malignant; dysgerminoma; embryonal carcinoma; teratoma, malignant; struma ovarii, malignant; choriocarcinoma; mesonephroma, malignant; hemangiosarcoma; hemangioendothelioma, malignant; Kaposi's sarcoma; hemangiopericytoma, malignant; lymphangiosarcoma; osteosarcoma; juxtacortical osteosarcoma; chondrosarcoma; chondroblastoma, malignant; mesenchymal chondrosarcoma; giant cell tumor of bone; Ewing's sarcoma; odontogenic tumor, malignant; ameloblastic odontosarcoma; ameloblastoma, malignant; ameloblastic fibrosarcoma; pinealoma, malignant; chordoma; glioma, malignant; ependymoma; astrocytoma; protoplasmic astrocytoma; fibrillary astrocytoma; astroblastoma; glioblastoma; oligodendroglioma; oligodendroblastoma; primitive neuroectodermal; cerebellar sarcoma; ganglioneuroblastoma; neuroblastoma; retinoblastoma; olfactory neurogenic tumor; meningioma, malignant; neurofibrosarcoma; neurilemmoma, malignant; granular cell tumor, malignant; malignant lymphoma; hodgkin's disease; hodgkin's; paragranuloma; malignant lymphoma, small lymphocytic; malignant lymphoma, large cell, diffuse; malignant lymphoma, follicular; mycosis fungoides; other specified non-hodgkin's

lymphomas; malignant histiocytosis; multiple myeloma; mast cell sarcoma; immunoproliferative small intestinal disease; leukemia; lymphoid leukemia; plasma cell leukemia; erythroleukemia; lymphosarcoma cell leukemia; myeloid leukemia; basophilic leukemia; eosinophilic leukemia; monocytic leukemia; mast cell leukemia; megakaryoblastic leukemia; myeloid sarcoma; and hairy cell leukemia. In some cases, solid cancers that are metastatic can be treated using the modified oncolytic viruses of this disclosure, such as a modified oncolytic vaccinia virus that is advantageous for systemic delivery. In some cases, solid cancers that are inaccessible or difficult to access, such as for purpose of intratumoral delivery of therapeutic agents, can be treated using the modified oncolytic viruses of this disclosure, such as a modified oncolytic vaccinia virus that is advantageous for systemic delivery. Cancers that are associated with increased expression of free fatty acids can, in some examples, be treated using the modified oncolytic viruses of this disclosure, such as a modified oncolytic vaccinia virus that is advantageous for systemic delivery and forms increased amounts of EEV.

[00240] This disclosure also contemplates methods for inhibiting or preventing local invasiveness or metastasis, or both, of any type of primary cancer. For example, the primary cancer can be melanoma, non-small cell lung, small-cell lung, lung, hepatocarcinoma, retinoblastoma, astrocytoma, glioblastoma, gum, tongue, leukemia, neuroblastoma, head, neck, breast, pancreatic, prostate, renal, bone, testicular, ovarian, mesothelioma, cervical, gastrointestinal, lymphoma, brain, colon, or bladder. In certain embodiments, the primary cancer can be lung cancer. For example, the lung cancer can be non-small cell lung carcinoma. Moreover, this disclosure can be used to prevent cancer or to treat pre-cancers or premalignant cells, including metaplasias, dysplasias, and hyperplasias. It can also be used to inhibit undesirable but benign cells, such as squamous metaplasia, dysplasia, benign prostate hyperplasia cells, hyperplastic lesions, and the like. In some embodiments, the progression to cancer or to a more severe form of cancer can be halted, disrupted, or delayed by methods of this disclosure involving the modified oncolytic virus as discussed herein.

[00241] Furthermore, the modified oncolytic virus as disclosed herein can be administered for treatment of tumors with high bioavailability of free fatty acids in the tumor microenvironment. In some instances, free fatty acids released by adipocytes in tumors in obese patients can feed and enhance the replication of the modified oncolytic virus within the tumor, and formation of EEV form of the virus. The advantage can also be realized in

non-obese patients, especially patients who have peritoneal cancer. For example, several peritoneal cancers can be targets for therapy using the modified oncolytic viruses of this disclosure as these tend to grow in omentum wall and can be fed by adipocytes, and as mentioned above free fatty acids released by adipocytes in tumors can feed and enhance the replication of the modified oncolytic virus within the tumor. The modified oncolytic virus as disclosed herein can form an increased titer of extracellular enveloped virus (EEV) in tumors with high bioavailability of free fatty acids.

Methods of Treatment and Assaying the Efficacy and Pharmacokinetics

[00242] This disclosure provides methods for treating a subject by administration of one or more modified oncolytic viruses, as disclosed herein. An "individual" or "subject," as used interchangeably herein, refers to a human or a non-human subject. Non-limiting examples of non-human subjects include non-human primates, dogs, cats, mice, rats, guinea pigs, rabbits, pigs, fowl, horses, cows, goats, sheep, cetaceans, etc. In some embodiments, the subject is human.

[00243] Provided is a method of producing a toxic effect in a cancer cell comprising administering, to the cancer cell, a therapeutically effective amount of a modified virus, such as an oncolytic vaccinia virus, as described above, or a pharmaceutical composition containing the same. This disclosure further provides a method of inhibiting at least one of growth and proliferation of a second cancer cell comprising administering, to a first cancer cell, a modified oncolytic virus as described above such that the first cancer cell is infected with said virus. Thus, in some embodiments of the methods disclosed here, it is contemplated that not every cancer or tumor cell is infected upon administering a therapeutically effective amount of an oncolytic vaccinia virus, as described herein, or a pharmaceutical composition containing the same, and growth of non-infected cells can be inhibited without direct infection.

[00244] In some examples, to induce oncolysis, kill cells, inhibit growth, inhibit metastases, decrease tumor size and otherwise reverse or reduce the malignant phenotype of tumor cells, using the methods and compositions of the present disclosure, a cancer cell or a tumor can be contacted with a therapeutically effective dose of an exemplary oncolytic vaccinia virus as described herein or a pharmaceutical composition containing the same. In certain embodiments, an effective amount of a modified oncolytic virus of the present disclosure, such as an oncolytic vaccinia virus as described herein or a pharmaceutical composition thereof, can include an amount sufficient to induce

oncolysis, the disruption or lysis of a cancer cell or the inhibition or reduction in the growth or size of a cancer cell. Reducing the growth of a cancer cell may be manifested, for example, by cell death or a slower replication rate or reduced growth rate of a tumor comprising the cell or a prolonged survival of a subject containing the cancer cell.

[00245] Provided, in some embodiments, is a method of treating a subject having a cancer or a tumor comprising administering, to the subject, an effective amount of a modified virus, as described above. An effective amount in such method can include an amount that reduces growth rate or spread of the cancer or that prolongs survival in the subject. This disclosure provides a method of reducing the growth of a tumor, which method can comprise administering, to the tumor, an effective amount of a modified oncolytic virus as described above. In certain embodiments, an effective amount of a modified virus, or a pharmaceutical composition thereof, can include an amount sufficient to induce the slowing, inhibition or reduction in the growth or size of a tumor and can include the eradication of the tumor. Reducing the growth of a tumor may be manifested, for example, by reduced growth rate or a prolonged survival of a subject containing the tumor.

[00246] This disclosure also provides a method of determining the infectivity or anti-tumor activity, or amount of tumor specific viral replication of an oncolytic vaccinia virus as described herein, which method can comprise; (i) administering to a subject a therapeutically effective amount of an oncolytic vaccinia virus or a pharmaceutical composition according to the present disclosure, which further expresses a luciferase reporter gene, alone or in combination with a further therapy; (ii) collecting a first biological sample from the subject immediately after administering the virus and determining the level of the luciferase reporter in the first biological sample (iii) collecting a second biological sample from the subject following the administration in step (ii) and (iii) detecting the level of the luciferase reporter in the second biological sample, wherein the oncolytic vaccinia virus is determined to be infective, demonstrate anti-tumor activity, exhibit tumor specific viral replication if the level of luciferase is higher in step (iii) than in step (ii). The second biological sample is collected about 30 mins, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 12 hours, about 15 hours, about 24 hours, about 36 hours, about 48 hours, about 72 hours, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, about 11 days, about 12 days, about 13 days, about 14 days, about 15 days, about 1 month, to about 2 months after the administration in step (i). In some embodiments, the method of mentioned above can further comprise, detecting in steps (i) and (iii), the level of one or more assaying

cytokine levels, *e.g.*, IL-2, IL-7, IL-8, IL-10, IFN-γ, GM-CSF, TNF-α, IL-6, IL-4, IL-5, and IL-13, in plasma samples collected from a subject after administering to said subject a therapeutically effective amount of a modified oncolytic virus of the present disclosure, such as an oncolytic vaccinia virus as described herein or a pharmaceutical composition comprising the same. In some embodiments of this disclosure, the increase in luciferase bioluminescence between steps (ii) and (iv) mentioned above is higher for a modified oncolytic virus as described herein, compared to that in an otherwise identical virus that does not comprise the modifications in the modified oncolytic virus. Other exemplary techniques for detecting and monitoring viral load after administration of the modified oncolytic viruses include real-time quantitative PCR.

[00247] Further provided is a method of monitoring the pharmacokinetics following administration of a therapeutically effective amount of modified oncolytic viruses according to the present disclosure, such as oncolytic vaccinia virus or a pharmaceutical composition containing the vaccinia virus, as described herein. An exemplary method for monitoring the pharmacokinetics can comprise the following steps: (i) administering to the subject a therapeutically effective amount of an oncolytic vaccinia virus or a pharmaceutical composition comprising the same, alone or in combination with a further therapy; (ii) collecting biological samples from the subject at one or more time points selected from about 15 minutes, about 30 minutes, about 45 mins, about 60 mins, about 75 mins, about 90 mins, about 120 mins, about 180 mins, and about 240 mins, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 12 hours, about 15 hours, about 24 hours, about 36 hours, about 48 hours, about 72 hours, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, about 11 days, about 12 days, about 13 days, about 14 days, about 15 days, about 1 month, to about 2 months after the administration in step (i) and (iii) detecting the quantity of the viral genome (or a reporter gene inserted within the viral genome, such as luciferase) in the biological samples collected at the above mentioned time points. In some instances, viral genome copies/mL can be highest in the sample collected at the 15 mins time point and further the sample collected at the 240 mins time point may not contain a detectable quantity of the viral genome. Therefore, in some instances, a viral peak can be observed at about 15 mins following administration and majority of the viruses can be cleared from the subject's system after about 240 mins (or 4 hours). In some instances, a first viral peak can be observed after about 15 mins following administration and a second viral peak can be observed in the biological samples collected in the subsequent time points, *e.g.*, at about 30 mins, about 45 mins, about 60 mins, or about 90

mins. The biological sample can be, in exemplar embodiments, blood, and the quantity of viral genome/mL can be determined by quantitative PCR or other appropriate techniques. In some examples, a first viral peak can be observed after about 15 mins following administration and a second viral peak can be observed after about 30 mins, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 12 hours, about 15 hours, about 24 hours, about 36 hours, about 48 hours, about 72 hours, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, about 11 days, about 12 days, about 13 days, about 14 days, about 15 days, about 1 month, to about 2 months following administration of a modified oncolytic virus of the present disclosure, such as an oncolytic vaccinia virus as described herein.

[00248] In some instances, tumor-selective replication of a modified virus, such as an oncolytic vaccinia virus can be measured through use of a reporter gene, such as a luciferase gene. In some embodiments, the luciferase gene can be inserted into the genome of a virus, and a tumor cell can be infected with the virus. Bioluminescence in infected tumor cells can be measured to monitor tumor-selective replication. Some examples show an increase in luciferase reporter bioluminescence in a modified oncolytic virus of this disclosure, compared to that in an otherwise identical oncolytic vaccinia virus that does not contain the modifications in the modified oncolytic virus.

Delivery of Modified Oncolytic Viruses

[00249] In some embodiments, amount of a modified oncolytic virus of this disclosure, such as an oncolytic vaccinia virus, administered to a subject can be between about 10^3 and 10^{12} infectious viral particles or plaque forming units (PFU), or between about 10^5 and 10^{10} PFU, or between about 10^5 and 10^8 PFU, or between about 10^8 and 10^{10} PFU. In some embodiments, the amount of a modified oncolytic virus of this disclosure, such as an oncolytic vaccinia virus administered to a subject can be between about 10^3 and 10^{12} viral particles or plaque forming units (PFU), or between about 10^5 and 10^{10} PFU, or between about 10^5 and 10^8 PFU, or between about 10^8 and 10^{10} PFU. In some embodiments, a modified oncolytic virus of this disclosure, such as an oncolytic vaccinia virus, can be administered at a dose that can comprise about 10^3 PFU/dose to about 10^4 PFU/dose, about 10^4 PFU/dose to about 10^5 PFU/dose, about 10^5 PFU/dose to about 10^6 PFU/dose, about 10^7 PFU/dose to about 10^8 PFU/dose, about 10^9 PFU/dose to about 10^{10} PFU/dose, about 10^{10} PFU/dose to about 10^{11} PFU/dose, about 10^{11} PFU/dose to about 10^{12} PFU/dose, about 10^{12}

PFU/dose to about 10^{13} PFU/dose, about 10^{13} PFU/dose to about 10^{14} PFU/dose, or about 10^{14} PFU/dose to about 10^{15} PFU/dose. In some embodiments, a modified oncolytic virus of this disclosure, such as an oncolytic vaccinia virus, can be administered at a dose that can comprise about 2 x 10^3 PFU/dose, 3 x 10^3 PFU/dose , 4 x 10^3 PFU/dose , 5 x 10^3 PFU/dose , 6 x 10^3 PFU/dose , 7 x 10^3 PFU/dose , 8 x 10^3 PFU/dose , 9 x 10^3 PFU/dose , about 10^4 PFU/dose, about 2 x 10^4 PFU/dose, about 3 x10^4 PFU/dose , about 4 x 10^4 PFU/dose , about 5 x 10^4 PFU/dose , about 6 x 10^4 PFU/dose , about 7 x 10^4 PFU/dose , about 8 x 10^4 PFU/dose , about 9 x 10^4 PFU/dose , about 10^5 PFU/dose, 2 x 10^5 PFU/dose , 3 x 10^5 PFU/dose , 4 x 10^5 PFU/dose , 5 x 10^5 PFU/dose , 6 x 10^5 PFU/dose , 7 x 10^5 PFU/dose , 8 x 10^5 PFU/dose , 9 x 10^5 PFU/dose , about 10^6 PFU/dose, about 2 x 10^6 PFU/dose , about 3 x 10^6 PFU/dose , about 4 x 10^6 PFU/dose , about 5 x 10^6 PFU/dose , about 6 x 10^6 PFU/dose , about 7 x 10^6 PFU/dose , about 8 x 10^6 PFU/dose , about 9 x 10^6 PFU/dose , about 10^7 PFU/dose, about 2 x 10^7 PFU/dose, about 3 x 10^7 PFU/dose, about 4 x 10^7 PFU/dose, about 5 x 10^7 PFU/dose, about 6 x 10^7 PFU/dose, about 7 x 10^7 PFU/dose, about 8 x 10^7 PFU/dose, about 9 x 10^7 PFU/dose, about 10^8 PFU/dose, about 2 x 10^8 PFU/dose, about 3 x 10^8 PFU/dose , about 4 x 10^8 PFU/dose , about 5 x 10^8 PFU/dose , about 6 x 10^8 PFU/dose , about 7 x 10^8 PFU/dose , about 8 x 10^8 PFU/dose , about 9 x 10^8 PFU/dose , about 10^9 PFU/dose, about 2 x 10^9 PFU/dose, about 3 x 10^9 PFU/dose, about 4 x 10^9 PFU/dose, about 5 x 10^9 PFU/dose, about 6 x 10^9 PFU/dose, about 7 x 10^9 PFU/dose, about 8 x 10^9 PFU/dose, about 9 x 10^9 PFU/dose, about 10^{10} PFU/dose, about 2 x 10^{10} PFU/dose, about 3 x 10^{10} PFU/dose, about 4 x 10^{10} PFU/dose, about 5 x10^{10} PFU/dose , about 6 x 10^{10} PFU/dose, about 7 x 10^{10} PFU/dose , about 8 x 10^{10} PFU/dose , about 9 x 10^{10} PFU/dose, about 10^{10} PFU/dose, about 2 x 10^{10} PFU/dose, about 3 x 10^{10} PFU/dose , about 4 x 10^{10} PFU/dose , about 5 x 10^{10} PFU/dose , about 6 x 10^{10} PFU/dose , about 7 x 10^{10} PFU/dose , about 8 x 10^{10} PFU/dose , about 9 x 10^{10} PFU/dose , about 10^{11} PFU/dose, about 2 x 10^{11} PFU/dose , about 3 x 10^{11} PFU/dose , about 4 x 10^{11} PFU/dose , about 5 x 10^{11} PFU/dose , about 6 x 10^{11} PFU/dose , about 7 x 10^{11} PFU/dose , about 8 x 10^{11} PFU/dose , about 9 x 10^{11} PFU/dose , or about 10^{12} PFU/dose, about 10^{12} PFU/dose to about 10^{13} PFU/dose, about 10^{13} PFU/dose to about 10^{14} PFU/dose, or about 10^{14} PFU/dose to about 10^{15} PFU/dose. In some embodiments, a modified oncolytic virus of this disclosure, such as an oncolytic vaccinia virus, can be administered at a dose that can comprise 5 x 10^9 PFU/dose. In some embodiments, a modified oncolytic virus of this disclosure, such as an oncolytic vaccinia virus, can be administered at a dose that can comprise up to 5 x 10^9 PFU/dose.

[00250] In some embodiments, a modified oncolytic virus of this disclosure, such as an oncolytic vaccinia virus, can be administered at a dose that can comprise about 10^3 viral particles/dose to about 10^4 viral particles /dose, about 10^4 viral particles /dose to about 10^5 viral particles /dose, about 10^5 viral particles /dose to about 10^6 viral particles /dose, about 10^7 viral particles /dose to about 10^8 viral particles /dose, about 10^9 viral particles /dose to about 10^{10} viral particles /dose, about 10^{10} viral particles /dose to about 10^{11} viral particles /dose, about 10^{11} viral particles /dose to about 10^{12} viral particles /dose, about 10^{12} viral particles /dose to about 10^{13} viral particles /dose, about 10^{13} viral particles /dose to about 10^{14} viral particles /dose, or about 10^{14} viral particles /dose to about 10^{15} viral particles /dose.

[00251] In some embodiments, a modified oncolytic virus of this disclosure can be administered at a dose that can comprise about 10^3 PFU/kg to about 10^4 PFU/kg, about 10^4 PFU/kg to about 10^5 PFU/kg, about 10^5 PFU/kg to about 10^6 PFU/kg, about 10^7 PFU/kg to about 10^8 PFU/kg, about 10^9 PFU/kg to about 10^{10} PFU/kg, about 10^{10} PFU/kg to about 10^{11} PFU/kg, about 10^{11} PFU/kg to about 10^{12} PFU/kg, about 10^{12} PFU/kg to about 10^{13} PFU/kg, about 10^{13} PFU/kg to about 10^{14} PFU/kg, or about 10^{14} PFU/kg to about 10^{15} PFU/kg. In some embodiments, a modified oncolytic virus of this disclosure, such as an oncolytic vaccinia virus, can be administered at a dose that can comprise about 2 x 10^3 PFU/kg, 3 x 10^3 PFU/kg , 4 x 10^3 PFU/kg , 5 x 10^3 PFU/kg , 6 x 10^3 PFU/kg , 7 x 10^3 PFU/kg , 8 x 10^3 PFU/kg , 9 x 10^3 PFU/kg , about 10^4 PFU/kg, about 2 x 10^4 PFU/kg, about 3 x10^4 PFU/kg , about 4 x 10^4 PFU/kg , about 5 x 10^4 PFU/kg , about 6 x 10^4 PFU/kg , about 7 x 10^4 PFU/kg , about 8 x 10^4 PFU/kg , about 9 x 10^4 PFU/kg , about 10^5 PFU/kg, 2 x 10^5 PFU/kg , 3 x 10^5 PFU/kg , 4 x 10^5 PFU/kg , 5 x 10^5 PFU/kg , 6 x 10^5 PFU/kg , 7 x 10^5 PFU/kg , 8 x 10^5 PFU/kg , 9 x 10^5 PFU/kg , about 10^6 PFU/kg, about 2 x 10^6 PFU/kg , about 3 x 10^6 PFU/kg , about 4 x 10^6 PFU/kg , about 5 x 10^6 PFU/kg , about 6 x 10^6 PFU/kg , about 7 x 10^6 PFU/kg , about 8 x 10^6 PFU/kg , about 9 x 10^6 PFU/kg , about 10^7 PFU/kg, about 2 x 10^7 PFU/kg, about 3 x 10^7 PFU/kg, about 4 x 10^7 PFU/kg, about 5 x 10^7 PFU/kg, about 6 x 10^7 PFU/kg, about 7 x 10^7 PFU/kg, about 8 x 10^7 PFU/kg, about 9 x 10^7 PFU/kg, about 10^8 PFU/kg, about 2 x 10^8 PFU/kg, about 3 x 10^8 PFU/kg , about 4 x 10^8 PFU/kg , about 5 x 10^8 PFU/kg , about 6 x 10^8 PFU/kg , about 7 x 10^8 PFU/kg , about 8 x 10^8 PFU/kg , about 9 x 10^8 PFU/kg , about 10^9 PFU/kg, about 2 x 10^9 PFU/kg, about 3 x 10^9 PFU/kg, about 4 x 10^9 PFU/kg, about 5 x 10^9 PFU/kg, about 6 x 10^9 PFU/kg, about 7 x 10^9 PFU/kg, about 8 x 10^9 PFU/kg, about 9 x 10^9 PFU/kg, about 10^{10} PFU/kg, about 2 x 10^{10} PFU/kg, about 3 x 10^{10} PFU/kg, about 4 x 10^{10} PFU/kg, about 5 x10^{10} PFU/kg , about 6 x 10^{10} PFU/kg, about 7 x 10^{10} PFU/kg , about 8 x 10^{10} PFU/kg , about 9 x 10^{10} PFU/kg, about 10^{10} PFU/kg, about 2 x 10^{10}

PFU/kg, about 3 x 10^{10} PFU/kg , about 4 x 10^{10} PFU/kg , about 5 x 10^{10} PFU/kg , about 6 x 10^{10} PFU/kg , about 7 x 10^{10} PFU/kg , about 8 x 10^{10} PFU/kg , about 9 x 10^{10} PFU/kg , about 10^{11} PFU/kg, about 2 x 10^{11} PFU/kg , about 3 x 10^{11} PFU/kg , about 4 x 10^{11} PFU/kg , about 5 x 10^{11} PFU/kg , about 6 x 10^{11} PFU/kg , about 7 x 10^{11} PFU/kg , about 8 x 10^{11} PFU/kg , about 9 x 10^{11} PFU/kg , or about 10^{12} PFU/kg, about 10^{12} PFU/kg to about 10^{13} PFU/kg, about 10^{13} PFU/kg to about 10^{14} PFU/kg, or about 10^{14} PFU/kg to about 10^{15} PFU/kg. In some embodiments, a modified oncolytic virus of this disclosure, such as an oncolytic vaccinia virus, can be administered at a dose that can comprise 5 x 10^{9} PFU/kg. In some embodiments, a modified oncolytic virus of this disclosure, such as an oncolytic vaccinia virus, can be administered at a dose that can comprise up to 5 x 10^{9} PFU/kg.

[00252] In some embodiments, a modified oncolytic virus of this disclosure can be administered at a dose that can comprise about 10^{3} viral particles/kg to about 10^{4} viral particles/kg, about 10^{4} viral particles/kg to about 10^{5} viral particles/kg, about 10^{5} viral particles/kg to about 10^{6} viral particles/kg, about 10^{7} viral particles/kg to about 10^{8} viral particles/kg, about 10^{9} viral particles/kg to about 10^{10} viral particles/kg, about 10^{10} viral particles/kg to about 10^{11} viral particles/kg, about 10^{11} viral particles/kg to about 10^{12} viral particles/kg, about 10^{12} viral particles/kg to about 10^{13} viral particles/kg, about 10^{13} viral particles/kg to about 10^{14} viral particles/kg, or about 10^{14} viral particles/kg to about 10^{15} viral particles/kg.

[00253] A liquid dosage form of an oncolytic vaccinia virus as described herein can comprise, in certain embodiments, a viral dose of about 10^{3} PFU/mL to about 10^{4} PFU/mL, about 10^{4} PFU/mL to about 10^{5} PFU/mL, about 10^{5} PFU/mL to about 10^{6} PFU/mL, about 10^{7} PFU/mL to about 10^{8} PFU/mL, about 10^{9} PFU/mL to about 10^{10} PFU/mL, about 10^{10} PFU/mL to about 10^{11} PFU/mL, about 10^{11} PFU/mL to about 10^{12} PFU/mL, about 10^{12} PFU/mL to about 10^{13} PFU/mL, about 10^{13} PFU/mL to about 10^{14} PFU/mL, or about 10^{14} PFU/mL to about 10^{15} PFU/mL. In some embodiments, a modified oncolytic virus of this disclosure, such as an oncolytic vaccinia virus, can be administered at a dose that can comprise about 2 x 10^{3} PFU/mL, 3 x 10^{3} PFU/mL , 4 x 10^{3} PFU/mL , 5 x 10^{3} PFU/mL , 6 x 10^{3} PFU/mL , 7 x 10^{3} PFU/mL , 8 x 10^{3} PFU/mL , 9 x 10^{3} PFU/mL , about 10^{4} PFU/mL, about 2 x 10^{4} PFU/mL, about 3 x10^{4} PFU/mL , about 4 x 10^{4} PFU/mL , about 5 x 10^{4} PFU/mL , about 6 x 10^{4} PFU/mL , about 7 x 10^{4} PFU/mL , about 8 x 10^{4} PFU/mL , about 9 x 10^{4} PFU/mL , about 10^{5} PFU/mL, 2 x 10^{5} PFU/mL , 3 x 10^{5} PFU/mL , 4 x 10^{5} PFU/mL , 5 x 10^{5} PFU/mL , 6 x 10^{5} PFU/mL , 7 x 10^{5} PFU/mL , 8 x 10^{5} PFU/mL , 9 x 10^{5} PFU/mL , about 10^{6} PFU/mL, about 2 x 10^{6} PFU/mL , about 3 x 10^{6} PFU/mL , about 4 x 10^{6} PFU/mL , about

5 x 10^6 PFU/mL , about 6 x 10^6 PFU/mL , about 7 x 10^6 PFU/mL , about 8 x 10^6 PFU/mL , about 9 x 10^6 PFU/mL , about 10^7 PFU/mL, about 2 x 10^7 PFU/mL, about 3 x 10^7 PFU/mL, about 4 x 10^7 PFU/mL, about 5 x 10^7 PFU/mL, about 6 x 10^7 PFU/mL, about 7 x 10^7 PFU/mL, about 8 x 10^7 PFU/mL, about 9 x 10^7 PFU/mL, about 10^8 PFU/mL, about 2 x 10^8 PFU/mL, about 3 x 10^8 PFU/mL , about 4 x 10^8 PFU/mL , about 5 x 10^8 PFU/mL , about 6 x 10^8 PFU/mL , about 7 x 10^8 PFU/mL , about 8 x 10^8 PFU/mL , about 9 x 10^8 PFU/mL , about 10^9 PFU/mL, about 2 x 10^9 PFU/mL, about 3 x 10^9 PFU/mL, about 4 x 10^9 PFU/mL, about 5 x 10^9 PFU/mL, about 6 x 10^9 PFU/mL, about 7 x 10^9 PFU/mL, about 8 x 10^9 PFU/mL, about 9 x 10^9 PFU/mL, about 10^{10} PFU/mL, about 2 x 10^{10} PFU/mL, about 3 x 10^{10} PFU/mL, about 4 x 10^{10} PFU/mL, about 5 x10^{10} PFU/mL , about 6 x 10^{10} PFU/mL, about 7 x 10^{10} PFU/mL , about 8 x 10^{10} PFU/mL , about 9 x 10^{10} PFU/mL, about 10^{10} PFU/mL, about 2 x 10^{10} PFU/mL, about 3 x 10^{10} PFU/mL , about 4 x 10^{10} PFU/mL , about 5 x 10^{10} PFU/mL , about 6 x 10^{10} PFU/mL , about 7 x 10^{10} PFU/mL , about 8 x 10^{10} PFU/mL , about 9 x 10^{10} PFU/mL , about 10^{11} PFU/mL, about 2 x 10^{11} PFU/mL , about 3 x 10^{11} PFU/mL , about 4 x 10^{11} PFU/mL , about 5 x 10^{11} PFU/mL , about 6 x 10^{11} PFU/mL , about 7 x 10^{11} PFU/mL , about 8 x 10^{11} PFU/mL , about 9 x 10^{11} PFU/mL , or about 10^{12} PFU/mL, about 10^{12} PFU/mL to about 10^{13} PFU/mL, about 10^{13} PFU/mL to about 10^{14} PFU/mL, or about 10^{14} PFU/mL to about 10^{15} PFU/mL. In some embodiments, a modified oncolytic virus of this disclosure, such as an oncolytic vaccinia virus, can be administered at a dose that can comprise 5 x 10^9 PFU/mL. In some embodiments, a modified oncolytic virus of this disclosure, such as an oncolytic vaccinia virus, can be administered at a dose that can comprise up to 5 x 10^9 PFU/mL.

[00254] In some instances, where the modified oncolytic virus is administered by an injection, the dosage can comprise about 10^3 viral particles per injection, 10^4 viral particles per injection, 10^5 viral particles per injection, 10^6 viral particles per injection, 10^7 viral particles per injection, 10^8 viral particles per injection, 10^9 viral particles per injection, 10^{10} viral particles per injection, 10^{11} viral particles per injection, 10^{12} viral particles per injection, 2 x 10^{12} viral particles per injection, 10^{13} viral particles per injection, 10^{14} viral particles per injection, or 10^{15} viral particles per injection. In further instances, where the modified oncolytic virus is administered by an injection, the dosage can comprise about 10^3 infectious viral particles per injection, 10^4 infectious viral particles per injection, 10^5 infectious viral particles per injection, 10^6 infectious viral particles per injection, 10^7 infectious viral particles per injection, 10^8 infectious viral particles per injection, 10^9 infectious viral particles per injection, 10^{10} infectious viral particles per injection, 10^{11}

infectious viral particles per injection, 10^{12} infectious viral particles per injection, 2 x 10^{12} infectious viral particles per injection, 10^{13} infectious viral particles per injection, 10^{14} infectious viral particles per injection, or 10^{15} infectious viral particles per injection. In additional embodiments, a modified oncolytic virus of this disclosure can be administered at a dose that can be about 10^3 Tissue Culture Inhibitor Dose 50% ($TCID_{50}$)/kg, 10^4 $TCID_{50}$/kg, 10^4 $TCID_{50}$/kg, 10^4 $TCID_{50}$/kg, 10^4 $TCID_{50}$/kg, 10^4 $TCID_{50}$/kg, 10^4 $TCID_{50}$/kg, 10^4 $TCID_{50}$/kg, 10^4 $TCID_{50}$/kg, 10^4 $TCID_{50}$/kg, 10^4 $TCID_{50}$/kg, 10^4 $TCID_{50}$/kg, $3x10^8$ $TCID_{50}$/kg, $4x10^8$ $TCID_{50}$/kg, $5x10^8$ $TCID_{50}$/kg, $3x10^9$ $TCID_{50}$/kg, $4x10^9$ $TCID_{50}$/kg, $5x10^9$ $TCID_{50}$/kg, $3x10^{10}$ $TCID_{50}$/kg, $4x10^{10}$ $TCID_{50}$/kg, or $4x10^{10}$ $TCID_{50}$/kg. Note that herein 10^x is alternatively expressed as 1 eX. In certain embodiments, the modified oncolytic virus can be administered in one or more doses. In certain embodiments, the virus can be administered in an amount sufficient to induce oncolysis in at least about 20% of cells in a tumor, in at least about 30% of cells in a tumor, in at least about 40% of cells in a tumor, in at least about 50% of cells in a tumor, in at least about 60% of cells in a tumor, in at least about 70% of cells in a tumor, in at least about 80% of cells in a tumor, or in at least about 90% of cells in a tumor. In certain embodiments, a single dose of virus can refer to the amount administered to a subject or a tumor over a 1, 2, 5, 10, 15, 20 or 24 hour period. In certain embodiments, the dose can be spread over time or by separate injection. In certain embodiments, multiple doses *(e.g.,* 2, 3, 4, 5, 6 or more doses) of the vaccinia virus can be administered to the subject, for example, where a second treatment can occur within 1, 2, 3, 4, 5, 6, 7 days or weeks of a first treatment. In certain embodiments, multiple doses of the modified oncolytic virus can be administered to the subject over a period of 1, 2, 3, 4, 5, 6, 7 or more days or weeks. In certain embodiments, the oncolytic vaccina virus or the pharmaceutical composition as described herein can be administered over a period of about 1 week to about 2 weeks, about 2 weeks to about 3 weeks, about 3 weeks to about 4 weeks, about 4 weeks to about 5 weeks, about 6 weeks to about 7 weeks, about 7 weeks to about 8 weeks, about 8 weeks to about 9 weeks, about 9 weeks to about 10 weeks, about 10 weeks to about 11 weeks, about 11 weeks to about 12 weeks, about 12 weeks to about 24 weeks, about 24 weeks to about 48 weeks, about 48 weeks or about 52 weeks, or longer. The frequency of administration of the oncolytic vaccinia virus or the pharmaceutical composition as described herein can be, in certain instances, once daily, twice daily, once every week, once every three weeks, once every four weeks (or once a month), once every 8 weeks (or once every 2 months), once every 12 weeks (or once every 3 months), or once every 24 weeks (once every 6 months). In

some embodiments of the methods disclosed herein, the oncolytic vaccinia virus or the pharmaceutical composition can be administered, independently, in an initial dose for a first period of time, an intermediate dose for a second period of time, and a high dose for a third period of time. In some embodiments, the initial dose is lower than the intermediate dose and the intermediate dose is lower than the high dose. In some embodiments, the first, second, and third periods of time are, independently, about 1 week to about 2 weeks, about 2 weeks to about 3 weeks, about 3 weeks to about 4 weeks, about 4 weeks to about 5 weeks, about 6 weeks to about 7 weeks, about 7 weeks to about 8 weeks, about 8 weeks to about 9 weeks, about 9 weeks to about 10 weeks, about 10 weeks to about 11 weeks, about 11 weeks to about 12 weeks, about 12 weeks to about 24 weeks, about 24 weeks to about 48 weeks, about 48 weeks or about 52 weeks, or longer.

[00255] In some examples, the subject can be put on a reduced carbohydrate diet, *e.g.*, a ketogenic diet prior to, concurrent with, and following administration of the modified oncolytic viruses, such as the oncolytic vaccinia viruses or the pharmaceutical composition comprising the same, as described herein, according to any of the methods of treatment described herein. In certain embodiments, the subject is put on a diet that can comprise consuming less than 500 grams of carbohydrates per day, less than 450 grams of carbohydrates per day, less than 450 grams of carbohydrates per day, less than 400 grams of carbohydrates per day, less than 350 grams of carbohydrates per day, less than 300 grams of carbohydrates per day, less than 250 grams of carbohydrates per day, less than 200 grams of carbohydrates per day, less than 150 grams of carbohydrates per day, less than 100 grams of carbohydrates per day, less than 90 grams of carbohydrates per day, less than 80 grams of carbohydrates per day, less than 70 grams of carbohydrates per day, less than 60 grams of carbohydrates per day, less than 50 grams of carbohydrates per day, less than 40 grams of carbohydrates per day, less than 30 grams of carbohydrates per day, less than 20 grams of carbohydrates per day, less or than 10 grams of carbohydrates per day.

[00256] An exemplary method for the delivery of a modified oncolytic virus of the present disclosure, such as an oncolytic vaccinia virus as described herein or a pharmaceutical composition comprising the same, to cancer or tumor cells can be *via* intratumoral injection. However, alternate methods of administration can also be used, *e.g*, intravenous, *via* infusion, parenteral, intravenous, intradermal, intramuscular, transdermal, rectal, intraurethral, intravaginal, intranasal, intrathecal, or intraperitoneal. The routes of administration can vary with the location and nature of the tumor. In certain embodiments, the route of administration can be intradental, transdermal, parenteral,

intravenous, intramuscular, intranasal, subcutaneous, regional *(e.g.,* in the proximity of a tumor, particularly with the vasculature or adjacent vasculature of a tumor), percutaneous, intrathecal, intratracheal, intraperitoneal, intraarterial, intravesical, intratumoral, inhalation, perfusion, by lavage or orally. An injectable dose of the oncolytic virus can be administered as a bolus injection or as a slow infusion. In certain embodiments, the modified oncolytic virus can be administered to the patient from a source implanted in the patient. In certain embodiments, administration of the modified oncolytic virus can occur by continuous infusion over a selected period of time. In some instances, an oncolytic vaccinia virus as described herein, or a pharmaceutical composition containing the same can be administered at a therapeutically effective dose by infusion over a period of about 15 mins, about 30 mins, about 45 mins, about 50 mins, about 55 mins, about 60 minutes, about 75 mins, about 90 mins, about 100 mins, or about 120 mins or longer. The oncolytic vaccina virus or the pharmaceutical composition of the present disclosure can be administered as a liquid dosage, wherein the total volume of administration is about 1 mL to about 5 mL, about 5 mL to 10 mL, about 15 mL to about 20 mL, about 25 mL to about 30 mL, about 30 mL to about 50 mL, about 50 mL to about 100 mL, about 100 mL to 150 mL, about 150 mL to about 200 mL, about 200 mL to about 250 mL, about 250 mL to about 300 mL, about 300 mL to about 350 mL, about 350 mL to about 400 mL, about 400 mL to about 450 mL, about 450 mL to 500 mL, about 500 mL to 750 mL, or about 750 mL to 1000 mL.

Pharmaceutical Compositions

[00257] Pharmaceutical compositions containing a modified virus, such as an oncolytic vaccinia virus, as described herein, can be prepared as solutions, dispersions in glycerol, liquid polyethylene glycols, and any combinations thereof in oils, in solid dosage forms, as inhalable dosage forms, as intranasal dosage forms, as liposomal formulations, dosage forms comprising nanoparticles, dosage forms comprising microparticles, polymeric dosage forms, or any combinations thereof. In some embodiments, a pharmaceutical composition as described herein can comprise a stabilizer and a buffer. In some embodiments, a pharmaceutical composition as described herein can comprise a solubilizer, such as sterile water, Tris-buffer. In some embodiments, a pharmaceutical composition as described herein can comprise an excipient. An excipient can be an excipient described in the Handbook of Pharmaceutical Excipients, American Pharmaceutical Association (1986). Non-limiting examples of suitable excipients can include a buffering agent, a preservative, a stabilizer, a

binder, a compaction agent, a lubricant, a chelator, a dispersion enhancer, a disintegration agent, a flavoring agent, a sweetener, a coloring agent.

[00258] In some embodiments an excipient can be a buffering agent. Non-limiting examples of suitable buffering agents can include sodium citrate, magnesium carbonate, magnesium bicarbonate, calcium carbonate, and calcium bicarbonate. As a buffering agent, sodium bicarbonate, potassium bicarbonate, magnesium hydroxide, magnesium lactate, magnesium gluconate, aluminium hydroxide, sodium citrate, sodium tartrate, sodium acetate, sodium carbonate, sodium polyphosphate, potassium polyphosphate, sodium pyrophosphate, potassium pyrophosphate, disodium hydrogen phosphate, dipotassium hydrogen phosphate, trisodium phosphate, tripotassium phosphate, potassium metaphosphate, magnesium oxide, magnesium hydroxide, magnesium carbonate, magnesium silicate, calcium acetate, calcium glycerophosphate, calcium chloride, calcium hydroxide and other calcium salts or combinations thereof can be used in a pharmaceutical formulation.

[00259] In some embodiments an excipient can comprise a preservative. Non-limiting examples of suitable preservatives can include antioxidants, such as alpha-tocopherol and ascorbate, and antimicrobials, such as parabens, chlorobutanol, and phenol. Antioxidants can further include but not limited to EDTA, citric acid, ascorbic acid, butylated hydroxytoluene (BHT), butylated hydroxy anisole (BHA), sodium sulfite, p-amino benzoic acid, glutathione, propyl gallate, cysteine, methionine, ethanol and N- acetyl cysteine. In some instances a preservatives can include validamycin A, TL-3, sodium ortho vanadate, sodium fluoride, N-a-tosyl-Phe- chloromethylketone, N-a-tosyl-Lys-chloromethylketone, aprotinin, phenylmethylsulfonyl fluoride, diisopropylfluorophosphate, kinase inhibitor, phosphatase inhibitor, caspase inhibitor, granzyme inhibitor, cell adhesion inhibitor, cell division inhibitor, cell cycle inhibitor, lipid signaling inhibitor, protease inhibitor, reducing agent, alkylating agent, antimicrobial agent, oxidase inhibitor, or other inhibitor.

[00260] In some embodiments a pharmaceutical composition as described herein can comprise a binder as an excipient. Non-limiting examples of suitable binders can include starches, pregelatinized starches, gelatin, polyvinylpyrolidone, cellulose, methylcellulose, sodium carboxymethylcellulose, ethylcellulose, polyacrylamides, polyvinyloxoazolidone, polyvinylalcohols, C_{12}-C_{18} fatty acid alcohol, polyethylene glycol, polyols, saccharides, oligosaccharides, and combinations thereof. The binders that can be used in a pharmaceutical formulation can be selected from starches such as potato starch, corn starch, wheat starch; sugars such as sucrose, glucose, dextrose, lactose, maltodextrin; natural and synthetic gums; gelatine; cellulose derivatives such as microcrystalline cellulose, hydroxypropyl cellulose,

hydroxyethyl cellulose, hydroxypropyl methyl cellulose, carboxymethyl cellulose, methyl cellulose, ethyl cellulose; polyvinylpyrrolidone (povidone); polyethylene glycol (PEG); waxes; calcium carbonate; calcium phosphate; alcohols such as sorbitol, xylitol, mannitol and water or a combination thereof.

[00261] In some embodiments a pharmaceutical composition as described herein can comprise a lubricant as an excipient. Non-limiting examples of suitable lubricants can include magnesium stearate, calcium stearate, zinc stearate, hydrogenated vegetable oils, sterotex, polyoxyethylene monostearate, talc, polyethyleneglycol, sodium benzoate, sodium lauryl sulfate, magnesium lauryl sulfate, and light mineral oil. The lubricants that can be used in a pharmaceutical formulation can be selected from metallic stearates (such as magnesium stearate, calcium stearate, aluminium stearate), fatty acid esters (such as sodium stearyl fumarate), fatty acids (such as stearic acid), fatty alcohols, glyceryl behenate, mineral oil, paraffins, hydrogenated vegetable oils, leucine, polyethylene glycols (PEG), metallic lauryl sulphates (such as sodium lauryl sulphate, magnesium lauryl sulphate), sodium chloride, sodium benzoate, sodium acetate and talc or a combination thereof.

In some embodiments a pharmaceutical formulation can comprise a dispersion enhancer as an excipient. Non-limiting examples of suitable dispersants can include starch, alginic acid, polyvinylpyrrolidones, guar gum, kaolin, bentonite, purified wood cellulose, sodium starch glycolate, isoamorphous silicate, and microcrystalline cellulose as high HLB emulsifier surfactants.

[00262] In some embodiments a pharmaceutical composition as described herein can comprise a disintegrant as an excipient. In some embodiments a disintegrant can be a non-effervescent disintegrant. Non-limiting examples of suitable non-effervescent disintegrants can include starches such as corn starch, potato starch, pregelatinized and modified starches thereof, sweeteners, clays, such as bentonite, micro-crystalline cellulose, alginates, sodium starch glycolate, gums such as agar, guar, locust bean, karaya, pectin, and tragacanth. In some embodiments a disintegrant can be an effervescent disintegrant. Non-limiting examples of suitable effervescent disintegrants can include sodium bicarbonate in combination with citric acid, and sodium bicarbonate in combination with tartaric acid.

[00263] In some embodiments an excipient can comprise a flavoring agent. Flavoring agents incorporated into an outer layer can be chosen from synthetic flavor oils and flavoring aromatics; natural oils; extracts from plants, leaves, flowers, and fruits; and combinations thereof. In some embodiments a flavoring agent can be selected from the group consisting of cinnamon oils; oil of wintergreen; peppermint oils; clover oil; hay oil; anise oil; eucalyptus;

vanilla; citrus oil such as lemon oil, orange oil, grape and grapefruit oil; and fruit essences including apple, peach, pear, strawberry, raspberry, cherry, plum, pineapple, and apricot.

[00264] In some embodiments an excipient can comprise a sweetener. Non-limiting examples of suitable sweeteners can include glucose (corn syrup), dextrose, invert sugar, fructose, and mixtures thereof (when not used as a carrier); saccharin and its various salts such as a sodium salt; dipeptide sweeteners such as aspartame; dihydrochalcone compounds, glycyrrhizin; Stevia Rebaudiana (Stevioside); chloro derivatives of sucrose such as sucralose; and sugar alcohols such as sorbitol, mannitol, sylitol, and the like.

[00265] In some instances, a pharmaceutical composition as described herein can comprise a coloring agent. Non-limiting examples of suitable color agents can include food, drug and cosmetic colors (FD&C), drug and cosmetic colors (D&C), and external drug and cosmetic colors (Ext. D&C). A coloring agents can be used as dyes or their corresponding lakes.

[00266] In some instances, a pharmaceutical composition as described herein can comprise a chelator. In some cases, a chelator can be a fungicidal chelator. Examples can include, but are not limited to: ethylenediamine-N,N,N',N'-tetraacetic acid (EDTA); a disodium, trisodium, tetrasodium, dipotassium, tripotassium, dilithium and diammonium salt of EDTA; a barium, calcium, cobalt, copper, dysprosium, europium, iron, indium, lanthanum, magnesium, manganese, nickel, samarium, strontium, or zinc chelate of EDTA; trans-1,2-diaminocyclohexane-N,N,N',N'-tetraaceticacid monohydrate; N,N-bis(2-hydroxyethyl)glycine; 1,3-diamino-2-hydroxypropane-N,N,N',N'-tetraacetic acid; 1,3-diaminopropane-N,N,N',N'-tetraacetic acid; ethylenediamine-N,N'-diacetic acid; ethylenediamine-N,N'-dipropionic acid dihydrochloride; ethylenediamine-N,N'-bis(methylenephosphonic acid) hemihydrate; N-(2-hydroxyethyl)ethylenediamine-N,N',N'-triacetic acid; ethylenediamine-N,N,N',N'-tetrakis(methylenephosponic acid); O,O'-bis(2-aminoethyl)ethyleneglycol-N,N,N',N'-tetraacetic acid; N,N-bis(2-hydroxybenzyl)ethylenediamine-N,N-diacetic acid; 1,6-hexamethylenediamine-N,N,N',N'-tetraacetic acid; N-(2-hydroxyethyl)iminodiacetic acid; iminodiacetic acid; 1,2-diaminopropane-N,N,N',N'-tetraacetic acid; nitrilotriacetic acid; nitrilotripropionic acid; the trisodium salt of nitrilotris(methylenephosphoric acid); 7,19,30-trioxa-1,4,10,13,16,22,27,33-octaazabicyclo[11,11,11] pentatriacontane hexahydrobromide; or triethylenetetramine-N,N,N',N",N'",N'"-hexaacetic acid.

[00267] Also contemplated are combination products that include one or more modified oncolytic viruses disclosed herein and one or more other antimicrobial or antifungal agents, for example, polyenes such as amphotericin B, amphotericin B lipid complex (ABCD),

liposomal amphotericin B (L-AMB), and liposomal nystatin, azoles and triazoles such as voriconazole, fluconazole, ketoconazole, itraconazole, pozaconazole and the like; glucan synthase inhibitors such as caspofungin, micafungin (FK463), and V-echinocandin (LY303366); griseofulvin; allylamines such as terbinafine; flucytosine or other antifungal agents, including those described herein. In addition, it is contemplated that a peptide can be combined with topical antifungal agents such as ciclopirox olamine, haloprogin, tolnaftate, undecylenate, topical nystatin, amorolfine, butenafine, naftifine, terbinafine, and other topical agents. In some instances, a pharmaceutical composition can comprise an additional agent. In some cases, an additional agent can be present in a therapeutically effective amount in a pharmaceutical composition.

[00268] Under ordinary conditions of storage and use, the pharmaceutical compositions as described herein can comprise a preservative to prevent the growth of microorganisms. In certain examples, the pharmaceutical compositions as described herein may not comprise a preservative. The pharmaceutical forms suitable for injectable use can include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. The pharmaceutical compositions can comprise a carrier which is a solvent or a dispersion medium containing, for example, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and/or vegetable oils, or any combinations thereof. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[00269] For parenteral administration in an aqueous solution, for example, the liquid dosage form can be suitably buffered if necessary and the liquid diluent rendered isotonic with sufficient saline or glucose. The liquid dosage forms are especially suitable for intravenous, intramuscular, subcutaneous, intratumoral, and intraperitoneal administration. In this connection, sterile aqueous media that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved

in 1mL to 20 mL of isotonic NaCl solution and either added to 100 mL to 1000 mL of a fluid, *e.g.*, sodium-bicarbonate buffered saline, or injected at the proposed site of infusion.

[00270] In certain embodiments, sterile injectable solutions can be prepared by incorporating a modified oncolytic virus according to the present disclosure, such as oncolytic vaccinia viruses as described herein or a pharmaceutical composition containing the same, in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. The compositions disclosed herein may be formulated in a neutral or salt form. Pharmaceutically-acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. Upon formulation, the pharmaceutical compositions can be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective.

[00271] In certain embodiments, a pharmaceutical composition of this disclosure can comprise an effective amount of a modified virus, disclosed herein, combined with a pharmaceutically acceptable carrier. "Pharmaceutically acceptable," as used herein, includes any carrier which does not interfere with the effectiveness of the biological activity of the active ingredients and/or that is not toxic to the patient to whom it is administered. Non-limiting examples of suitable pharmaceutical carriers include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents and sterile solutions. Additional non-limiting examples of pharmaceutically compatible carriers can include gels, bioadsorbable matrix materials, implantation elements containing the modified oncolytic virus or any other suitable vehicle, delivery or dispensing means or material. Such carriers can be formulated by conventional methods and can be administered to the subject at an effective amount.

Methods of Production

[00272] The modified oncolytic viruses of this disclosure can be produced by methods known to one of skill in the art. In certain embodiments, the modified oncolytic virus can

be propagated in suitable host cells, *e.g.*, HeLa cells, 293 cells, or Vero cells, isolated from host cells and stored in conditions that promote stability and integrity of the virus, such that loss of infectivity over time is minimized. In certain exemplary methods, the modified oncolytic viruses are propagated in host cells using cell stacks, roller bottles, or perfusion bioreactors. In some examples, downstream methods for purification of the modified oncolytic viruses can comprise filtration (*e.g.*, depth filtration, tangential flow filtration, or a combination thereof), ultracentrifugation, or chromatographic capture. The modified oncolytic virus can be stored, *e.g.*, by freezing or drying, such as by lyophilization. In certain embodiments, prior to administration, the stored modified oncolytic virus can be reconstituted (if dried for storage) and diluted in a pharmaceutically acceptable carrier for administration.

[00273] Some embodiments provide that the modified oncolytic virus as described herein, exhibit a higher titer in HeLa cells and 293 cells compared to an otherwise identical virus that does not comprise the modifications in the modified oncolytic virus. In certain instances, a higher titer in HeLa cells and 293 cells is seen in modified oncolytic virus.

Combination Therapies

[00274] In certain embodiments, the methods of this disclosure comprise administering a modified oncolytic virus as disclosed herein or a pharmaceutical composition containing the same, followed by, and preceded by or in combination with one or more further therapy. Examples of the further therapy can include, but are not limited to, chemotherapy, radiation, oncolytic viral therapy with an additional virus, treatment with immunomodulatory proteins, an anti-cancer agent, or any combinations thereof. The further therapy can be administered concurrently or sequentially with respect to administration of the modified virus, such as oncolytic vaccinia virus. In certain embodiments, the methods of this disclosure can comprise administering a modified oncolytic virus as disclosed herein, followed by, preceded by, or in combination with one or more anti-cancer agents or cancer therapies. Anti-cancer agents can include, but are not limited to, chemotherapeutic agents, radiotherapeutic agents, cytokines, immune checkpoint inhibitors, anti-angiogenic agents, apoptosis-inducing agents, anti-cancer antibodies and/or anti-cyclin-dependent kinase agents. In certain embodiments, the cancer therapies can include chemotherapy, biological therapy, radiotherapy, immunotherapy, hormone therapy, anti-vascular therapy, cryotherapy, toxin therapy and/or surgery or combinations thereof. In certain embodiments, the methods of this disclosure can include administering a modified virus, disclosed herein, followed by, preceded by or in combination with an modified

oncolytic virus of this disclosure. Combination of the modified oncolytic vaccinia virus with chemotherapy achieves a synergistic effect which is not seen in modified oncolytic viruses that do not comprise the modifications in the modified oncolytic virus. The synergistic effect of the above combination can be advantageously used to lower the dose of chemotherapy, such as Taxol®. Thus, the treatment method disclosed here, with the modified virus, can reduced toxicities associated with chemotherapy, *e.g.*, patients who respond to chemotherapy but suffer side effects at therapeutic doses. The synergistic effect, can, in certain cases, results in a decrease in tumor growth compared to chemotherapy alone or oncolytic vaccinia virus alone. Exemplary decrease in tumor growth can be from about 2% to about 50%, such as about 5%, about 10%, about 20%, about 25%, about 35%, about 45% or about 50%.

[00275] In certain embodiments, treatment using a modified oncolytic virus can be used alone or in combination with one or immunomodulatory agents. An immunomodulatory agent can include any compound, molecule or substance capable of suppressing antiviral immunity associated with a tumor or cancer. In certain embodiments, the immunomodulatory agent can be capable of suppressing innate immunity or adaptive immunity to the modified virus. Non-limiting examples of immunomodulatory agents include anti-CD33 antibody or variable region thereof, an anti-CD11b antibody or variable region thereof, a COX2 inhibitor, *e.g.,* celecoxib, cytokines, such as IL-12, GM-CSF, IL-2, IFN3 and 1FNγ, and chemokines, such as MIP-1, MCP-1 and IL-8. In certain embodiments, the immunomodulatory agent can include immune checkpoint modulators such as, but not limited to, anti-CTLA4, anti-PD-1, and anti-PD-L1 and TLR agonists *(e.g.,* Poly I:C). In some examples, the immunomodulatory agent can include an immune checkpoint inhibitor, such as an antagonist of PD-1 (e.g., an antagonist antibody that binds to PD-1), an antagonist of PD-L1 (*e.g.*, an antagonist antibody that binds to PD-L1), an antagonist of CTLA-4 (*e.g.*, an antagonist antibody that binds to CTLA-4), an antagonist of A2AR (*e.g.*, an antagonist antibody that binds to A2AR), an antagonist of B7-H3 (e.g., an antagonist antibody that binds to B7-H3), an antagonist of B7-H4 (*e.g.*, an antagonist antibody that binds to B7-H4), an antagonist of BTLA (*e.g.*, an antagonist antibody that binds to BTLA), an antagonist of IDO (*e.g.*, an antagonist antibody that binds to IDO), an antagonist of KIR (*e.g.*, an antagonist antibody that binds to KIR), an antagonist of LAG3 (*e.g.*, an antagonist antibody that binds to LAG3), an antagonist of TIM-3 (*e.g.*, an antagonist antibody that binds to TIM3). In some embodiments, the further therapy can comprise administering an immune checkpoint regulator. In one example, the immune

checkpoint regulator can be TGN1412. In one example, the immune checkpoint regulator can be NKTR-214. In one example, the immune checkpoint regulator can be MEDI0562. In one example, the immune checkpoint regulator can be MEDI6469. In one example, the immune checkpoint regulator can be MEDI6383. In one example, the immune checkpoint regulator can be JTX-2011. In one example, the immune checkpoint regulator can be Keytruda (pembrolizumab). In one example, the immune checkpoint regulator can be Opdivo (nivolumab). In one example, the immune checkpoint regulator can be Yervoy (ipilimumab). In one example, the immune checkpoint regulator can be tremelimumab. In one example, the immune checkpoint regulator can be Tecentriq (atezolizumab). In one example, the immune checkpoint regulator can be MGA271. In one example, the immune checkpoint regulator can be indoximod. In one example, the immune checkpoint regulator can be Epacadostat. In one example, the immune checkpoint regulator can be lirilumab. In one example, the immune checkpoint regulator can be BMS-986016. In one example, the immune checkpoint regulator can be MPDL3280A. In one example, the immune checkpoint regulator can be avelumab. In one example, the immune checkpoint regulator can be durvalumab. In one example, the immune checkpoint regulator can be MEDI4736. In one example, the immune checkpoint regulator can be MEDI4737. In one example, the immune checkpoint regulator can be TRX518. In one example, the immune checkpoint regulator can be MK-4166. In one example, the immune checkpoint regulator can be urelumab (BMS-663513). In one example, the immune checkpoint regulator can be PF-05082566 (PF-2566)

[00276] In certain examples, where the further therapy is radiation exemplary doses can be 5,000 Rads (50 Gy) to 100,000 Rads (1000 Gy), or 50,000 Rads (500 Gy), or other appropriate doses within the recited ranges. Alternatively, the radiation dose can be about 30 to 60 Gy, about 40 to about 50 Gy, about 40 to 48 Gy, or about 44 Gy, or other appropriate doses within the recited ranges, with the dose determined, example, by means of a dosimetry study as described above. “Gy” as used herein can refer to a unit for a specific absorbed dose of radiation equal to 100 Rads. Gy is the abbreviation for “Gray.”

[00277] In certain examples, where the further therapy is chemotherapy, exemplary chemotherapeutic agents can include without limitation alkylating agents (*e.g.*, nitrogen mustard derivatives, ethylenimines, alkylsulfonates, hydrazines and triazines, nitrosureas, and metal salts), plant alkaloids (*e.g.*, vinca alkaloids, taxanes, podophyllotoxins, and camptothecan analogs), antitumor antibiotics (*e.g.*, anthracyclines, chromomycins, and the like), antimetabolites (*e.g.*, folic acid antagonists, pyrimidine antagonists, purine antagonists,

and adenosine deaminase inhibitors), topoisomerase I inhibitors, topoisomerase II inhibitors, and miscellaneous antineoplastics (*e.g.*, ribonucleotide reductase inhibitors, adrenocortical steroid inhibitors, enzymes, antimicrotubule agents, and retinoids). Exemplary chemotherapeutic agents can include, without limitation, anastrozole (Arimidex®), bicalutamide (Casodex®), bleomycin sulfate (Blenoxane®), busulfan (Myleran®), busulfan injection (Busulfex®), capecitabine (Xeloda®), N4-pentoxycarbonyl-5-deoxy-5-fluorocytidine, carboplatin (Paraplatin®), carmustine (BiCNU®), chlorambucil (Leukeran®), cisplatin (Platinol®), cladribine (Leustatin®), cyclophosphamide (Cytoxan® or Neosar®), cytarabine, cytosine arabinoside (Cytosar-U®), cytarabine liposome injection (DepoCyt®), dacarbazine (DTIC-Dome®), dactinomycin (Actinomycin D, Cosmegan), daunorubicin hydrochloride (Cerubidine®), daunorubicin citrate liposome injection (DaunoXome®), dexamethasone, docetaxel (Taxotere®), doxorubicin hydrochloride (Adriamycin®, Rubex®), etoposide (Vepesid®), fludarabine phosphate (Fludara®), 5-fluorouracil (Adrucil®, Efudex®), flutamide (Eulexin®), tezacitibine, Gemcitabine (difluorodeoxycitidine), hydroxyurea (Hydrea®), Idarubicin (Idamycin®), ifosfamide (IFEX®), irinotecan (Camptosar®), L-asparaginase (ELSPAR®), leucovorin calcium, melphalan (Alkeran®), 6-mercaptopurine (Purinethol®), methotrexate (Folex®), mitoxantrone (Novantrone®), mylotarg, paclitaxel (Taxol®), phoenix (Yttrium90/MX-DTPA), pentostatin, polifeprosan 20 with carmustine implant (Gliadel®), tamoxifen citrate (Nolvadex®), teniposide (Vumon®), 6-thioguanine, thiotepa, tirapazamine (Tirazone®), topotecan hydrochloride for injection (Hycamptin®), vinblastine (Velban®), vincristine (Oncovin®), and vinorelbine (Navelbine®), Ibrutinib, idelalisib, and brentuximab vedotin.

[00278] Exemplary alkylating agents can include, without limitation, nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas and triazenes): uracil mustard (Aminouracil Mustard®, Chlorethaminacil®, Demethyldopan®, Desmethyldopan®, Haemanthamine®, Nordopan®, Uracil nitrogen Mustard®, Uracillost®, Uracilmostaza®, Uramustin®, Uramustine®), chlormethine (Mustargen®), cyclophosphamide (Cytoxan®, Neosar®, Clafen®, Endoxan®, Procytox®, Revimmune™), ifosfamide (Mitoxana®), melphalan (Alkeran®), Chlorambucil (Leukeran®), pipobroman (Amedel®, Vercyte®), triethylenemelamine (Hemel®, Hexalen®, Hexastat®), triethylenethiophosphoramine, Temozolomide (Temodar®), thiotepa (Thioplex®), busulfan (Busilvex®, Myleran®), carmustine (BiCNU®), lomustine (CeeNU®), streptozocin (Zanosar®), and Dacarbazine (DTIC-Dome®). Additional exemplary alkylating agents include, without limitation,

Oxaliplatin (Eloxatin®); Temozolomide (Temodar® and Temodal®); Dactinomycin (also known as actinomycin-D, Cosmegen®); Melphalan (also known as L-PAM, L-sarcolysin, and phenylalanine mustard, Alkeran®); Altretamine (also known as hexamethylmelamine (HMM), Hexalen®); Carmustine (BiCNU®); Bendamustine (Treanda®); Busulfan (Busulfex® and Myleran®); Carboplatin (Paraplatin®); Lomustine (also known as CCNU, CeeNU®); Cisplatin (also known as CDDP, Platinol® and Platinol®-AQ); Chlorambucil (Leukeran®); Cyclophosphamide (Cytoxan® and Neosar®); Dacarbazine (also known as DTIC, DIC and imidazole carboxamide, DTIC-Dome®); Altretamine (also known as hexamethylmelamine (HMM), Hexalen®); Ifosfamide (Ifex®); Prednumustine; Procarbazine (Matulane®); Mechlorethamine (also known as nitrogen mustard, mustine and mechloroethamine hydrochloride, Mustargen®); Streptozocin (Zanosar®); Thiotepa (also known as thiophosphoamide, TESPA and TSPA, Thioplex®); Cyclophosphamide (Endoxan®, Cytoxan®, Neosar®, Procytox®, Revimmune®); and Bendamustine HCl (Treanda®).

[00279] Exemplary anthracyclines can include, without limitation, *e.g.*, doxorubicin (Adriamycin® and Rubex®); bleomycin (Lenoxane®); daunorubicin (dauorubicin hydrochloride, daunomycin, and rubidomycin hydrochloride, Cerubidine®); daunorubicin liposomal (daunorubicin citrate liposome, DaunoXome®); mitoxantrone (DHAD, Novantrone®); epirubicin (Ellence™); idarubicin (Idamycin®, Idamycin PFS®); mitomycin C (Mutamycin®); geldanamycin; herbimycin; ravidomycin; and desacetylravidomycin.

[00280] Exemplary vinca alkaloids can include, but are not limited to, vinorelbine tartrate (Navelbine®), Vincristine (Oncovin®), and Vindesine (Eldisine®)); vinblastine (also known as vinblastine sulfate, vincaleukoblastine and VLB, Alkaban-AQ® and Velban®); and vinorelbine (Navelbine®).

[00281] Exemplary proteasome inhibitors can, but are not limited to, bortezomib (Velcade®); carfilzomib (PX-171-007, (S)-4-Methyl-N—((S)-1-(((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)-2-((S)-2-(2-morpholinoac etamido)-4-phenylbutanamido)-pentanamide); marizomib (NPI-0052); ixazomib citrate (MLN-9708); delanzomib (CEP-18770); and O-Methyl-N-[(2-methyl-5-thiazolyl)carbonyl]-L-seryl-O-methyl-N-[(1S)-2-[(2R)-2-methyl-2-oxiranyl]-2-oxo-1-(phenylmethyl)ethyl]-L-serinamide (ONX-0912).

[00282] "In combination with," as used herein, means that the modified virus, such as an oncolytic vaccinia virus as described herein or a pharmaceutical composition comprising

the same, and the further therapy, such as a further therapy comprising one or more agents are administered to a subject as part of a treatment regimen or plan. In certain embodiments, being used in combination does not require that the modified oncolytic virus and the one or more agents are physically combined prior to administration or that they be administered over the same time frame. For example, and not by way of limitation, the modified oncolytic virus and the one or more agents can be administered concurrently to the subject being treated, or can be administered at the same time or sequentially in any order or at different points in time.

[00283] The further therapy can be administered, in various embodiments, in a liquid dosage form, a solid dosage form, a suppository, an inhalable dosage form, an intranasal dosage form, in a liposomal formulation, a dosage form comprising nanoparticles, a dosage form comprising microparticles, a polymeric dosage form, or any combinations thereof. In certain embodiments, the further therapy is administered over a period of about 1 week to about 2 weeks, about 2 weeks to about 3 weeks, about 3 weeks to about 4 weeks, about 4 weeks to about 5 weeks, about 6 weeks to about 7 weeks, about 7 weeks to about 8 weeks, about 8 weeks to about 9 weeks, about 9 weeks to about 10 weeks, about 10 weeks to about 11 weeks, about 11 weeks to about 12 weeks, about 12 weeks to about 24 weeks, about 24 weeks to about 48 weeks, about 48 weeks or about 52 weeks, or longer. The frequency of administration of the further therapy can be, in certain instances, once daily, twice daily, once every week, once every three weeks, once every four weeks (or once a month), once every 8 weeks (or once every 2 months), once every 12 weeks (or once every 3 months), or once every 24 weeks (once every 6 months). In certain embodiments, a method of treating a subject having a cancer can include administering, to the subject, an effective amount of a modified oncolytic virus, *e.g.,* modified vaccinia virus, of this disclosure. In certain embodiments, the methods of this disclosure can further include administering to the subject an effective amount of one or more agents. For example, and not by way of limitation, the agent can be an anti-cancer agent, an immunomodulatory agent, or any combinations thereof, as described above.

Kits

[00284] In embodiments, this disclosure provides for a kit for administering a modified oncolytic virus as described herein. In certain embodiments, a kit of this disclosure can include a modified oncolytic virus or a pharmaceutical composition comprising a modified oncolytic virus as described above. In certain embodiments, a kit of this disclosure can further include one or more components such as instructions for use,

devices and additional reagents, and components, such as tubes, containers and syringes for performing the methods disclosed above. In certain embodiments, a kit of this disclosure can further include one or more agents, *e.g.,* at least one of an anti-cancer agent, an immunomodulatory agent, or any combinations thereof, that can be administered in combination with a modified virus.

[00285] In certain embodiments, a kit of this disclosure can comprise one or more containers containing a modified virus, disclosed herein. For example, and not by way of limitation, a kit of this disclosure can comprise one or more containers that contain a modified oncolytic virus of this disclosure.

[00286] In certain embodiments, a kit of this disclosure can include instructions for use, a device for administering the modified oncolytic virus to a subject, or a device for administering an additional agent or compound to a subject. For example, and not by way of limitation, the instructions can include a description of the modified oncolytic virus and, optionally, other components included in the kit, and methods for administration, including methods for determining the proper state of the subject, the proper dosage amount and the proper administration method for administering the modified virus. Instructions can also include guidance for monitoring the subject over duration of the treatment time.

[00287] In certain embodiments, a kit of this disclosure can include a device for administering the modified oncolytic virus to a subject. Any of a variety of devices known in the art for administering medications and pharmaceutical compositions can be included in the kits provided herein. For example, and not by way of limitation, such devices include, a hypodermic needle, an intravenous needle, a catheter, a needle-less injection device, an inhaler and a liquid dispenser, such as an eyedropper. In certain embodiments, a modified oncolytic virus to be delivered systemically, for example, by intravenous injection, an intratumoral injection, an intraperitoneal injection, can be included in a kit with a hypodermic needle and syringe.

EXAMPLES

[00288] The examples below further illustrate the described embodiments without limiting the scope of this disclosure.

[00289] **EXAMPLE 1: EXEMPLARY MODIFIED VACCINIA VIRUS HAVING A MUTATION IN B5R GENE SHOWS ENHANCED THERAPEUTIC EFFECTS IN MURINE TUMOR MODELS**

[00290] The aim of this study was to explore the effects of an exemplary modified vaccinia virus according to this disclosure, where an exemplary mutation was introduced to the SCR3 and SCR4 regions of the viral B5R gene and the viral thymidine kinase (TK) gene was deleted (referred to herein as WR.B5RmutTK-), in murine tumor models, in comparison with vaccinia viruses that do not have the exemplary B5R mutation.

[00291] In one experiment, the WR.B5RmutTK- virus was compared with a vehicle (buffered saline) control, and another modified vaccinia virus where TK gene was deleted and an exogenous nucleic acid encoding GMCSF was added (referred to herein as WR.TK-GMCSF). A single dose of either one of the three viruses (1×10^7 PFU) was administered to treat BALB/c mice implanted subcutaneously with pre-established RENCA tumors (**FIG. 1**). Tumor volumes were monitored by caliper measurement as shown in **FIG. 1** (n = 10-15 per group). It was noted that WR.TK-GMCSF virus administered intratumorally ("IT") at the given dose, as expected, did not delay the tumor volume increase over time, as compared to the control virus administrated intravenously ("IV"). However, the treatment of WR.B5RmutTK- virus at the same dose via either intratumoral or intravenous delivery both led to significant delay in tumor volume growth, demonstrating an enhanced therapeutic effect of the exemplary modified vaccinia virus having the exemplary B5R mutation.

[00292] In another experiment, a different mouse tumor model, C57/BL6 mice bearing subcutaneous B16 tumors, was used to test the effects of the exemplary vaccinia virus having the exemplary B5R mutation (**FIGS. 2A-2B**). The mice were split into two groups, one group received immunization of vaccinia virus through one injection of WR. TK- virus (where TK gene was deleted from the genome) 3 weeks prior to tumor implantation ("Immunized mice") (**FIG. 2B**), and the other group were not immunized ("Non-immunized mice") (**FIG. 2A**). WR.B5RmutTK- virus (referred to as B5R in the figure) was examined in comparison with another modified vaccinia virus WR.TK-A34R K151E, where TK was deleted and the viral A34R gene was mutated with an amino acid change, K151 to E (referred to WI in the figure). A single intravenous injection of either virus was given to the mice (1×10^8 PFU/mouse, n = 5 per group/timepoint) 96 hours after the tumor implantation, and then the mice were sacrificed 1 day or 2 days after the injection. Tumors were collected for quantification of the number of viral genomes per gram of tumor by Q-PCR. It was observed that WR.B5RmutTK- virus exhibited significantly enhanced accumulation in the tumors in both immunized and non-immunized mice, suggesting the exemplary B5R mutation may promote delivery and spreading of the modified vaccinia virus in tumor. Increased viral replication in tumor were also observed in a plaque assay (**FIGS. 3A-3B**), which quantified the plaque

forming capability of the viruses in the collected tumors, suggesting that the exemplary B5R mutation can promote replication of the modified vaccinia virus in tumor.

[00293] **EXAMPLE 2: EXEMPLARY MODFIED VACCINIA VIRUS HAVING AN EXEMPLARY EXOGENOUS NUCLEIC ACID THAT ENCODES CHEMOKINE RECEPTOR SHOWS ENHANCED THERAPEUTIC EFFECTS IN CANCER CELL LINES AND MURINE TUMOR MODELS**

[00294] The aim of this study was to explore the effects of several exemplary modified vaccinia viruses according to this disclosure, in each of which an exemplary exogenous nucleic acid encoding a chemokine receptor was added, in cancer cell lines and murine tumor models, in comparison with vaccinia viruses that do not have the exemplary exogenous nucleic acids.

[00295] In one experiment, three different modified vaccinia viruses were tested in mice bearing orthotopic (mammary fat pad) 4T1 tumors subcutaneously. In one vaccinia virus, as termed mCCR5/TK- virus herein, TK gene was deleted from the genome and an exogenous nucleic acid encoding mouse CCR5 was added. In another vaccinia virus, as termed mCXCR4/TK- virus herein, TK gene was deleted and an exogenous nucleic acid encoding mouse CXCR4 was added. In a third vaccinia virus, as termed TK- virus herein, TK gene was deleted. All three viruses were also engineered to express luciferase as a reporter. Each mouse was treated with a single injection of either one of the three viruses at a dose of 1×10^8 PFU. Number of viruses in the tumors was quantified by bioluminescence imaging and measurement of the luciferase activity *in vivo*, every day after the injection for three days (**FIG. 4**). It was observed that mCXCR4/TK- virus receiving mice showed significantly more photon intensity in the tumors, as compared to mice receiving mCCR5/TK- virus or TK- virus, suggesting expression of CXCR4 may promote viral delivery to the tumor. In contrast, CCR5/TK- showed relatively low photon intensity among the three over all three day measurement, suggesting expression of CCR5 may reduce viral delivery to the tumor.

[00296] The therapeutic effects of the viruses were also examined. Tumor volume was monitored as described above (**FIG. 5A**), showing that mCXCR4/TK- virus significantly delayed the tumor volume growth as compared to other two viruses and PBS sham control. Moreover, as shown in **FIG. 5B**, mouse survival percentage was also significantly improved by the administration of mCXCR4/TK- virus (identified in **FIG. 5B** as CXCR4).

[00297] In another experiment, the same three viruses were tested in a different tumor model, C57/BL6 mice bearing B16 tumors subcutaneously. Increased photon intensity was observed in mCXCR4/TK- virus-treated mice, as compared to in mice treated with TK- virus or

mCCR5/TK- virus, as can be seen in both the quantification plot in **FIG. 6A** and the representative photos from the bioluminescence imaging in **FIG. 6B**. Similarly, mCXCR4/TK- virus administration significantly delayed the tumor volume growth (**FIG. 7**). These results, as well as the results above, suggest that the addition of the exogenous nucleic acid that encodes CXCR4 to the vaccinia virus resulted in enhanced therapeutic effects against tumor.

[00298] Next, viral replication capability for the three viruses in different cancer cell lines in vitro was examined with plaque assays. Plaque-forming was quantified every 24 hours after addition of the viruses to the cell line. As shown in **FIGS. 8A-8D**, mCCR5/TK- virus, but not mCXCR4/TK- virus, showed increased replication in both cancer cell lines tested (4T1 and B16), at two different multiplicity of infections (MOI 0.1, as in **FIGS. 8A** and **8C** and MOI 1, as in **FIG. 8B** and **8D**), suggesting that expression of CCR5, but not CXCR4, can promote viral replication in cancer cells. This further can suggest that CXCR4 can display an enhanced effect *in vivo*, despite not increasing replication *in vitro* and the enhanced effect of the exemplary modified oncolytic vaccinia virus can be attributed to the delivery, such as, improved systemic delivery.

[00299] In an effort to start to understand the mechanisms underlying the enhanced therapeutic effects conferred by CXCR4 expression, experiments were conducted using transgenic mice with B-cells depleted (referred to herein as JH). Viruses as described above were administered to Balb/C mice having B-Cells and JH mice without B-cells, both of which had been implanted subcutaneously with pre-established 4T1 tumors. As shown in **FIGS. 9A-9C**, consistent with the data above, in Balb/C mice, mCXCR4/TK- virus displayed increased accumulation in the tumors as measured by the bioluminescence imaging radiance. However, such an increase was not observed in JH mice, suggesting the enhanced accumulation of mCXCR4/TK- in tumors was at least partially dependent on B cells. On the other hand, flow cytometry experiments showed that mCXCR4/TK- virus administration led to increased entry of B cells into the tumors, but not other organs, like spleen or lymph nodes (LN), in BALB/c mice bearing 4T1 tumor subcutaneously (**FIGS. 10A-10C**). These data indicate that B cells may serve as a vehicle for vaccinia virus to be delivered to the tumors *in vivo*.

[00300] Moreover, enhanced T cell function was also observed in mCXCR4/TK- virus-treated mice. As shown in **FIGS. 11A-11B**, T cells were collected from the mice that had been implanted with 4T1 tumors subcutaneously and subsequently treated as described above, and their immune activity was examined by ELISpot assays that tested their

interferon-γ (IFNgamma) release in response to different immunogens. The tested T cells were recovered from spleens 14 days after the virus injection. T cells from TK- virus-treated mice displayed some immune response to tumor immunogens (4T1-cell lysate), as compared to T cells from PBS-treated mice, whereas T cells from mCXCR4/TK- virus-treated mice displayed enhanced immune response (**FIG. 11A**). In contrast, CXCR4 expression did not seem to enhance T cell function against the vaccinia virus, as **FIG. 11B** shows that the interferon- γ release, in response to inactivated vaccinia virus (VV) mixed with tumor cell 4T1 lysate, was not significantly different between T cells from TK- virus-treated mice and mCXCR4/TK- virus-treated mice. These data indicate that CXCR4 expression can result in enhanced immune response against the tumor, thereby contributing, at least partially, to the enhanced therapeutic benefits of the exemplary modified vaccinia virus.

[00301] Additional experiments were conducted using another exemplary modified vaccinia virus encoding CXCR4. In these experiments, the vaccinia viruses used were as follows: (i) a modified virus with the A52R gene deleted and an exogenous nucleic acid encoding mouse CXCR4 added, termed A52R- CXCR4+; (ii) a modified virus with the TK gene deleted and an exogenous nucleic acid encoding mouse GMCSF added, termed TK- GMCSF+; (iii) a modified virus with the TK gene deleted and no exogenous nucleic acid added, termed TK-, and (iv) a vehicle formulated buffer, termed VFB. In one experiment, BALB/c mice bearing RENCA tumors subcutaneously were treated with a single intravenous injection (1×10^7 PFU) of one of the four modified viruses. Tumors were harvested 24 hours later, and the number of viral genomes per milligram of tissue quantified by qPCR (**FIG. 16**). Higher numbers of A52R- CXCR4+ genomes were found in the tumors compared to the other modified viruses, supporting the above conclusion that viral CXCR4 expression can enhance viral delivery to tumors.

[00302] In another experiment, BALB/c mice bearing RENCA tumors subcutaneously were treated with intravenous injections (1×10^7 PFU) of one of the same four modified viruses on day 1 and 4, and tumor volume was monitored as described above. Intravenously-delivered A52R- CXCR4+ virus significantly delayed tumor volume growth (**FIG. 17**), further supporting the notion that the addition of an exogenous nucleic acid encoding CXCR4 can result in enhanced anti-cancer therapeutic effects.

[00303] **EXAMPLE 3: EXEMPLARY VACCINIA VIRUSES HAVING EXEMPLARY EXOGENOUS NUCLEIC ACIDS THAT ENCODE EXTRACELLULAR MATRIX-**

DEGRADING ENZYMES SHOW ENHANCED THERAPEUTIC EFFECTS IN MURINE TUMOR MODELS

[00304] The aim of this study was to explore the effects of exemplary modified vaccinia virus according to this disclosure, where exemplary exogenous nucleic acids encoding extracellular matrix-degrading enzymes (such as hyaluronidases) were added, in murine tumor models, in comparison with vaccinia viruses that do not have an exemplary exogenous nucleic acid.

[00305] In one experiment, an exemplary modified vaccinia virus having an exogenous nucleic acid that encodes hyaluronidase PH-20 and having TK gene deleted (referred to herein as WR. TK-PH20) was tested in comparison with a WR.TK- only virus where there was only deletion of TK gene but no addition of PH-20 gene, together with another exemplary vaccinia virus having an exogenous nucleic acid that encodes a MMP8 and having TK gene deleted (referred to herein as WR.TK-mmp8). All three viruses were also engineered to express luciferase as a reporter. A single injection of either virus was given to BALB/c mice bearing RENCA tumors subcutaneously. The subsequent accumulation and spread of the virus was followed through measurement of the viral luciferase activity via bioluminescence imaging. As shown in **FIGS. 12A-12B**, it was found that WR.TK-Ph20 virus displayed significantly more accumulation in the tumors *in vivo* as compared to the other two viruses, while WR.TK-mmp8 virus was also relatively high compared to WR.TK-only. These data indicate that expression of PH-20 can enhance viral delivery and spread to the tumors through degradation of ECM and reduction of IFP.

[00306] Next, the therapeutic effects against tumor were also examined for the viral expression of PH-20. In the first experiment, BALB/c mice subcutaneously implanted with 4T1 tumors were treated intravenously with WR.TK- virus or another exemplary vaccinia virus WR.TK-PH20DCK, where TK gene was deleted, and an exogenous nucleic acid encoding PH20 and an exogenous nucleic acid encoding deoxycytidine kinase (DCK) were both added. Both viruses expressed luciferase, and the viral luciferase expression inside the tumor was measured with bioluminescence imaging. As shown in **FIG. 13A**, similar as WR.TK-PH20 virus in the RENCA model, WR.TK-PH20DCK virus-injected mice showed significantly higher luciferase activity inside the tumor (n = 10), suggesting an enhanced viral accumulation of WR.TK-PH20DCK virus in the tumor as compared to WR.TK- virus.

[00307] In the second experiment, combined therapies were tested on BALB/c mice bearing the 4T1 tumors subcutaneously. Six groups of mice were used. Each group of mice received a single intravenous injection of WR.TK- virus or WR.TK-PH20DCK virus, or control PBS,

and then received an intraperitoneal injection of chemotherapeutic drug gemcitabine or water on day3 and day 7 after the virus injection. As shown in **FIG. 13B** and **FIG.13C**, there were a control group receiving water for all three injections, a Gemcitabine group receiving no virus injection but gemcitabine injections, a TK-w group receiving injection of WR.TK- but no gemcitabine, a TK-PH20DCK w group receiving injection of WR.TK-PH20DCK but no gemcitabine, a TK-+gem group receiving injections of both WR.TK- and gemcitabine, and a TK-PH20DCK+gem group receiving injections of both WR.TK-PH20CK and gemcitabine. Continued monitoring of tumor volume (n = 10, monitored until more than 4 mice died in the group) and survival curves (*, $P<0.05$ compared with PBS group; #, $P<0.05$ compared with TK- vv + gem group; **, $P<0.05$ compared with TK- group) both showed that the combined treatment of WR.TK-PH20DCK virus and gemcitabine yielded the best result, suggesting that the PH20-mediated reduced interstitial fluid pressure may enhance the chemotherapeutic-delivery, thereby yielding better therapeutic effects.

[00308] In the third experiment, exemplary vaccinia virus having an exogenous nucleic acid that encodes PH-20 with GPI-anchor and vaccinia virus having an exogenous nucleic acid that encodes PH-20 without GPI-anchor were examined and compared. BALB/c mice bearing RENCA tumors subcutaneously were treated with a single dose of 1×10^7 PFU of virus *via* intratumoral (IT) or intravenous (IV) delivery, and their tumor volumes was measured 45 days after the virus injection. Seven groups of mice were examined at this point (**FIG. 14**): one control group of mice receiving intravenous injection of no virus but sham control; WR.TK-PH20+(IT) group receiving intratumoral injection of WR.TK-PH20+ virus, where the PH20 expressed by the virus had a GPI-anchor; WR.TK-GMCSF(IT) group receiving intratumoral injection of WR.TK-GMCSF virus, where no PH20 was expressed but GMCSF; WR.TK-PH20+(no-gpi)(IT) group receiving intratumoral injection of WR.TK-PH20+(no-gpi) virus, where PH20 expressed by the virus had no GPI; WR.TK-PH20+(IV) group receiving intravenous injection of WR.TK-PH20+ virus, WR.TK-GMCSF(IV) group receiving intravenous injection of WR.TK-GMCSF virus, and WR.TK-PH20+(no-gpi)(IV) group receiving intravenous injection of WR.TK-PH20+(no-gpi) virus. Remarkably, at the late time such as 45 days after the virus injection, every PH-20-expressing virus-treated animal (IT or IV) was still alive, whereas controls and WR.TK-GMCSF virus-treated mice had all been sacrificed as their tumors had grown larger than $1500mm^3$. As shown in **FIG. 14**, strikingly, the inhibitory effect on tumor growth seemed more enhanced by the presence of the GPI anchor in the WR.TK-PH20+ virus-treated mice as compared to no GPI in the case of WR.TK-PH20+(no-gpi) virus. This is an unexpected result, given that GPI anchors

PH-20 to cell membranes, and secretory PH-20 without the GPI anchor that exists more freely in the ECM is believed to be more effective in degrading ECM and reducing IFP.

[00309] Additional experiments were performed using exemplary modified vaccinia viruses expressing secreted hyaluronidases to determine how these enzymes affected viral delivery, spread, cell killing, and therapeutic efficacy.

[00310] Modified viruses were constructed wherein the TK gene was deleted and an exogenous nucleic acid encoding a hyaluronidase (HysA, lin, or sko) was added; these modified viruses are referred to as TK-HysA, TK-lin, and TK-sko, respectively

[00311] In an initial experiment, a hyaluronidase activity ELISA was used to determine the activity of the secreted hyaluronidases expressed by the modified viruses described above. HysA showed the most activity, as shown in Table 3.

Table 3

	% protein degraded	
Virus/control	**24h**	**48h**
TK-HysA	98.55	100
TK-lin	7.29	0
TK-sko	97.96	98.45
rv	0	0
WR	0.69	0
No virus	16.61	0
Buffer	0	0

[00312] In another experiment, cells in culture were infected with one of: the TK- modified virus, the TK- HysA modified virus, or the TK-MMP8 modified virus described above, each of which also expressed GFP. In infected cultures of MC38 cancer cells, the TK-HysA and TK-MMP8 viruses replicated, spread, and prevented cancer cell expansion more so than the TK- virus, suggesting that HysA or MMP8 expression can enhance oncolytic virus replication, spread, and cancer cell killing (**FIGS. 18A-18D**). In infected cultures of HCT116 cancer cells, the TK-HysA and TK-MMP8 viruses replicated and spread more than the TK- virus, and the TK-HysA virus prevented cancer cell expansion more effectively than the TK- virus, supporting a role for HysA in enhancing oncolytic viral replication, spread, and cancer cell killing (**FIGS. 19A-19D**).

[00313] Additional experiments were performed using the following viruses: (i) the TK-HysA modified virus (ii) the TK-MMP8 modified virus, (iii) the TK-PH20-expressing modified virus described above (TK-PH20/TK-SPAM1), (iv) the TK- modified virus without an exogenous enzyme added, and (v) a vehicle formulated buffer (VFB). BALB/c mice

bearing RENCA tumors subcutaneously were treated with a single intravenous injection (1 x 10^7 PFU) of one of the five viruses. Tumors were harvested 24 hours later, and the number of viral genomes per milligram of tissue quantified by qPCR (**FIG. 20**). Higher numbers of TK-HysA genomes were found in the tumors compared to the other viruses, suggesting that HysA expression can result in enhanced delivery of a modified vaccinia virus to tumors.

[00314] In another experiment, BALB/c mice bearing RENCA tumors subcutaneously were treated with one intratumoral injection (1 x 10^7 PFU) of the TK-HysA, TK-MMP8, TK-PH20 (labeled in **FIG. 21** as SPAM1), or TK- modified virus, and tumor volume was monitored as described above. Intratumorally-delivered TK-HysA, TK-MMP8 and TK-PH20 delayed tumor growth more the TK- virus, suggesting that expression of HysA, MMP8 or PH20 can enhance the anti-tumoral efficacy of oncolytic viruses (**FIG. 21**).

[00315] To further investigate the potential utility of HysA in oncolytic viruses, modified viruses were generated wherein the A52R gene was deleted, and an exogenous nucleic acid encoding HysA inserted to the A52R locus (A52R- HysA) (labeled in **FIG. 22A-D** as WR. A52R- EL hysA GFP 1MOI). Like the TK- HysA (labeled in **FIG. 22A-D** as WR. TK- EL hysA GFP 1MOI) modified virus, the A52R-HysA modified virus successfully spread in cultures of LLC or MC38 cancer cells, and limited expansion of the cancer cells (**FIGS. 22A-22D**). These results further support a role for HysA in enhancing oncolytic viral replication, spread, and cancer cell killing, and demonstrate that modified viruses with A52R deleted can be used as onclytic viruses.

[00316] Collectively, these data demonstrate that expression of an extracellular matrix-degrading enzyme such as PH20, HysA, or MMP8 can enhance the replication, spread, cancer killing, and therapeutic efficacy of oncolytic viruses.

[00317] **EXAMPLE 4: EXEMPLARY VACCINIA VIRUS HAVING AN EXEMPLARY MODIFIED VH1 GENE SHOWS ENHANCED THERAPEUTIC EFFECTS IN MURINE TUMOR MODELS**

[00318] The aim of this study was to explore the effects of an exemplary modified vaccinia virus according to this disclosure, where the vaccinia virus VH1 gene was replaced with a mutated VH1 gene from a different poxvirus, in murine tumor models, in comparison with vaccinia viruses that do not have the exemplary modifications.

[00319] In one experiment, BALB/c mice bearing RENCA tumors subcutaneously were treated with a single intratumoral injection (10 x 10^7 PFU) of WR.TK-GMCSF virus, WR.TK-VH1mut virus, or sham control. In this case, the WR.TK-VH1mut virus had TK gene deleted and VH1 gene replaced with a VH1 gene from a different species of poxvirus,

which was also modified with certain mutations to be more active. The WR.TK-GMCSF virus had TK gene deleted and had an exogenous nucleic acid encoding GMCSF, but had no modification of VH1 gene. Tumor volume growth was monitored as shown in **FIG. 15** over the time after the virus injection by caliper measurement. WR.TK-VH1mut virus significantly delayed the tumor growth as compared to both other two groups, suggesting that the exemplary modifications of the viral VH1 gene can result in enhanced therapeutic effects against the tumor.

[00320] **EXAMPLE 5: CLINICAL STUDY OF AN EXEMPLARY MODIFIED VACCINIA VIRUS IN PATIENTS WITH A METASTATIC CANCER**

[00321] A Phase 1B/2, open-label, study of an exemplary vaccinia virus (WR.TK-PH20.CXCR4) as disclosed herein is carried out in patients with a metastatic cancer.

Study Design:

[00322] The study is an open-label, Phase 1B/2 study evaluating the modified vaccinia virus WR.TK-PH20.CXCR4 in patients with a metastatic cancer. The study has 2 phases: a Dose Escalation/Confirmation Phase (Phase 1b) and an Expansion Phase (Phase 2), with the Expansion Phase utilizing a Simon 2-stage design for each cohort.

[00323] The modified vaccinia virus WR.TK-PH20.CXCR4 is a Western Reserve vaccinia virus, comprising a deletion of TK gene, an exogenous nucleic acid coding for PH20 and CXCR4. As implicated in animal studies, the deletion of TK and expression of PH20 and CXCR4 in vaccinia virus can result in greater therapeutic effects against cancer.

Phase 1B (Dose escalation phase)

Objectives:

Dose

[00324] Determine the dose-limiting toxicities (DLT) and maximum tolerated dose (MTD) or recommended Phase 2 dose (RP2D) of WR.TK-PH20.CXCR4 virus.

[00325] Evaluate safety and the tolerability of WR.TK-PH20.CXCR4 virus, as measured by clinical adverse events (AEs) and laboratory parameters.

[00326] The starting dose (dose level 1) for WR.TK-PH20.CXCR4 virus is up to 5 $x10^9$ PFU by intravenous injection once.

[00327] If dose level 1 is not tolerated, dose level -1 for WR.TK-PH20.CXCR4 virus is set at up to 5 x10^7 PFU once.

[00328] Each dose level in the dose escalation phase enrolls between 6 and 12 evaluable patients.

Table 1. Dose Escalation Schematic

Cohort	Number of Subjects	Exemplary WR.TK-PH20.CXCR4 Dose
-1	6-12	5X10^9 PFU
1	6-12	5X10^8 PFU

[00329] *Safety*

[00330] Safety is assessed during the study by documentation of AEs, clinical laboratory tests, physical examination, vital sign measurements, electrocardiograms (ECGs), and other relevant procedures.

[00331] Any detected cumulative toxicity may require later dose reductions and/or other changes to the dosing schedule, as appropriate, including further refinement of the RP2D.

[00332] If the dose of up to 5 x 10^9 PFU exceeds the MTD, then a lower dose is evaluated. Toxicities are assessed by the study Investigator using the United States (US) National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 4.03. The decision regarding whether to proceed to the next dose level is made by the Medical Monitor in consultation with the study Investigators after the majority of the safety assessments for each cohort are completed.

[00333] After completion of the Dose Escalation/Confirmation Phase of the study, with identification of the MTD/RP2D, the Phase 2 portion of the study commences.

[00334] *Phase 2*

[00335] Phase 2 (Expansion): In the Expansion Phase, WR.TK-PH20.CXCR4 virus is evaluated using the RP2D identified in the Dose Escalation/Confirmation Phase in patients with a metastatic cancer. In the phase 2 component, patients with the metastatic cancer are randomized in a 2:1 ratio to receive WR.TK-PH20.CXCR4 virus (at the RP2D) or placebo. The primary endpoint of this randomized component is progression free survival (PFS) as

assessed by iRECIST. Secondary endpoints include durable remission rate (DRR), treatment free interval (TFI), quality of life (QoL), overall response rate (ORR), overall survival (OS), and safety. The sample size in the Phase 2 component is 100-150 patients, which can result in 90% power with a one-sided p = 0.1 to detect a PFS benefit with a hazard ratio (HR) of about 0.5. The duration of treatment for the test arm is 6 months, 9 months, 12 months, or 18 months, and that for the control arm is 4-6 months. Accrual is estimated to take 12 months, and the duration of the trial is projected to be 2-3 years.

[00336] **EXAMPLE 6: CLINICAL STUDY OF AN EXEMPLARY MODIFIED VACCINIA VIRUS IN PATIENTS WITH A CANCER**

[00337] A Phase 1B/2, open-label, study of an exemplary vaccinia virus (WR.TK-PH20.CXCR4) as disclosed herein is carried out in patients with a cancer.

Study Design:

[00338] The study is an open-label, Phase 1B/2 study evaluating the modified vaccinia virus WR.TK-PH20.CXCR4 in patients with a cancer. The study has 2 phases: a Dose Escalation/Confirmation Phase (Phase 1b) and an Expansion Phase (Phase 2), with the Expansion Phase utilizing a Simon 2-stage design for each cohort.

[00339] The modified vaccinia virus WR.TK-PH20.CXCR4 is a Western Reserve vaccinia virus, comprising a deletion of TK gene, an exogenous nucleic acid coding for PH20 and CXCR4. As implicated in animal studies, the deletion of TK and expression of PH20 and CXCR4 in vaccinia virus can result in greater therapeutic effects against cancer.

Phase 1B (Dose escalation phase)

Objectives:

Dose

[00340] Determine the dose-limiting toxicities (DLT) and maximum tolerated dose (MTD) or recommended Phase 2 dose (RP2D) of WR.TK-PH20.CXCR4 virus.

[00341] Evaluate safety and the tolerability of WR.TK-PH20.CXCR4 virus, as measured by clinical adverse events (AEs) and laboratory parameters.

[00342] The starting dose (dose level 1) for WR.TK-PH20.CXCR4 virus is up to 5 $x10^9$ PFU by intravenous injection once.

[00343] If dose level 1 is not tolerated, dose level -1 for WR.TK-PH20.CXCR4 virus is set at up to 5 x10^7 PFU once.

[00344] Each dose level in the dose escalation phase enrolls between 6 and 12 evaluable patients.

Table 2. Dose Escalation Schematic

Cohort	Number of Subjects	WR.TK-PH20.CXCR4 Dose
-1	6-12	5X10^9 PFU
1	6-12	5X10^8 PFU

[00345] *Safety*

[00346] Safety is assessed during the study by documentation of AEs, clinical laboratory tests, physical examination, vital sign measurements, electrocardiograms (ECGs), and other relevant procedures.

[00347] Any detected cumulative toxicity may require later dose reductions and/or other changes to the dosing schedule, as appropriate, including further refinement of the RP2D.

[00348] If the dose of up to 5 x 10^9 PFU exceeds the MTD, then a lower dose is evaluated. Toxicities are assessed by the study Investigator using the United States (US) National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 4.03. The decision regarding whether to proceed to the next dose level is made by the Medical Monitor in consultation with the study Investigators after the majority of the safety assessments for each cohort are completed.

[00349] After completion of the Dose Escalation/Confirmation Phase of the study, with identification of the MTD/RP2D, the Phase 2 portion of the study commences.

[00350] *Phase 2*

[00351] Phase 2 (Expansion): In the Expansion Phase, WR.TK-PH20.CXCR4 virus is evaluated using the RP2D identified in the Dose Escalation/Confirmation Phase in patients with a cancer. In the phase 2 component, patients with the cancer are randomized in a 2:1 ratio to receive WR.TK-PH20.CXCR4 virus (at the RP2D) or placebo. The primary endpoint of this randomized component is progression free survival (PFS) as assessed by iRECIST. Secondary endpoints include durable remission rate (DRR), treatment free interval (TFI),

quality of life (QoL), overall response rate (ORR), overall survival (OS), and safety. The sample size in the Phase 2 component is 100-150 patients, which can result in 90% power with a one-sided p = 0.1 to detect a PFS benefit with a hazard ratio (HR) of about 0.5. The duration of treatment for the test arm is 6 months, 9 months, 12 months, or 18 months, and that for the control arm is 4-6 months. Accrual is estimated to take 12 months, and the duration of the trial is projected to be 2-3 years.

[00352] **EXAMPLE 7: EXEMPLARY VACCINIA VIRUSES WITH EXEMPLARY MUTATIONS TO PROMOTE NK CELL ACTIVITY SHOW ENHANCED THERAPEUTIC EFFECTS IN MURINE TUMOR MODELS**

[00353] In order to test whether oncolytic viruses with mutations designed to promote NK cell activity displayed anti-cancer effects, the following modified viruses were generated: (i) a virus with K7R deleted, and exogenous nucleic acids encoding both IL15 and CCL5 inserted, referred to as K7R- IL15 CCL5, (ii) a virus with TK deleted, and an exogenous nucleic acid encoding LIGHT inserted, referred to as TK-LIGHT, (iii) a virus with TK deleted, and exogenous nucleic acids encoding both ITAC and fractalkine (also known as CX3CL1) inserted, referred to as TK-ITAC fractalkine, (iv) a virus with A52R deleted, and exogenous nucleic acids encoding both IL15 and IL15-Rα inserted, referred to as A52R-IL15/IL15-Rα, and (v) a virus with TK deleted, A52R deleted, and exogenous nucleic acids encoding ITAC, LIGHT, IL15, and IL15Rα inserted, referred to as TK- ITAC LIGHT A52R-IL15/IL15-Rα.

[00354] In one experiment, BALB/c mice bearing RENCA tumors subcutaneously were treated with a single intratumoral injection (1×10^8 PFU) of K7R- IL15+ CCL5+, TK-LIGHT+, or TK-GMCSF+ viruses. Control mice received an intravenous injection of a control buffer. Tumor volume was quantified on day 34 after treatment. Tumor volume was smaller in mice having received the K7R-IL15+CCL5+ or TK-LIGHT+ modified viruses, suggesting that mutations in oncolytic viruses designed to promote NK cell activity can result in enhanced anti-cancer therapeutic effects (**FIG. 23**).

[00355] In another experiment, BALB/c mice bearing RENCA tumors subcutaneously were treated with a single intratumoral injection (1×10^7 PFU) of TK-LIGHT+, TK-GMCSF+, TK-ITAC+ fractalkine+, A52R- IL15+/IL15R-α+, TK- ITAC+ LIGHT+ A52R- IL15+/IL15-Rα, or a vehicle formulated buffer, termed VFB, and tumor volume was monitored over time as described above. Intratumorally-delivered TK-LIGHT+, TK-ITAC+ fractalkine+, A52R-IL15+/IL15-Rα, and TK- ITAC+ LIGHT+ A52R- IL15+/IL15-Rα+ delayed tumor growth,

suggesting that mutations in oncolytic viruses designed to promote NK cell activity resulted in enhanced therapeutic effects against tumor (**FIG. 24**).

[00356] **EXAMPLE 8: EXEMPLARY VACCINIA VIRUS WITH EXEMPLARY EXOGENOUS NUCLEIC ACIDS ENCODING A CHEMOKINE RECEPTOR AND AN EXTRACELLULAR MATRIX-DEGRADING ENZYME SHOWS SPREAD BETWEEN AND KILLING OF CANCER CELLS**

[00357] In order to test the feasibility of combining elements of the modified oncolytic viruses disclosed above, a modified virus was developed wherein the A52R gene and TK gene were deleted, and exogenous nucleic acids encoding HysA and CXCR4 were inserted (TK- HysA A52R- CXCR4). LLC cancer cells in culture were infected with the TK- (labeled in **FIGS. 25A-25B** as WR TK- GFP 1MOI), A52R-HysA (labeled in **FIGS. 25A-25B** as WR A52R- EL HysA GFP 1MOI), or TK- HysA A52R- CXCR4 (labeled in **FIGS. 25A-25B** as WR A52R- 7.5 CXCR4 TK- EL HysA) modified viruses, each of which also expressed GFP. The A52R-HysA and TK- HysA A52R- CXCR4 viruses were capable of spreading between cancer cells and reducing cancer cell expansion (**FIGS. 25A-25B**). These data provide a non-limiting example demonstrating that oncolytic viruses comprising multiple modified elements disclosed herein can be generated, and can have anti-cancer activity, including spread between and killing of cancer cells.

[00358] **EXAMPLE 9: AN EXEMPLARY MODIFIED HIGH EEV-EXPRESSING VACCINIA VIRUS HAVING AN EXEMPLARY DELETION OF SCR1 SHOWS SPREAD BETWEEN AND KILLING OF CANCER CELLS**

[00359] IHDJ was selected as an exemplary high EEV-producing virus to be evaluated for oncolytic potential. A modified IHDJ virus was developed wherein the neutralizing antibody binding site on the surface of the EEV (B5R SCR1) was deleted (IHDJ-B5RcoΔSCR1). Infection of cultures of MC38 and HCT116 cancer cells demonstrated that the IHDJ and IHDJ-B5RcoΔSCR1 (both expressing GFP) were capable of spreading between cancer cells and reducing cancer cell expansion (**FIGS. 26A-26B**). A plaque assay further supported the ability of the IHDJ and IHDJ-B5RcoΔSCR1 viruses to spread between and kill cancer cells (**FIG. 26C**). In another experiment, a comet tail assay was performed, wherein cancer cell monolayers were infected at a low multiplicity of infection and incubated under liquid media. In this assay, spread of the virus results in elongated "comet tail"-shaped plaques. The IHDJ and IHDJ-B5RcoΔSCR1 viruses produced prominent comet tails, consistent with effective EEV production (**FIG. 26D**). These experiments demonstrate that high EEV-expressing vaccinia viruses, including an exemplary modified virus with the neutralizing antibody

binding site on the surface of the EEV deleted, can have anti-cancer activity, including spread between and killing of cancer cells.

[00360] Exemplary sequences for the viral backbone genes and viral proteins are provided in Table 4. Further provided are exemplary sequences for proteins expressed by various exogenous nucleic acid sequences that can be inserted into the modified oncolytic viruses described herein. In some cases, the above described viral backbones genes and viral proteins, in the modified oncolytic viruses described herein, can comprise sequences that are about 70%, about 75%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identical to the exemplary sequences of viral backbones genes and viral proteins provided in Table 4. In some cases, the above described proteins expressed from the exogenous nucleic acid sequences in the modified oncolytic viruses described herein, can comprise sequences that are about 70%, about 75%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identical to the exemplary sequences of proteins provided in Table 4.

Table 4

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
1	B5R	>tr|Q80KX4|Q80KX4_9POXV B5R OS=Vaccinia virus GN=B5R PE=4 SV=1 MKTISVVTLLCVLPAVVYSTCTVPTMNNAKLTSTETSFNDKQKVTFTCDQGY HSLDPNAVCETDKWKYENPCKKMCTVSDYVSELYDKPLYEVNSTMTLSCNGE TKYFRCEEKNGNTSWNDTVTCPNAECQPLQLEHGSCQPVKEKYSFGEYITIN CDVGYEVIGASYISCTANSWNVIPSCQQKCDMPSLSNGLISGSTFSIGGVIH LSCKSGFILTGSPSSTCIDGKWNPILPTCVRSNKEFDPVDDGPDDETDLSKL SKDVVQYEQEIESLEATYHIIIVALTIMGVIFLISVIVLVCSCDKNNDQYKF HKLLP
2	F13L	>tr|Q1M1R8|Q1M1R8_9POXV EEV phospholipase OS=Vaccinia virus GN=F13L PE=4 SV=1 MWPFASVPAGAKCRLVETLPENMDFRSDHLTTFECFNEIITLAKKYIYIASF CCNPLSTTRGALIFDKLKEASEKGIKIIVLLDERGKRNLGELQSHCPDINFI TVNIDKKNNVGLLLGCFWVSDDERCYVGNASFTGGSIHTIKTLGVYSDYPPL ATDLRRRFDTFKAFNSAKNSWLNLCSAACCLPVSTAYHIKNPIGGVFFTDSP EHLLGYSRDLDTDVVIDKLRSAKTSIDIEHLAIVPTTRVDGNSYYWPDIYNS IIEAAINRGVKIRLLVGNWDKNDVYSMATARSLDALCVQNDLSVKVFTIQNN TKLLIVDDEYVHITSANFDGTHYQNHGFVSFNSIDKQLVSEAKKIFERDWVS SHSKSLKI
3	A36R	>sp|P68618|A36_VACCC Protein A36 OS=Vaccinia virus (strain Copenhagen) GN=A36R PE=3 SV=1

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
		MMLVPLITVTVVAGTILVCYILYICRKKIRTVYNDNKIIMTKLKKIKSSNSS KSSKSTDSESDWEDHCSAMEQNNDVDNISRNEILDDDSFAGSLIWDNESNVM APSTEHIYDSVAGSTLLINNDRNEQTIYQNTTVVINETETVEVLNEDTKQNP NYSSNPFVNYNKTSICSKSNPFITELNNKFSENNPFRRAHSDDYLNKQEQDH EHDDIESSVVSLV
4	A34R	>sp|P21057|A34_VACCC Protein A34 OS=Vaccinia virus (strain Copenhagen) GN=A34R PE=3 SV=1 MKSLNRQTVSMFKKLSVPAAIMMILSTIISGIGTFLHYKEELMPSACANGWI QYDKHCYLDTNIKMSTDNAVYQCRKLRARLPRPDTRHLRVLFSIFYKDYWVS LKKTNNKWLDINNDKDIDISKLTNFKQLNSTTDAEACYIYKSGKLVKTVCKS TQSVLCVKKFYK
5	A33R	>tr|Q71TT1|Q71TT1_9POXV A33R OS=Vaccinia virus GN=A33R PE=1 SV=1 MMTPENDEEQTSVFSATVYGDKIQGKNKRKRVIGLCIRISMVISLLSMITMS AFLIVRLNQCMSANEAAITDAAVAVAAASSTHRKVASSTTQYDHKESCNGLY YQGSCYILHSDYQLFSDAKANCTAESSTLPNKSDVLITWLIDYVEDTWGSDG NPITKTTSDYQDSDVSQEVRKYFCVKTMN
6	B8R	>sp|P21004|B8_VACCC Soluble interferon gamma receptor B8 OS=Vaccinia virus (strain Copenhagen) GN=B8R PE=3 SV=1 MRYIIILAVLFINSIHAKITSYKFESVNFDSKIEWTGDGLYNISLKNYGIKT WQTMYTNVPEGTYDISAFPKNDFVSFWVKFEQGDYKVEEYCTGLCVEVKIGP PTVTLTEYDDHINLYIEHPYATRGSKKIPIYKRGDMCDIYLLYTANFTFGDS EEPVTYDIDDYDCTSTGCSIDFATTEKVCVTAQGATEGFLEKITPWSSEVCL TPKKNVYTCAIRSKEDVPNFKDKMARVIKRKFNKQSQSYLTKFLGSTSNDVT TFLSMLNLTKYS
7	B18R	>tr|Q9DUN2|Q9DUN2_9POXV Interferon-alpha/beta receptor OS=Vaccinia virus GN=B18R PE=4 SV=1 MTMKMMVHIYFVSLSLLLLLFHSYAIDIENEITEFFNKMRDTLPAKDSKWLN PACMFGGTMNDIAALGEPFSAKCPPIEDSLLSHRYKDYVVKWERLEKNRRRQ VSNKRVKHGDLWIANYTSKFSNRRYLCTVTTKNGDCVQGIVRSHIRKPPSCI PKTYELGTHDKYGIDLYCGILYAKHYNNITWYKDNKEINIDDIKYSQTGKKL IIHNPELEDSGRYDCYVHYDDVRIKNDIVVSRCKILTVLPSQDHRFKLKRNC GYASN
8	SPI-1	>sp|P15058|SPI1_VACCW Serine proteinase inhibitor 1 OS=Vaccinia virus (strain Western Reserve) GN=SPI-1 PE=2 SV=1 MDIFKELILKHTDENVLISPVSILSTLSILNHGAAGSTAEQLSKYIENMNEN TPDDNNDMDVDIPYCATLATANKIYGSDSIEFHASFLQKIKDDFQTVNFNNA NQTKELINEWVKTMTNGKINSLLTSPLSINTRMTVVSAVHFKAMWKYPFSKH LTYTDKFYISKNIVTSVDMMVSTENNLQYVHINELFGGFSIIDIPYEGNSSM VIILPDDIEGIYNIEKNITDEKFKKWCGMLSTKSIDLYMPKFKVEMTEPYNL VPILENLGLTNIFGYYADFSKMCNETITVEKFLHTTFIDVNEEYTEASAVTG VFMTNFSMVYRTKVYINHPFMYMIKDNTGRILFIGKYCYPQ
9	SPI-2	>sp|P15059|SPI2_VACCW Serine proteinase inhibitor 2 OS=Vaccinia virus (strain Western Reserve) GN=SPI-2 PE=2 SV=2 MDIFREIASSMKGENVFISPASISSVLTILYYGANGSTAEQLSKYVEKEENM DKVSAQNISFKSINKVYGRYSAVFKDSFLRKIGDKFQTVDFTDCRTIDAINK CVDIFTEGKINPLLDEPLSPDTCLLAISAVYFKAKWLTPFEKEFTSDYPFYV SPTEMVDVSMMSMYGKAFNHASVKESFGNFSIIELPYVGDTSMMVILPDKID GLESIEQNLTDTNFKKWCNSLEATFIDVHIPKFKVTGSYNLVDTLVKSGLTE VFGSTGDYSNMCNSDVSVDAMIHKTYIDVNEEYTEAAAATCALVSDCASTIT NEFCVDHPFIYVIRHVDGKILFVGRYCSPTTNC

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
10	B15R	>tr|L7QJF6|L7QJF6_9POXV Uncharacterized protein OS=Vaccinia virus GN=B15R PE=4 SV=1 MTANFSTHVFSPQHCGCDRLTSIDDVRQCLTEYIYWSSYAYRNRQCAGQLYS TLLSFRDDAESVFIDIRELVKNMPWDDVKDCTEIIRCYIPDEQKTIREISAI IGLCAYAATYWGGEDHPTSNSLNALFVMLEMLNYVDYNIIFRRMN
11	B14R (*Vaccinia virus* Copenhagen strain)	>sp|P21089|B14_VACCC Protein B14 OS=Vaccinia virus (strain Copenhagen) GN=B15R PE=3 SV=1 MTANFSTHVFSPQHCGCDRLTSIDDVKQCLTEYIYWSSYAYRNRQCAGQLYS TLLSFRDD AELVFIDIRELVKNMPWDDVKDCTEIIRCYIPDEQKTIREISAIIGLCAYAA TYWGGEDH PTSNSLNALFVMLEMLNYVDYNIIFRRMN
12	VGF	>sp|P01136|VGF_VACCW Pro-vaccinia growth factor OS=Vaccinia virus (strain Western Reserve) GN=VGF-1 PE=1 SV=1 MSMKYLMLLFAAMIIRSFADSGNAIETTSPEITNATTDIPAIRLCGPEGDGY CLHGDCIHARDIDGMYCRCSHGYTGIRCQHVVLVDYQRSENPNTTTSYIPSP GIMLVLVGIIIITCCLLSVYRFTRRTKLPIQDMVVP
13	E3L	>tr|Q86638|Q86638_9POXV Double-stranded RNA-binding protein OS=Vaccinia virus GN=E3L PE=1 SV=1 MSKIYIDERSNAEIVCEAIKTIGIEGATAAQLTRQLNMEKREVNKALYDLQR SAMVYSSDDIPPRWFMTTEADEADADAMSDVIIDDVSREKSMREDHKSFDDV IPAKKIIDWKGANPVTVINEYCQITRRDWSFRIESVGPSNSPTFYACVDIDG RVFDKADGKSKRDAKNNAAKLAVDKLLGYVIIRF
14	K3L	>sp|P20639|K3_VACCC Protein K3 OS=Vaccinia virus (strain Copenhagen) GN=K3L PE=1 SV=1 MLAFCYSLPNAGDVIKGRVYEKDYALYIYLFDYPHSEAILAESVKMHMDRYV EYRDKLVGKTVKVKVIRVDYTKGYIDVNYKRMCRHQ
15	A41L	>sp|P21064|A41_VACCC Protein A41 OS=Vaccinia virus (strain Copenhagen) GN=A41L PE=3 SV=1 MYSLLFIILMCIPFSFQTVYDDKSVCDSDNKEYMGIEVYVEATLDEPLRQTT CESEIHKYGASVSNGGLNISVDLLNCFLNFHTVGVYTNRDTVYAKFASLDPW TTEPINSMTHDDLVKLTEECIVDIYLKCEVDKTKDFMKTNGNRLKPRDFKTV PPSDVGSMIELQSDYCVNDVTAYVKIYDECGNIKQHSIPTLRDYFTTKNGQP RKILKKKFDNC
16	K7R	>sp|P68467|K7_VACCC Protein K7 OS=Vaccinia virus (strain Copenhagen) GN=K7R PE=1 SV=1 MATKLDYEDAVFYFVDDDKICSRDSIIDLIDEYITWRNHVIVFNKDITSCGR LYKELMKFDDVAIRYYGIDKINEIVEAMSEGDHYINFTKVHDQESLFATIGI CAKITEHWGYKKISESRFQSLGNITDLMTDDNINILILFLEKKLN
17	N1L	>sp|P21054|N1_VACCC Protein N1 OS=Vaccinia virus (strain Copenhagen) GN=N1L PE=1 SV=1 MRTLLIRYILWRNDNDQTYYNDDFKKLMLLDELVDDGDVCTLIKNMRMTLSD GPLLDRLNQPVNNIEDAKRMIAISAKVARDIGERSEIRWEESFTILFRMIET YFDDLMIDLYGEK
18	A52R	>sp|Q01220|A52_VACCW Protein A52 OS=Vaccinia virus (strain Western Reserve) GN=VACWR178 PE=1 SV=1 MDIKIDISISGDKFTVTTRRENEERKKYLPLQKEKTTDVIKPDYLEYDDLLD RDEMFTILEEYFMYRGLLGLRIKYGRLFNEIKKFDNDAEEQFGTIEELKQKL RLNSEEGADNFIDYIKVQKQDIVKLTVYDCISMIGLCACVVDVWRNEKLFSR WKYCLRAIKLFINDHMLDKIKSILQNRLVYVEMS

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
19	B5R	>NC_006998.1:168374-169327 Vaccinia virus, complete genome ATGAAAACGATTTCCGTTGTTACGTTGTTATGCGTACTACCTGCTGTTGTTT ATTCAACATGTACTGTACCCACTATGAATAACGCTAAATTAACGTCTACCGA AACATCGTTTAATGATAAACAGAAAGTTACGTTTACATGTGATCAGGGATAT CATTCTTCGGATCCAAATGCTGTCTGCGAAACAGATAAATGGAAATACGAAA ATCCATGCAAAAAAATGTGCACAGTTTCTGATTACATCTCTGAATTATATAA TAAACCGCTATACGAAGTGAATTCCACCATGACACTAAGTTGCAACGGCGAA ACAAAATATTTTCGTTGCGAAGAAAAAAATGGAAATACTTCTTGGAATGATA CTGTTACGTGTCCTAATGCGGAATGTCAACCTCTTCAATTAGAACACGGATC GTGTCAACCAGTTAAAGAAAAATACTCATTTGGGGAATATATGACTATCAAC TGTGATGTTGGATATGAGGTTATTGGTGCTTCGTACATAAGTTGTACAGCTA ATTCTTGGAATGTTATTCCATCATGTCAACAAAAATGTGATATGCCGTCTCT ATCTAATGGATTAATTTCCGGATCTACATTTTCTATCGGTGGCGTTATACAT CTTAGTTGTAAAAGTGGTTTTACACTAACGGGGTCTCCATCATCCACATGTA TCGACGGTAAATGGAATCCCGTACTCCCAATATGTGTACGAACTAACGAAGA ATTTGATCCAGTGGATGATGGTCCCGACGATGAGACAGATTTGAGCAAACTC TCGAAAGACGTTGTACAATATGAACAAGAAATAGAATCGTTAGAAGCAACTT ATCATATAATCATAGTGGCGTTAACAATTATGGGCGTCATATTTTTAATCTC CGTTATAGTATTAGTTTGTTCCTGTGACAAAAATAATGACCAATATAAGTTC CATAAATTGCTACCGTAA
20	F13L	>NC_006998.1:c41949-40831 Vaccinia virus, complete genome ATGTGGCCATTTGCATCGGTACCTGCGGGAGCAAAATGTAGGCTGGTAGAAA CACTACCAGAAAATATGGATTTTAGATCCGATCATTTAACAACATTTGAATG TTTTAACGAAATTATCACTCTAGCTAAGAAATATATATACATAGCATCTTTT TGTTGTAATCCTCTGAGTACGACTAGGGGAGCGCTTATTTTTGATAAACTAA AAGAGGCATCTGAAAAAGGGATTAAAATAATAGTTTTGCTAGATGAACGAGG GAAAAGAAATCTGGGAGAGCTACAAAGTCACTGCCCGGATATAAATTTTATA ACCGTTAATATAGATAAAAAAAATAATGTGGGACTACTACTCGGTTGTTTTT GGGTGTCAGATGATGAAAGATGTTATGTAGGAAACGCGTCATTTACTGGAGG ATCTATACATACGATTAAAACGTTAGGTGTATATTCTGATTATCCCCCGCTG GCCACAGATCTTCGTAGAAGATTTGATACTTTTAAAGCCTTTAATAGCGCAA AAAATTCATGGTTGAATTTATGCTCTGCGGCTTGTTGTTTGCCAGTTAGCAC TGCGTATCATATTAAGAATCCTATAGGTGGAGTGTTCTTTACTGATTCTCCG GAACACCTATTGGGATATTCTAGAGATCTAGATACCGATGTAGTTATTGATA AACTCAAGTCGGCTAAGACTAGTATAGATATTGAACATTTGGCCATAGTTCC CACTACACGTGTCGACGGTAATAGCTACTATTGGCCCGACATTTACAACTCC ATTATAGAAGCAGCCATTAATAGAGGAGTTAAGATCAGACTTCTAGTTGGTA ATTGGGATAAGAACGACGTATATTCTATGGCAACCGCCAGAAGTCTAGACGC GTTGTGTGTTCAAAATGATCTATCTGTGAAGGTTTTCACTATTCAGAATAAT ACAAAATTGTTGATAGTCGACGACGAATATGTTCATATCACTTCGGCAAATT TCGACGGAACCCATTACCAAAATCACGGATTCGTCAGTTTTAATAGTATAGA TAAACAGCTTGTAAGCGAGGCTAAAAAAATATTTGAGAGAGATTGGGTATCT AGCCACAGTAAATCGTTAAAAATTTAA
21	A36R	>NC_006998.1:145059-145724 Vaccinia virus, complete genome ATGATGCTGGTACCTCTTATCACGGTGACCGTAGTTGCGGGAACAATATTAG TATGTTATATATTATATATTTGTAGGAAAAAGATACGTACTGTCTATAATGA CAATAAAATTATCATGACAAAATTAAAAAAGATAAAGAGTTCTAATTCCAGC AAATCTAGTAAATCAACTGATAGCGAATCAGACTGGGAGGATCACTGTAGTG CTATGGAACAAAACAATGACGTAGATAATATTTCTAGGAATGAGATATTGGA CGATGATAGCTTCGCTGGTAGTTTAATATGGGATAACGAATCCAATGTCATG GCGCCTAGCACAGAACACATTTACGATAGTGTTGCTGGAAGCACGCTGCTAA TAAATAATGATCGTAATGAACAGACTATTTATCAGAACACTACAGTAGTAAT TAATGAGACGGAGACTGTTGAAGTACTTAATGAAGATACCAAACAGAATCCT

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
		AACTATTCATCCAATCCTTTCGTAAATTATAATAAAACCAGTATTTGTAGCA AGTCAAATCCGTTCATTACAGAACTCAACAATAAATTTAGTGAGAATAATCC GTTTAGACGAGCACATAGCGATGATTATCTTAATAAGCAAGAACAAGATCAT GAACACGATGATATAGAATCATCGGTCGTATCATTGGTGTGA
22	A34R	>NC_006998.1:143912-144418 Vaccinia virus, complete genome ATGAAATCGCTTAATAGACAAACTGTAAGTAGGTTTAAGAAGTTGTCGGTGC CGGCCGCTATAATGATGATACTCTCAACCATTATTAGTGGCATAGGAACATT TCTGCATTACAAAGAAGAACTGATGCCTAGTGCTTGCGCCAATGGATGGATA CAATACGATAAACATTGTTATTTAGATACTAACATTAAAATGTCTACAGATA ATGCGGTTTATCAGTGTCGTAAATTACGAGCCAGATTGCCTAGACCGGATAC TAGACATCTGAGAGTATTGTTTAGTATTTTTTATAAAGATTATTGGGTAAGT TTAAAAAAGACCAATGATAAATGGTTAGATATTAATAATGATAAAGATATAG ATATTAGTAAATTAACAAATTTTAAACAACTAAACAGTACGACGGATGCTGA AGCGTGTTATATATACAAGTCTGGAAAACTGGTTAAAACAGTATGTAAAAGT ACTCAATCTGTACTATGTGTTAAAAAATTCTACAAGTGA
23	A33R	>NC_006998.1:143331-143888 Vaccinia virus, complete genome ATGATGACACCAGAAAACGACGAAGAGCAGACATCTGTGTTCTCCGCTACTG TTTACGGAGACAAAATTCAAGGAAAGAATAAACGCAAACGCGTGATTGGTCT ATGTATTAGAATATCTATGGTTATTTCACTACTATCTATGATTACCATGTCC GCGTTTCTCATAGTGCGCCTAAATCAATGCATGTCTGCTAACGAGGCTGCTA TTACTGACGCCGCTGTTGCCGTTGCTGCTGCATCATCTACTCATAGAAAGGT TGCGTCTAGCACTACACAATATGATCACAAAGAAAGCTGTAATGGTTTATAT TACCAGGGTTCTTGTTATATATTACATTCAGACTACCAGTTATTCTCGGATG CTAAAGCAAATTGCACTGCGGAATCATCAACACTACCCAATAAATCCGATGT CTTGATTACCTGGCTCATTGATTATGTTGAGGATACATGGGGATCTGATGGT AATCCAATTACAAAAACTACATCCGATTATCAAGATTCTGATGTATCACAAG AAGTTAGAAAGTATTTTTGTGTTAAAACAATGAACTAA
24	B8R	>NC_006998.1:170571-171389 Vaccinia virus, complete genome ATGAGATATATTATAATTCTCGCAGTTTTGTTCATTAATAGTATACACGCTA AAATAACTAGTTATAAGTTTGAATCCGTCAATTTTGATTCCAAAATTGAATG GACTGGGGATGGTCTATACAATATATCCCTTAAAAATTATGGCATCAAGACG TGGCAAACAATGTATACAAATGTACCAGAAGGAACATACGACATATCCGCAT TTCCAAAGAATGATTTCGTATCTTTCTGGGTTAAATTTGAACAAGGCGATTA TAAAGTGGAAGAGTATTGTACGGGACTATGCGTCGAAGTAAAAATTGGACCA CCGACTGTAACATTGACTGAATACGACGACCATATCAATTTGTACATCGAGC ATCCGTATGCTACTAGAGGTAGCAAAAAGATTCCTATTTACAAACGCGGTGA CATGTGTGATATCTACTTGTTGTATACGGCTAACTTCACATTCGGAGATTCT AAAGAACCAGTACCATATGATATCGATGACTACGATTGCACGTCTACAGGTT GCAGCATAGACTTTGTCACAACAGAAAAAGTGTGCGTGACAGCACAGGGAGC CACAGAAGGGTTTCTCGAAAAAATTACTCCATGGAGTTCGAAAGTATGTCTG ACACCTAAAAAGAGTGTATATACATGCGCAATTAGATCCAAAGAAGATGTTC CCAATTTCAAGGACAAAATGGCCAGAGTTATCAAGAGAAAATTTAATAAACA GTCTCAATCTTATTTAACTAAATTTCTCGGTAGCACATCAAATGATGTTACC ACTTTTCTTAGCATGCTTAACTTGACTAAATATTCATAA
25	B18R	>NC_006998.1:177306-179030 Vaccinia virus, complete genome ATGAGTCGTCGTCTGATTTATGTTTTAAATATCAACCGCGAATCAACTCATA AAATACAAGAGAATGAAATATATACATATTTTAGTCATTGCAATATAGACCA TACTTCTACAGAACTTGATTTTGTAGTTAAAAACTATGATCTAAACAGACGA CAACCTGTAACTGGGTATACTGCACTACACTGCTATTTGTATAATAATTACT TTACAAACGATGTACTGAAGATATTATTAAATCATGGAGTGGATGTAACGAT GAAAACCAGTAGCGGACGTATGCCTGTTTATATATTGCTTACTAGATGTTGC AATATTTCACATGATGTAGTGATAGATATGATAGACAAAGATAAAAACCACT

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
		TATTACATAGAGACTATTCCAACCTATTACTAGAGTATATAAAATCTCGTTA CATGTTATTAAAGGAAGAGGATATCGATGAGAACATAGTATCCACTTTATTA GATAAGGGAATCGATCCTAACTTTAAACAAGACGGATATACAGCGTTACATT ATTATTATTTGTGTCTCGCACACGTTTATAAACCAGGTGAGTGTAGAAAACC GATAACGATAAAAAAGGCCAAGCGAATTATTTCTTTGTTTATACAACATGGA GCTAATCTAAACGCGTTAGATAATTGTGGTAATACACCATTCCATTTGTATC TTAGTATTGAAATGTGTAATAATATTCATATGACTAAAATGCTGTTGACTTT TAATCCGAATTTCGAAATATGTAATAATCATGGATTAACGCCTATACTATGT TATATAACTTCCGACTACATACAACACGATATTCTTGTTATGTTAATACATC ACTATGAAACAAATGTTGGAGAAATGCCGATAGATGAGCGTCGTATAATCGT ATTCGAGTTTATCAAAACATATTCTACACGTCCTGCAGATTCGATAACTTAT TTGATGAATAGGTTTAAAAATATAGATATTTATACCCGCTATGAAGGAAAGA CATTATTACACGTAGCATGTGAATATAATAATACACACGTAATAGATTATCT TATACGTATCAACGGAGATATAAATGCGTTAACCGACAATAACAAACACGCT ACACAACTCATTATAGATAACAAAGAAAATTCCCCATATACCATTAATTGTT TACTGTATATACTTAGATATATTGTAGATAAGAATGTGATAAGATCGTTGGT GGATCAACTTCCATCTCTACCTATCTTCGATATAAAATCATTTGAGAAATTC ATATCCTACTGTATACTTTTAGATGACACATTTTACAATAGACACGTTAGGA ATCGCGATTCTAAAACGTATCGATACGCATTTTCAAAATACATGTCGTTTGA TAAATACGATGGTATAATAACTAAATGTCATAAAGAAACAATATTGCTCAAA CTATCCACTGTTCTAGACACTACACTATATGCAGTTTTAAGATGCCATAATT CGAAAAAGTTAAGAAGATACCTCACCGAGTTAAAAAAATATAATAACGATAA GTCCTTTAAAATATATTCTAATATTATGAATGAGAGATACCTTAATGTATAT TATAAAGATATGTACGTGTCAAAGGTATATGATAAACTATTTCCTGTTTTCA CAGATAAAAATTGTCTACTAACATTACTACCTTCAGAAATTATATACGAAAT ATTATACATGCTGACAATTAACGATCTTTATAATATATCGTATCCACCTACC AAAGTATAG
26	SPI-1	>M24217.1 Vaccinia virus serine protease inhibitor superfamily gene SPI-1 TCACATAATCTATTTAGAGATCGAGTCATGCACGATTATATAAGTAATACAT ATATTGATCTTGAGTGTTTAGATATTATTAGATCGTTGGATGGATTCGATAT CAATGGTTACTTTGAAGGACGTACACCACTTCATTGCGCTATACAACATAAC TTCACTCAGATTGCTAAGTACTTATTAGATCGAGGAGCTGATATAGTCGTAC CCAACACATTGATTATACATCAGTACATACAGTAAATAGCATAGATATGGAG GAGGATACAAATATTTCAAATAAAGTTATAAGGTACAACACTGTCAATAATA TATGGGAAACATTACCTAACTTCTGGACTGGAACTATAAATCCAGGCGTGGT CTCGCATAAAGATGATATATATGTTGTATGCGACATCAAAGATGAAAAAAAT GTTAAAACTTGTATATTTAGATATAACACGAATACGTATAACGGATGGGAAT TGGTCACGACGACAGAAAGCAGATTATCAGCTCTGCATACTATTCTTTATAA CAATACCATAATGATGTTACATTGTTATGAATCGTATATGTTACAAGATACA TTTAATGTGTACACTCGCGAATGGAATCATATGTGTCATCAACATTCGAATA GTTATATCATGTACAATATACTACCCATCTACTAAATATAATAGAATAAAAT AAATGAGTATGATCATTTTAGATAACGATTGATTTTATCATTACCGCTTCAT TCTTATATTCTTTGCTTACGGAACCTATATTTAGAAACATCTACTAACGATT TTTTATGCTTGCATTATTAATGGTATGTAATATGATTGATTGTGTACGCAAT ACCAATTTGTTAAGTATGAATACGGGGTACAAACATAAACTGAAGTTTAACA TTATTTATTTATGATATATATCGTTATTGTTTGGTCTATACCATGGATATCT TTAAAGAACTAATCTTAAAACACACGGATGAAAATGTTTTGATTTCTCCAGT TTCTATTTTATCTACTTTATCTATTCTAAATCATGGAGCAGCTGGTTCTACA GCTGAACAACTATCAAAATATATAGAGAATATGAATGAGAATACACCCGATG ACAATAATGACATGGACGTAGATATTCCGTATTGTGCGACACTAGCTACCGC AAATAAAATATACGGTAGCGATAGTATCGAGTTCCACGCCTCCTTCCTACAA AAAATAAAAGACGATTTTCAAACTGTAAACTTTAATAATGCTAACCAAACAA AGGAACTAATCAACGAATGGGTTAAGACAATGACAAATGGTAAAATTAATTC CTTATTGACTAGTCCGCTATCCATTAATACTCGTATGACAGTTGTTAGCGCC GTCCATTTTAAAGCAATGTGGAAATATCCATTTTCTAAACATCTTACATATA CAGACAAGTTTTATATTTCTAAGAATATAGTTACCAGTGTTGATATGATGGT GAGCACTGAGAATAACTTGCAATATGTACATATTAATGAATTATTCGGAGGA

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
		TTCTCTATTATCGATATTCCATACGAGGGAAACTCTAGTATGGTAATTATAC TACCGGACGACATAGAAGGTATATATAACATAGAAAAAAATATAACAGATGA AAAATTTAAAAAATGGTGTGGTATGTTATCTACTAAAAGTATAGACTTGTAT ATGCCAAAGTTTAAAGTGGAAATGACAGAACCGTATAATCTGGTACCGATTT TAGAAAATTTAGGACTTACTAATATATTCGGATATTATGCAGATTTTAGCAA GATGTGTAATGAAACTATCACTGTAGAAAAATTTCTACATACGACGTTTATA GATGTTAATGAGGAGTATACAGAAGCATCGGCCGTTACAGGAGTATTTATGA CTAACTTTTCGATGGTATATCGTACGAAGGTCTACATAAACCATCCATTCAT GTACATGATTAAAGACAACACAGGACGTATACTTTTTATAGGGAAATACTGC TATCCGCAATAAATATAAACAAATAGACTTTTATCACGTTTATCTATGTCTA AATATTACAAATAGTAATAGTATAAACTAAAGCTGATAATACTTAAAAAAAT AATAATATCATTTACAATTAATAGTATAAACTAAAAATTAAACAAATCGTTA TTATAAGTAATATCAAAATGATGATATACGGATTAATAGCGTGTCTTATATT CGTGACTTCATCCATCGCTAGTCCACTTTATATTCCCGTTATTCCACCCATT TCGGAAGATAAATCGTTCAATAGTGTAGAGGTATTAGTTTCCTTGTTTAGAG ATGACCAAAAAGACTATACGGTAACTTCTCAGTTCAATAACTACACTATCGA TACCAAAGACTGGACTATCGGCGTACTATCCACACCTGATGGTTTGGATATA CCATTGACTAATATAACTTATTGGTCACGGTTTACTATAGGTCGTGCATTGT TCAAATCAGAGTCTGAGGATATTTTCCAAAAGAAAATGAGTATTCTAGGTGT TTCTATAGAATGTAAGAAGTCGTCGACATTACTTACTTTTTTGACCGTGCGT AAAATGACTCGAGTATTTAATAAATTTCCAGATATGGCTTATTATCGAGGAG ACTGTTTAAAAGCCGTTTATGTAACAATGACTTATAAAAATACTAAAACTGG AGAGACTGATTACACGTACCTCTCTAATGGGGGGTTGCCTGCATACTATCGT AATGGGGTCGATGGTTGATTATTGATTAGTATATTCCTTATTCTTTTTATTC ACACAAAAAGAACATTTTTATAAACATGAAACCACTGTCTAAATGTAATTAT GATCTTGATTTATAGATGAAGATCAGCCTTTAGAGGATTTTAACCAGTATGT TTAATATGAAAAAAATAAACATAACATATTTTGAGATTAAGCGCTATTGTGC AAGATTATATTAGAATCAAATTAATCTTTCATACGAGAAAAATAACGACATA CGTCGTCAACAAATTAAACTTTTTATTTATTAGTTA ACTAGCTTATAGAACTTGCTCATTGTTATGTTTCTAAAACGGG
27	SPI-2	>M24218.1 Vaccinia virus serine protease inhibitor superfamily gene SPI-2 TCCATGGAAAAACGAAAGTAGTATAAAAGTAATAAAACAAAAAAAAGAATAT AAAAAATTTATAGCTACTTTCTTTGAGGACTGTTTTCCTGAAGGAAATGAAC CTCTGGAATTAGTTAGATATATAGAATTAGTATACACGTTAGATTATTCTCA AACTCCTAATTATGACAGACTACGTAAACTGTTTATACAAGATTGAAATTAT ATTCTTTTTTTTTATAGAGTGTGGTAGTGTTACGGATATTTAATATTAGACTA TCTCTATCGCGCTACACGACCAATATCGATTACTATGGATATCTTCAGGGAA ATCGCATCTTCTATGAAAGGAGAGAATGTATTCATTTCTCCAGCGTCAATCT CGTCAGTATTGACAATACTGTATTATGGAGCTAATGGATCCACTGCTGAACA GCTATCAAAATATGTAGAAAAGGAGGAGAACATGGATAAGGTTAGCGCTCAA AATATCTCATTCAAATCCATAAATAAAGTATATGGGCGATATTCTGCCGTGT TTAAAGATTCCTTTTTGAGAAAAATTGGCGATAAGTTTCAAACTGTTGACTT CACTGATTGTCGCACTATAGATGCAATCAACAAGTGTGTAGATATCTTTACT GAGGGGAAAATCAATCCACTATTGGATGAACCATTGTCTCCTGATACCTGTC TCCTAGCAATTAGTGCCGTATACTTTAAAGCAAAATGGTTGACGCCATTCGA AAAGGAATTTACCAGTGATTATCCCTTTTACGTATCTCCGACGGAAATGGTA GATGTAAGTATGATGTCTATGTACGGCAAGGCATTTAATCACGCATCTGTAA AGGAATCATTCGGCAACTTTTCAATCATAGAACTGCCATATGTTGGAGATAC TAGTATGATGGTCATTCTTCCAGACAAGATTGATGGATTAGAATCCATAGAA CAAAATCTAACAGATACAAATTTTAAGAAATGGTGTAACTCTCTGGAAGCTA CGTTTATCGATGTTCACATTCCCAAGTTTAAGGTAACAGGCTCGTATAATCT GGTGGATACTCTAGTAAAGTCAGGACTGACAGAGGTGTTCGGTTCAACTGGA GATTATAGCAATATGTGTAATTCAGATGTGAGTGTCGACGCTATGATCCACA AAACGTATATAGATGTCAATGAAGAGTATACAGAAGCAGCTGCAGCAACTTG TGCACTGGTGTCAGACTGTGCATCAACAATTACAAATGAGTTCTGTGTAGAT CATCCGTTCATCTATGTGATTAGGCATGTTGATGGAAAAATTCTTTTCGTTG GTAGATATTGCTCTCCGACAACTAATTGTTAACCATTTTTTTTAAAAAATAG

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
		AAAAAACATGTGGTATTAGTGCAGGTCGTTATTCTTCCAATTGCAATTGGTA AGATGACGGCCAACTTTAGTACCCACGTCTTTTCACCACAGCACTGTGGATG TGACAGACTGACCAGTATT
28	B15R	>NC_006998.1:174585-175034 Vaccinia virus, complete genome ATGACGGCCAACTTTAGTACCCACGTCTTTTCACCACAGCACTGTGGATGTG ACAGACTGACCAGTATTGATGACGTCAGACAATGTTTGACTGAATATATTTA TTGGTCGTCCTATGCATACCGCAACAGGCAATGCGCTGGACAATTGTATTCC ACACTCCTCTCTTTTAGAGATGATGCGGAATTAGTGTTCATCGACATTCGCG AGCTGGTAAAAAATATGCCGTGGGATGATGTCAAAGATTGTGCAGAAATCAT CCGTTGTTATATACCGGATGAGCAAAAAACCATCAGAGAGATTTCGGCCATC ATCGGACTTTGTGCATATGCTGCTACTTACTGGGGAGGTGAAGACCATCCCA CTAGTAACAGTCTGAACGCATTGTTTGTGATGCTTGAGATGCTCAATTACGT GGATTATAACATCATATTCCGGCGTATGAATTGA
29	VGF	>S61049.1 VGF=growth factor [vaccinia virus, LIVP, Genomic, 423 nt] ATGTCGATGAAATATCTGATGTTGTTGTTCGCTGCTATGATAATCAGATCAT TCGCCGATAGTGGTAACGCTATCGAAACGACATTGCCAGAAATTACAAACGC TACAACAGATATTCCAGCTATCAGATTATGCGGTCCAGAGGGAGATGGATAT TGTTTACACGGTGACTGTATCCACGCTAGAGATATTGACGGTATGTATTGTA GATGCTCTCATGGTTATACAGGCATTAGATGTCAGCATGTAGTATTAGTAGA CTATCAACGTTCAGAAAACCCAAACACTACAACGTCATATATCCCATCTCCC GGTATTGTGCTTGTATTAGTAGGCATTATTATTATTACGTGTTGTTCATTAT CTGTTTATAGGTTCACTCGACGAACTAAACTACCTATACAAGATATGGTTGT GCCATAA
30	E3L	>NC_006998.1:c48352-47780 Vaccinia virus, complete genome ATGTCTAAAATCTATATCGACGAGCGTTCTAACGCAGAGATTGTGTGTGAGG CTATTAAAACCATTGGAATCGAAGGAGCTACTGCTGCACAACTAACTAGACA ACTTAATATGGAGAAGCGAGAAGTTAATAAAGCTCTGTACGATCTTCAACGT AGTGCTATGGTGTACAGCTCCGACGATATTCCTCCTCGTTGGTTTATGACAA CGGAGGCGGATAAGCCGGATGCTGATGCTATGGCTGACGTCATAATAGATGA TGTATCCCGCGAAAAATCAATGAGAGAGGATCATAAGTCTTTTGATGATGTT ATTCCGGCTAAAAAAATTATTGATTGGAAAGGTGCTAACCCTGTCACCGTTA TTAATGAGTACTGCCAAATTACTAGGAGAGATTGGTCTTTTCGTATTGAATC AGTGGGGCCTAGTAACTCTCCTACATTTTATGCCTGTGTAGACATCGACGGA AGAGTATTCGATAAGGCAGATGGAAAATCTAAACGAGATGCTAAAAATAATG CAGCTAAATTGGCAGTAGATAAACTTCTTGGTTACGTCATCATTAGATTCTG A
31	K3L	>NC_006998.1:c27572-27306 Vaccinia virus, complete genome ATGCTTGCATTTTGTTATTCGTTGCCCAATGCGGGTGATGTAATAAAGGGCA GAGTATACGAGAAGGATTATGCTCTATATATTTATCTTTTTGACTATCCTCA CTTTGAAGCTATCTTGGCAGAGAGTGTTAAGATGCATATGGATAGATATGTT GAATATAGGGATAAACTGGTAGGGAAAACTGTAAAAGTTAAAGTGATTAGAG TTGATTATACAAAAGGATATATAGATGTCAATTACAAAAGGATGTGTAGACA TCAATAA
32	A41L	>NC_006998.1:c150164-149505 Vaccinia virus, complete genome ATGTACTCGTTAGTATTTGTTATTTTGATGTGTATACCATTTAGTTTTCAAA CAGTGTATGATGATAAATCGGTATGCGATTCTGACAATAAAGAATATATGGG AATAGAAGTTTATGTAGAAGCAACGCTAGACGAACCCCTCAGACAAACAACG TGTGAATCCAAAATCCATAAATATGGTGCATCTGTATCAAACGGAGGATTAA

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
		ATATTTCTGTTGATCTATTAAACTGTTTTCTTAATTTTCATACAGTTGGTGT ATACACTAATCGCGATACCGTATACGCGAAGTTTGCTAGTTTGGATCCATGG ACTACGGAACCTATAAATTCTATGACCCATGACGATCTAGTAAAATTAACAG AAGAATGTATAGTGGACATTTATTTAAAATGTGAAGTGGATAAAACAAAGGA TTTCATGAAAACTAACGGTAATAGATTAAAACCAAGAGACTTTAAAACTGTT CCTCCTTCTAATGTAGGAAGCATGATAGAACTACAGTCTGACTATTGCGTAA ACGATGTGACTACATACGTCAAAATATACGATGAGTGTGGAAACATTAAACA GCATTCCATTCCAACACTAAGAGATTATTTTACCACCAAGAATGGTCAACCA CGTAAAATATTAAAGAAAAAATTTGATAATTGTTAA
33	K7R	>NC_006998.1:29832-30281 Vaccinia virus, complete genome ATGGCGACTAAATTAGATTATGAGGATGCTGTTTTTTACTTTGTGGATGATG ATAAAATATGTAGTCGCGACTCCATCATCGATCTAATAGATGAATATATTAC GTGGAGAAATCATGTTATAGTGTTTAACAAAGATATTACCAGTTGTGGAAGA CTGTACAAGGAATTGATGAAGTTCGATGATGTCGCTATACGGTACTATGGTA TTGATAAAATTAATGAGATTGTCGAAGCTATGAGCGAAGGAGACCACTACAT CAATTTTACAAAAGTCCATGATCAGGAAAGTTTATTCGCTACCATAGGAATA TGTGCTAAAATCACTGAACATTGGGGATACAAAAAGATTTCAGAATCTAGAT TCCAATCATTGGGAAACATTACAGATCTGATGACCGACGATAATATAAACAT CTTGATACTTTTTCTAGAAAAAAAATTGAATTGA
34	N1L	>NC_006998.1:c22172-21819 Vaccinia virus, complete genome ATGAGGACTCTACTTATTAGATATATTCTTTGGAGAAATGACAACGATCAAA CCTATTATAATGATGATTTTAAAAAGCTTATGTTGTTGGATGAATTGGTAGA TGACGGCGATGTATGTACATTGATTAAGAACATGAGAATGACGCTGTCCGAC GGTCCATTGCTAGATAGATTGAATCAACCAGTTAATAATATAGAAGACGCTA AGCGAATGATCGCTATTAGTGCCAAAGTGGCTAGAGACATTGGTGAACGTTC AGAAATTAGATGGGAAGAGTCATTCACCATACTCTTTAGGATGATTGAAACA TATTTTGATGATCTAATGATTGATCTATATGGTGAAAAATAA
35	A52R	>NC_006998.1:158743-159315 Vaccinia virus, complete genome ATGGACATAAAGATAGATATTAGTATTTCTGGTGATAAATTTACGGTGACTA CTAGGAGGGAAAATGAAGAAAGAAAAAAATATCTACCTCTCCAAAAAGAAAA AACTACTGATGTTATCAAACCTGATTATCTTGAGTACGATGACTTGTAGATA GAGATGAGATGTTTACTATTCTAGAGGAATATTTTATGTACAGAGGTCTATT AGGCCTCAGAATAAAATATGGACGACTCTTTAACGAAATTAAAAAATTCGAC AATGATGCGGAAGAACAATTCGGTACTATAGAAGAACTCAAGCAGAAACTTA GATTAAATTCTGAAGAGGGAGCAGATAACTTTATAGATTATATAAAGGTACA AAAACAGGATATCGTCAAACTTACTGTATACGATTGCATATCTATGATAGGA TTGTGTGCATGCGTGGTAGATGTTTGGAGAAATGAGAAACTGTTTTCTAGAT GGAAATATTGTTTACGAGCTATTAAACTGTTTATTAATGATCACATGCTTGA TAAGATAAAATCTATACTGCAGAATAGACTAGTATATGTGGAAATGTCATAG
36	PH-20	MGVLKFKHIFFRSFVKSSGVSQIVFTFLLIPCCLTLNFRAPPVIPNVPFLWA WNAPSEFCLGKFDEPLDMSLFSFIGSPRINATGQGVTIFYVDRLGYYPYIDS ITGVTVNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWAR NWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGK LLRPNHLWGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALY PSIYLNTQQSPVAATLYVRNRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQV LKFLSQDELVYTFGETVALGASGIVIWGTLSIMRSMKSCLLLDNYMETILNP YIINVTLAAKMCSQVLCQEQGVCIRKNWNSSDYLHLNPDNFAIQLEKGGKFT VRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAVDVCIADGVCIDAF LKPPMETEEPQIFYNASPSTLSATMFIVSILFLIISSVASL
37	Hyaluronidase (*Bos taurus*)	MRPFSLEVSLHLPWAMAAHLLPVCTLFLNLLSMTQGSRDPVVPNQPFTTIWN ANTEWCMKKHGVDVDISIFDVVTNPGQTFRGPNMTIFYSSQLGTYPYYTSAG EPVFGGLPQNASLNAHLARTFQDILAAMPEPRFSGLAVIDWEAWRPRWAFNW

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
		DTKDIYRQRSRALVQKQHPDWLAPRVEAAAQDQFEGAAEEWMAGTLKLGQAL RPQGLWGFYNFPECYNYDFKSPNYTGRCPLNICAQNDQLGWLWGQSRALYPS IYLPAALEGTKKTQMFVQHRVAEAFRVAAGAGDPKLPVLPYMQLFYDMTNHF LPAEELEHSLGESAAQGAAGVVLWVSWLSTSTKESCQAIKEYVDTTLGPSIL NVTSGARLCSQVLCSGHGRCARRPSYPKARLILNSTSFSIKPTPGGGPLTLQ GALSLEDRLRMAVEFECRCYRGWRGTRCEQWGMW
38	Hyaluronidase (*Bos taurus*)	MRMLRRHHISFRSFAGSSGTPQAVFTFLLLPCCLALDFRAPPLISNTSFLWA WNAPVERCVNRRFQLPPDLRLFSVKGSPQKSATGQFITLFYADRLGYYPHID EKTGKTVFGGIPQLGNLKSHMEKAKNDIAYYIPNDSVGLAVIDWENWRPTWA RNWKPKDVYRDESVELVLQKNPQLSFPEASKIAKVDFETAGKSFMQETLKLG KLLRPNHLWGYYLFPDCYNHNHNQPTYNGNCPDVEKRRNDDLEWLWKESTAL FPSVYLNIRLKSTQNAALYVRNRVQEAIRLSKIASVESPLPVFVYARPVFTD GSSTYLSQGDLVNSVGEIVSLGASGIIMWGSLNLSLSMQSCMNLGTYLNTTL NPYIINVTLAAKMCSQVLCHNEGVCTRKHWNSSDYLHLNPMNFAIQTGEGGK YTVPGTVTLEDLQKFSDTFYCSCYANIHCKKRVDIKNVHSVNVCMAEDICID SPVKLQPSDHSSSQEASTTTFSSISPSTTTATVSPCTPEKHSPECLKVRCSE VIPNVTQKACQSVKLKNISYQSPIQNIKNQTTY
39	Hyaluronidase (*Bos taurus*)	MGMFRRHHISFRSFAGSSGTPQAVFTFLLLPCCLALDFRAPPLISNTSFLWA WNAPVERCVNRRFQLPPDLRLFSVKGSPQKSATGQFITLFYADRLGYYPHID EKTGKTVFGGIPQLGNLKSHLEKAKNDIAYYIPNDSVGLAVIDWENWRPTWA RNWKPKDVYRDESVELVLQKNPQLSFPEASKIAKVDFETAGKSFMQETLKLG KLLRPNHLWGYYLFPDCYNHNHNQPTYNGNCPDVEKRRNDDLEWLWKESTAL FPSVYLNIRLKSTQNAALYVRNRVQEAIRLSKIASVESPLPVFVYARPVFTD GSSTYLSQGDLVNSVGEIVSLGASGIIMWGSLNLSLSVQSCMNLGTYLNTTL NPYIINVTLAAKMCSQVLCHDGGVCTRKHWNSSDYLHLNPMNFAIQTGEGGK YTVPGTLTLEDLQKFSDTFYCSCYSNLSCKKRVDIKNVHSVDVCMAEDVCID AFLKPP
40	Hyaluronidase (*Vespula vulgaris*)	SERPKRVFNIYWNVPTFMCHQYDLYFDEVTNFNIKRNSKDDFQGDKIAIFYD PGEFPALLSLKDGKYKKRNGGVPQEGNITIHLQKFIENLDKIYPNRNFSGIG VIDFERWRPIFRQNWGNMKIHKNFSIDLVRNEHPTWNKKMIELEASKRFEKY ARFFMEETLKLAKKTRKQADWGYYGYPYCFNMSPNNLVPECDVTAMHENDKM SWLFNNQNVLLPSVYVRQELTPDQRIGLVQGRVKEAVRISNNLKHSPKVLSY WWYVYQDETNTFLTETDVKKTFQEIVINGGDGIIIWGSSSDVNSLSKCKRLQ DYLLTVLGPIAINVTEAVN
41	Hyaluronidase (*Vespula vulgaris*)	DRTIWPKKGFSIYWNIPTHFCHNFGVYFKELKQFNIKYNSMNNFRGETISLF YDPGNFPSMVLLKNGTYEIRNEGVPQKGNLTIHLEQFTKELDEIYPKKIAGG IGVIHFHNWRPIFRRNVDNLKINKDISIDLVRKEHPKWDKSMIEKEASNRFE TSAKIFMEKTLKLAKEIRKKTEWGYHGYPHCLSGSTDKPSFDCDALSMSEND KMSWLFNNQNVLLPSIYLKNVLKPDEKIHLVQERLKEAIRISKNFKHLPKVL PYWWYTYQDKESIFLTEADVKNTFKEILTNGADGIIIWGVSYELTDRKRCEK LKEYLMKILGPIAFKVTKAVKENTPLNF
42	Hyaluronidase (*Apis mellifera*)	MSRPLVITEGMMIGVLLMLAPINALLLGFVQSTPDNNKTVREFNVYWNVPTF MCHKYGLRFEEVSEKYGILQNWMDKFRGEEIAILYDPGMFPALLKDPNGNVV ARNGGVPQLGNLTKHLQVFRDHLINQIPDKSFPGVGVIDFESWRPIFRQNWA SLQPYKKLSVEVVRREHPFWDDQRVEQEAKRRFEKYGQLFMEETLKAAKRMR PAANWGYYAYPYCYNLTPNQPSAQCEATTMQENDKMSWLFESEDVLLPSVYL RWNLTSGERVGLVGGRVKEALRIARQMTTSRKKVLPYYWYKYQDRRDTDLSR ADLEATLRKITDLGADGFIIWGSSDDINTKAKCLQFREYLNNELGPAVKRIA LNNNANDRLTVDVSVDQV
43	Hyaluronidase *Dolichovespula maculata*	SERPKRVFNIYWNVPTFMCHQYGLYFDEVTNFNIKHNSKDDFQGDKISIFYD PGEFPALLPLKEGNYKIRNGGVPQEGNITIHLQRFIENLDKTYPNRNFNGIG VIDFERWRPIFRQNWGNMMIHKKFSIDLVRNEHPFWDKKMIELEASKRFEKY ARLFMEETLKLAKKTRKQADWGYYGYPYCFNMSPNNLVPDCDATAMLENDKM SWLFNNQNVLLPSVYIRHELTPDQRVGLVQGRVKEAVRISNNLKHSPKVLSY WWYVYQDDTNTFLTETDVKKTFQEIAINGGDGIIIWGSSSDVNSLSKCKRLR EYLLTVLGPITVNVTETVN

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
44	Hyaluronidase *Polistes annularis*	YVSLSPDSVFNIITDDISHQILSRSNCERSKRPKRVFSIYWNVPTFMCHQYG MNFDEVTDFNIKHNSKDNFRGETISIYYDPGKFPALMPLKNGNYEERNGGVP QRGNITIHLQQFNEDLDKMTPDKNFGGIGVIDFERWKPIFRQNWGNTEIHKK YSIELVRKEHPKWSESMIEAEATKKFEKYARYFMEETLKLAKKTRKRAKWGY YGFPYCYNVTPNNPGPDCDAKATIENDRLSWMYNNQEILFPSVYVRHEQKPE ERVYLVQGRIKEAVRISNNLEHSPSVLAYWWYVYQDKMDIYLSETDVEKTFQ EIVTNGGDGIIIWGSSSDVNSLSKCKRLREYLLNTLGPFAVNVTETVNGRSS LNF
45	Hyaluronidase *Mus musculus*	MRAGLGPIITLALVLEVAWAGELKPTAPPIFTGRPFVVAWNVPTQECAPRHK VPLDLRAFDVKATPNEGFFNQNITTFYYDRLGLYPRFDAAGTSVHGGVPQNG SLCAHLPMLKESVERYIQTQEPGGLAVIDWEEWRPVWVRNWQEKDVYRQSSR QLVASRHPDWPSDRVMKQAQYEFEFAARQFMLNTLRYVKAVRPQHLWGFYLF PDCYNHDYVQNWESYTGRCPDVEVARNDQLAWLWAESTALFPSVYLDETLAS SVHSRNFVSFRVREALRVAHTHHANHALPVYVFTRPTYTRGLTGLSQVDLIS TIGESAALGSAGVIFWGDSEDASSMETCQYLKNYLTQLLVPYIVNVSWATQY CSWTQCHGHGRCVRRNPSANTFLHLNASSFRLVPGHTPSEPQLRPEGQLSEA DLNYLQKHFRCQCYLGWGGEQCQRNYKGAAGNASRAWAGSHLTSLLGLVAVA LTWTL
46	Hyaluronidase *Mus musculus*	MRAGLGPIITLALVLEVAWAGELKPTAPPIFTGRPFVVAWNVPTQECAPRHK VPLDLRAFDVKATPNEGFFNQNITTFYYDRLGLYPRFDAAGTSVHGGVPQNG SLCAHLPMLKESVERYIQTQEPGGLAVIDWEEWRPVWVRNWQEKDVYRQSSR QLVASRHPDWPSDRVMKQAQYEFEFAARQFMLNTLRYVKAVRPQHLWGFYLF PDCYNHDYVQNWESYTGRCPDVEVARNDQLAWLWAESTALFPSVYLDETLAS SVHSRNFVSFRVREALRVAHTHHANHALPVYVFTRPTYTRGLTGLSQVDLIS TIGESAALGSAGVIFWGDSEDASSMETCQYLKNYLTQLLVPYIVNVSWATQY CSWTQCHGHGRCVRRNPSANTFLHLNASSFRLVPGHTPSEPQLRPEGQLSEA DLNYLQKHFRCQCYLGWGGEQCQRNYKGAAGNASRAWAGSHLTSLLGLVAVA LTWTL
47	Hyaluronidase *Mus musculus*	MIMHLGLMMVVGLTLCLMHGQALLQVPEHPFSVVWNVPSARCKAHFGVHLPL DALGIVANHGQHFHGQNISIFYKNQFGLYPYFGPRGTAHNGGIPQAVSLDHH LARAAHQILHSLGSSFAGLAVLDWEEWYPLWAGNWGPHRQVYLAASWVWTQQ MFPGLDPQEQLHKAHTSFEQAARALMEYTLQLGRTLRPSGLWGFYRYPACGN GWHKMASNYTGHCHAAITTQNTQLRWLWAASSALFPSIYLPPRLPLAYRQAF VRHRLEEAFRVALLEHSHPLPVLAYSRLTHRSSGRFLSLDDLMQTIGVSAAL GTAGVVLWGDLSFSSSEEKCWRLHDYLVGTLGPYVINVTKADMACSHQRCHG HGRCARKDPGQMEAFLHLQPDDSLGAWNSFRCHCYSGWAGPTCLEPKP
48	Hyaluronidase *Rattus norvegicus*	MGELQFKWLFWRSFAESGGTFQTVLIFLFIPYSLTVDYRATPVLSDTTFVWV WNVPTEACVENVTEPIDLSFFSLIGSPRKTAIGQPVTLFYVDRLGNYPHIDA QQTEHHGGIPQKGDLTTHLVKAKEDVERYIPTDKLGLAIIDWEEWRPTWMRN WTPKDIYRNKSIELVQAADPAINITEATVRAKAQFEGAAKEFMEGTLKLGKH IRPKHLWGFYLFPDCYNNKFQVDNYDGQCPDVEKKRNDDLDWLWKESTGLYP SVYLKKDLKSSRKATLYVRYRVLESIRVSKVSDESNPVPIFVYIRLVFTDHV SEYLLEDDLVNTIGEIVAQGTSGIIIWDAMSLAQRSAGCPILRQYMKTTLNP YIVNVTLAAKMCSQTLCKEKGMCSRKTESSDAYLHLDPSSFSINVTEAGKYE VLGKPEVKDLEYFSEHFKCSCFSKMTCEETSDMRSIQDVNVCMGDNVCIKAT LGPNSAFHLLPGKGLLLMTTLAHILHHLPHDIFVFPWKMLVSTP
49	Hyaluronidase *Sus scrofa*	MAAHLLPICTLFLNLLSVAQGSRDPVVLNRPFTTIWNANTQWCLKRHGVDVD VSVFEVVVNPGQTFRGPNMTIFYSSQLGTYPYYTSAGEPVFGGLPQNASLDV HLNRTFKDILAAMPESNFSGLAVIDWEAWRPRWAFNWDAKDIYRQRSRALVQ KQHPDWPAPWVEAAAQDQFQEAAQTWMAGTLKLGQTLRPHGLWGFYGFPDCY NYDFQSSNYTGQCPPGVSAQNDQLGWLWGQSRALYPSIYLPSALEGTNKTQL YVQHRVNEAFRVAAAAGDPNLPVLPYAQIFHDMTNRLLSREELEHSLGESAA QGAAGVVLWVSWENTRTKESCQSIKEYVDTTLGPFILNVTSGALLCSQAVCS GHGRCVRRPSHTEALPILNPSSFSIKPTPGGGPLTLQGALSLKDRVQMAEEF

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
		QCRCYPGWRGTWCEQQGTR
50	Hyaluronidase *Sus scrofa*	MTMQLGLALVLGVAMCLGCGQPLLRAPERPFCVLWNVPSARCKARFGVHLPL EALGITANHGQRFHGQNITIFYKSQLGLYPYFGPRGTAHNGGIPQAVSLDHH LARAAYQIHRSLRPGFTGLAVLDWEEWCPLWAGNWGRRQAYQAASCAWAQRV YPNLDPQEQLCKARAGFEEAARALMEDTLRLGRMLRPHGLWGFYHYPACGNG WHGTASNYTGHCHAAALARNTQLYWLWAASSALFPSIYLPPGLPPAYHQAFV RYRLEEAFRVALVGHPHPLPVLAYARLTHRNSGRFLSQDELVQTIGVSAALG ASGVVLWGDLSFSSSEEECWHLRGYLVGTLGPYVINVTRAAMACSHQRCHGH GRCAWQDPGQLKVFLHLHPGGSPGAWESFSCRCYWGWAGPTCQEPRPELGPE EAT
51	Hyaluronidase *Rattus norvegicus*	MKPFSPEVSPDPCPATAAHLLRTYTLFLTLLELAQGCRGSMVSNRPFITVWN ADTHWCLKDHGVDVDVSVFDVVANKEQNFQGPNMTIFYREELGTYPYYTPTG EPVFGGLPQNASLVTHLAHAFQDIKAAMPEPDFSGLAVIDWEAWRPRWAFNW DSKDIYQQRSMELVRAEHPDWPETLVEAEAQGQFQEAAEAWMAGTLQLGQVL RPRGLWGYYGFPDCYNYDFLSPNYTGQCSLSIHDQNDQLGWLWNQSYALYPS IYLPAALMGTGKSQMYVRYRVQEAFRLALVSRDPHVPIMPYVQIFYEKTDYL LPLEELEHSLGESAAQGAAGAVLWISSEKTSTKESCQAIKAYMDSTLGPFIL NVTSAALLCSEALCSGRGRCVRHPSYPEALLTLSPASFSIEPTHDGRPLSLK GTLSLKDRAQMAMKFKCRCYRGWSGEWCKKQDM
52	Hyaluronidase *Rattus norvegicus*	MRAGLGPIITLALVLEVAWASELKPTAPPIFTGRPFVVAWNVPTQECAPRHK VPLDLRAFDVEATPNEGFFNQNITTFYYDRLGLYPRFDAAGMSVHGGVPQNG SLCAHLPMLKEAVERYIQTQEPAGLAVIDWEEWRPVWVRNWQEKDVYRQSSR QLVASRHPDWPSDRIVKQAQYEFEFAARQFMLNTLRYVKAVRPQHLWGFYLF PDCYNHDYVQNWDSYTGRCPDVEVAQNDQLAWLWAENTALFPSVYLDKTLAS SKHSRNFVSFRVQEALRVAHTHHANHALPVYVFTRPTYTRRLTELNQMDLIS TIGESAALGSAGVIFWGDSVYASSMENCQNLKKYLTQTLVPYIVNVSWATQY CSWTQCHGHGRCVRRNPSASTFLHLSPSSFRLVPGRTPSEPQLRPEGELSED DLSYLQMHFRCHCYLGWGGEQCQWNHKRAAGDASRAWAGAHLASLLGLVAMT LTWTL
53	Hyaluronidase *Rattus norvegicus*	MITQLGLTLVVGLTLCLVHVQALLQVPEFPFSVLWNVPSARCKTRFGVHLPL DALGIIANHGQRFHGQNITIFYKNQFGLYPYFGPRGTAHNGGIPQAVSLDHH LAQAAHQILHNLGSSFAGLAVLDWEEWYPLWAGNWGTHRQVYQAASWAWAQQ MFPDLNPQEQLHKAQTGFEQAARALMEHTLRLGQMLRPHGLWGFYRYPVCGN GWHNMASNYTGHCHPAIITRNTQLRWLWAASSALFPSIYLPPRLPPAYHQTF VRHRLEEAFRVALTGHAHPLPVLAYVRLTHRSSGRFLSLDDLMQTIGVSAAL GAAGVVLWGDLSVSSSEEECWRLHDYLVGTLGPYVINVTKAATACSHQRCHG HGRCSWKDPGQMEAFLHLQPDDNLGAWKSFRCRCYLGWSGPTCLEPKP
54	Hyaluronidase *Cavia porcellus*	MGAFTFKHSFFGSFVECSGVLQTVFIFLLIPCCLADKRAPPLIPNVPLLWVW NAPTEFCIGGTNQPLDMSFFSIVGTPRKNITGQSITLYYVDRLGYYPYIDPH TGAIVHGGLPQLMNLQQHLRKSRQDILFYMPTDSVGLAVIDWEEWRPTWTRN WRPKDIYRNKSIELVKSQHPQYNHSYAVAVAKRDFERTGKAFMLETLKLGKS LRPSSLWGYYLFPDCYNTHFTKPNYDGHCPPIELQRNNDLQWLWNDSTALYP SVYLTSRVRSSQNGALYVRNRVHESIRVSKLMDDKNPLPIYVYIRLVFTDQT TTFLELDDLVHSVGEIVPLGVSGIIIWGSLSLTRSLVSCIGLENYMKGTLLP YLINVTLAAKMCGQVLCKNQGICTRKDWNTNTYLHLNATNFDIELQQNGKFV VHGKPSLEDLQEFSKNFHCSCYTNVACKDRLDVHNVRSVNVCTANNICIDAV LNFPSLDDDDEPPITDDTSQNQDSISDITSSAPPSSHILPKDLSWCLFLLSI FSQHWKYLL
55	Hyaluronidase *Oryctolagus cuniculus*	MGVLKFKHIFFGSAVELSGVFQIVFIFLLIPCCLTANFRAPPVIPNVPFLWA WNAPTEFCLGKSGEPLDMSLFSLFGSPRKNKTGQGITIFYVDRLGYYPYIDP HTGAIVHGRIPQLGPLQQHLTKLRQEILYYMPKDNVGLAVIDWEEWLPTWLR NWKPKDIYRIKSIELVKSQHPQYNHSYATEKAKRDFEKAGKDFMEETLKLGR LLRPNHLWGYYLFPDCYNHHYDKPNLYKGSCFDIEKKRNDDLSWLWKESTAL

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
		FPSVYLTSRARSATALSKLYVVRNRVHEAIRVSKIPDDKSPLPNFVYTRLVF TDQIFQFLSHHDLVYTIGEIVALGASGIVVWGSQSLARSMKSCLHLDNYMKT ILNPYLINVTLAAKMCNQVLCQEQGVCTRKNWNPNDYLHLNPGNFAIQLGSN GTYKVDGKPTLTDLEQFSKNFQCSCYTNLNCKERTDMNNVRTVNVCAVENVC IDTNVGPQAVTYAPKEKKDVAHILSNTTSINSSTTMSLPFPRKHVSGCLLVL CMYSQYLNICYRLVAIGIQHGYYLK
56	Hyaluronidase *Ovis aries*	MWTGLGPAVTLALVLVVAWATELKPTAPPIFTGRPFVVAWDVPTQDCGPRHK MPLDPKDMKAFDVQASPNEGFVNQNITIFYRDRLGMYPHFNSVGRSVHGGVP QNGSLWVHLEMLKGHVEHYIRTQEPAGLAVIDWEDWRPVWVRNWQDKDVYRR LSRQLVASHHPDWPPERIVKEAQYEFEFAARQFMLETLRFVKAFRPRHLWGF YLFPDCYNHDYVQNWETYTGRCPDVEVSRNDQLSWLWAESTALFPSVYLEET LASSTHGRNFVSFRVQEALRVADVHHANHALPVYVFTRPTYSRGLTGLSEMD LISTIGESAALGAAGVILWGDAGFTTSNETCRRLKDYLTRSLVPYVVNVSWA AQYCSWAQCHGHGRCVRRDPNAHTFLHLSASSFRLVPSHAPDEPRLRPEGEL SWADRNHLQTHFRCQCYLGWGGEQCQWDRRRAAGGASGAWAGSHLTGLLAVA VLAFTWTS
57	Hyaluronidase *Ovis aries*	LYVRNRVREAIRLSKIASVESPLPVFVYHRPVFTDGSSTYLSQGDLVNSVGE IVALGASGIIMWGSLNLSLTMQSCMNLGNYLNTTLNPYIINVTLAAKMCSQV LCQEQGVCIR
58	Hyaluronidase *Ovis aries*	LDFPAPPLISNTSFLWAWNAPAERCVKIFKLPPDLRLFSVKGSPQKSATGQF ITLFYADRLGYYPHIDEKTGNTVYGGIPQLGNLKNHLEKAKKDIAYYIPNDS VGLAVIDWENWRPTWARNWKPKDVYRDESVELVLQKNPQLSFPEASKIAKVD FETAGKSFMQETLKLGKLLRPNHLWGYYLFPDCYNHNYNQPTYNGNCSDLEK RRNDDLDWLWKESTALFPSVYLNIKLKSTPKAAFYVRNRVQEAIRLSKIASV ESPLPVFVYHRPVFTDGSSTYLSQGDLVNSVGEIVALGASGIIMWGSLNLSL TMQSCMNLGNYNTTLNPYIINVTLAAKMCSQVLCHDEGVCTRKQWNSSDYLH LNPIMNFAIQTGKGGKYTVPGKVTLEDLQTFSDKFYCSCYANINCKKRVDIK NVHSVNVCMAEDICIEGPVKLQPSDHSSSQNEASTTTVSSISPSTTATTVVS PCTPEKQSPECLKVRCLEAIANVTQTGCQGVKWKNTSSQSQSSIQNIKNQTT
59	Hyaluronidase *Ovis aries*	DFRAPPLISNTSFLWAWNAPAERCIKIFKLPPDLRLFSVKGSPQKSATGQFI TLFYADRLGYYPHIDEKTGNTVYGGIPQLGNLKNHLEKAKKDIAYYIPNDSV GLAVIDWENWRPTWARNWKPKDVYRDESVELVLQKNPQLSFPEASKIAKVDF ETAGKSFMQETLKLGKLLRPNHLWGYYLFPDCYNHNYNQPTYNGNCSDLEKR RNDDLDWLWKESTALFPSVYLNIKLKSTPKAAFYVRNRVQEAIRLSKIASVE SPLPVFVYHRPVFTDGSSTYLSQGDLVNSVGEIVALGASGIIMWGSLNLSLT MQSCMNLGNYLNTTLNPYIINVTLAAKMCSQVLCHDEGVCTRKQWNSSDYLH LNPMNFAIQTGKGGKYTVPGKVTLEDLQTFSDKFYCSCYANINCKKRVDIKN VHSVNVCMAEDICIEGPVKLQPSDHSSSQNEASTTTVSSISPSTTATTVSPC TPEKQSPECLKVRCLEAIANVTQTGCQGVKWKNTSSQSSIQNIKNQTTY
60	Hyaluronidase *Pan troglodytes*	MGVLKFKHIFFRSFVKSSGVSQIVFTFLLIPCCLTLNFRAPPVIPNVPFLWA WNAPSEFCLGKFDEPLDMSLFSFIGSPRINVTGQDVTIFYVDRLGYYPYIDS ITGVTVNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWAR NWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGK LLRPNHLWGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALY PSIYLNTQQSPVAATLYVRNRVQEAIRVSKIPDAKSPLPVFVYTRIVFTDQV LKFLSQDELVYTFGETVALGASGIVIWGTLSIMRSMKSCLLLDNYMETILNP YIINVTLAAKMCSQVLCQEQGVCIRKNWNSSDYLHLNPDNFAIQLEKGGKFT VRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAVDVCIADGVCIDAF LKPPMETEESQIFYNASPSTLSATMFIDLCDLYLVPTSYLIL
61	Hyaluronidase *Pan troglodytes*	MGVLKFKHIFFRSFVKSSGVSQIVFTFLLIPCCLTLNFRAPPIIPNVPFL WAWNAPSEFCLGKFNEPLDMSLFTLMGSPRINITGQGVTIFYVDRLGYYP YIDLTTGVTVHGGIPQKVSLQDHLDKSKQDILFYMPVDNLGMAVIDWEEW RPTWARNWKPKDVYKNRSIELVQQQNVQLSLPQATDKAKQEFEKAGKDFM

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
		LETIKLGRSLRPNHLWGYYLFPDCYNHHYRKPGYNGSCFDVEIKRNDDLS WLWNESTALYPSIYLNTQQSVVVATLYVRNRVREAIRVSKIPDAKNPLPV FVYARLVFTDQVLKFLSREELVSTLGETVALGASGIVIWGSLSITRSMKS CLLLDTYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKDWNSSDYLHL NPDNFDIRLEKGGKFTVHGKPTVEDLEEFSEKFYCSCYTNLSCKEKADVK DTDAVDVCIADGVCIDASLKPPVETEGSPPIFYNTSSSTVSTTMFIWRLE VWDQGISRIGFF
62	Hyaluronidase *Pongo pygmaeus*	MTTRLGPALVLGVALCLGCGQPLPQVPERPFSVLWNVPSAHCKSRFGVHL PLNALGIIANRGQHFHGQNMTIFYKNQLGLYPYFGPKGTAHNGGIPQALP LDRHLALAAYQIHHSLRPGFAGPAVLDWEEWCPLWAGNWGRRRAYQAASW AWAQQVFPDLDPQEQLYKAYTGFEQAARALMEDTLRVAQALRPHGLWGFY HYPACGNGWHSMASNYTGRCHAATLARNTQLHWLWAASSALFPSIYLPPR LPPAHHQAFVRHRLEEAFRVALVGHLPVLAYVRLTHRRSGRFLSQDDLVQ TIGVSAALGAAGVVLWGDLSLSSSEEECWHLHDYLVDTLGPYGINVTRAA MACSHQRCHGHGRCARRDPGQMEAFLHLWPDGSLGDWKSFSCHCYWGWAG PTCQEPRLGPKEAV
63	Hyaluronidase *Macaca fascicularis*	MGVLKFKHIFFRSFVKSSGVSQIVFTFLLIPCCLTLNFRAPPIIPNVPFL WAWNAPSEFCLGKFNEPLDMSLFTLMGSPRINVTGQGVTIFYVDRLGYYP YIDLTTGVTVHGGIPQKVSLQDHLDKSKQDILFYMPVDNLGMAVIDWEEW RPTWARNWKPKDVYKNRSIELVQQQNVQLSLPQATDKAKQEFEKAGKDFM LETIKLGRSLRPNHLWGYYLFPDCYNHHYRKPGYNGSCFDVEIKRNDDLS WLWNESTALYPSIYLNTQQSVVVATLYVRNRVREAIRVSKIPDAKNPLPV FVYARLVFTDQVLKFLSREELVSTLGETVALGASGIVIWGSLSITRSMKS CLLLDTYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKDWNSSDYLHL NPDNFDIRLEKGGKFTVHGKPTVEDLEEFSEKFYCSCYTNLSCKEKADVK DTDAVDVCIADGVCIDASLKPPVETEGSPPIFYNTSSSTVSTTMFIVNIL FLIISSVASL
64	Hyaluronidase *Mus musculus*	MGELRFKHLFWGSFVESGGTFQTVLIFLLIPCSLTVDYRAAPILSNTTFL WIWNVPTERCVGNVNDPIDLSFFSLIGSPRKTATGQPVTLFYVDRLGLYP HIDANQAEHYGGIPQRGDYQAHLRKAKTDIEHYIPDDKLGLAIIDWEEWR PTWLRNWKPKDNYRNKSIELVQSTNPGLSITEATQKAIQQFEEAGRKFME GTLHLGKFLRPNQLWGYYLFPDCYNNKFQDPKYDGQCPAVEKKRNDNLKW LWKASTGLYPSVYLKKDLKSNRQATLYVRYRVVEAIRVSKVGNASDPVPI FVYIRLVFTDRTSEYLLEDDLVNTIGEIVALGTSGIIIWDAMSLAQRAAG CPILHKYMQTTLNPYIVNVTLAAKMCSQTLCNEKGMCSRRKESSDVYLHL NPSHFDIMLTETGKYEVLGNPRVGDLEYFSEHFKCSCFSRMTCKETSDVK NVQDVNVCVGDNVCIKAKVEPNPAFYLLPGKSLLFMTTLGHVLYHLPQDI FVFPRKTLVSTP
65	Hyaluronidase *Arthrobacter sp.*	MTREFSRRTALKGAALSGLLLAMVHGPAHAAATANATLTPADFAGLRQRWVD QITGRKVLVAGDNDFVTALAALDKKARTAIDLLERSAGRLTVFSDLSL AKDTDLVTTHTRLATMATAWATPGSEHFADAGLLAAIRAGLADANSLCYN ASKEEQGNWWSWEIGTPKALADTMVLLHAELTAAERAAYCAAIDHFVPDP WQQFPPKRGKITSVGANRVDLCQAVTIRSLVDEDAEKLTHAVAGLSEVWQ YVSAGNGFFTDGSFIQHSTTPYTGSYGVVLLTGLSKLFALLGGTGAEVSD PSRDILFKTVEGSFAPFMVAGAMADSVRGRSISRESNTGFDLGASTIESI LLLARAVDPVTARRWRSLCLAWINQNRKAPILADAGVGRTALVKELLAMG LTETDLPGGHYLFPAMDRTMHHSQGWTLSTAMASSRIAWYECGNGENNRG YHTGSGMTYVYDGDLGQYDDAFWATANHCRLPGITVDTSSLPDKVEGEWG AATPANEWTGSTAYGDVAAVGQHLIGPGGTGLTARKSWFVSKDVIVCLGA DIRTGSGSRIETVVDHRNLHAGFNAMGTAAGTVAATPGHPEVLTVDRWVH LEGFGGYVVLDAAPLQVLREQREGSWSEVNVKGSAARQTRNYATLYFDHG HEPEAASYAYLVAPGASASMTSSLSGQSFHTVLRNDEVAQAVKFKKEKTT AATFWRPGTVGDLALSGPACVVVKEVGDRLSIAVSDPTQNASTLTLRLKT KRFFRIIEGQGASLSHGADGFTVLEVDIANHAGRTKQIELSAE
66	Hyaluronida	MTKFFFLLTLISATAFAQSEPDWTAGVPVPPGGRSNIYSWNDFDFQATLN

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
	se *Bdellovibrio bacteriovorus*	KGKIHAQVYPVTVTGMLPPYEPVRRLIEEKNSNPLRKWIQSLMKGLSGFR SFEDVLKNLGLHKYPLENERGVYAVPYPNEIRPDTLMGFGLIERNGAEGF TFSCAACHSSNLFGKTVLGMTNRFPRANEFFIKAKKVMPLMDPHIFQAYT RATDAETALLVESKERLKSVALKQPIALGLDTSLAQVSLSLNRRAKDGYA NYSDKAARSPRADAYLDNKPADSKPAVWWNVKYKNRWLSDGSVLSGNPIF TNLIWNEIGRGADLHELEQWLADNDHIIKELTTAVFASEAPHITDFYPAE KIDLGRAKAGEQIFKNTCAKCHGHYEKAWNLPQALVLSAAERLKTVEVRY KEKTPVVNVGTDPFRRQGMKSLEQLNDLEISKKNGIVIKAQEGYVPPPLV GIWARWPYMHNNSIPNLCVLLTPAKKRPSIYYSGEALNKDTDYDFSCGGY PIGDKTPKAWKTREHLYDTRNPGMGNMGHDEGIFIKDGKEILSAEDKYNL IQFLQTL
67	Hyaluronidase *Propionibacterium acnes*	MFGTPSRRTFLTASALSAMALAASPTVTDAIAAPGPDSWSALCERWIDII TGRRAARTSDPRARAIIAKTDRKVAEILTDLVSGSSRQTVLISADLRKEQ SPFITKTARAIESMACAWATPGSSYHKDPEILSACIEGLRDFCRLRYNPS QDEYGNWWDWEDGASRAVADVMCILHDVLPPEVMSAAAAGIDHFIPDPWF QQPASVKPTANPVQPVVSTGANRMDLTRAVMCRSIATGDEKRLRHAVDGL PDAWRVTTEGDGFRADGGFIQHSHIPYTGGYGDVLFSGLAMLFPLVSGMR FDIVESARKAFHDQVERGFIPVMYNGQILDDVRGRSISRINESAAMHGIS IARAMLMMADALPTHRAEQWRGIVHGWMARNTFDHLSEPSTLVDISLFDA AAKARPVPESSTPSYFASMDRLVHRTADWLITVSNCSDRIAWYEYGNGEN EWASRTSQGMRYLLLPGDMGQYEDGYWATVDYSAPTGTTVDSTPLKRAVG ASWAAKTPTNEWSGGLASGSWSAAASHITSQDSALKARRLWVGLKDAMVE LTTDVTTDASRAITVVEHRKVASSSTKLLVDGNRVSSATSFQNPRWAHLD GVGGYVFATDTDLSADVATRKGTWIDVNPSRKVKGADEVIERAYASLHVT HHDRPVAWALLPTASRSHTMALATRPGVEPFTVLRNDATVQAVRSAGALL TKDPTVVTTLAFWKPATCGGVAVNRPALVQTRESANQMEVVIVEPTQKRG SLTVTIEGSWKVKTADSHVDVSCENAAGTLHVDTAGLGGQSVRVTLARQV TQTPSGGGRHDRA
68	Hyaluronidase *Streptococcus agalactiae*	MEIKKKYRIMLYSALILGTILVNNSYQAKAEELTKTTSTSQIRDTQTNNI EVLQTESTTVKETSTTTTQQDLSNPTASTATATATHSTMKQVVDNQTQNK ELVKNGDFNQTNPVSGSWSHTSAREWSAWIDKENTADKSPIIQRTEQGQV SLSSDKGFRGAVTQKVNIDPTKKYEVKFDIETSNKAGQAFLRIMEKKDNN TRLWLSEMTSGTTNKHTLTKIYNPKLNVSEVTLELYYEKGTGSATFDNIS MKAKGPKDSEHPQPVTTQIEESVNTALNKNYVFNKADYQYTLTNPSLGKI VGGILYPNATGSTTVKISDKSGKIIKEVPLSVTASTEDKFTKLLDKWNDV TIGNHVYDTNDSNMQKINQKLDETNAKNIKTIKLDSNHTFLWKDLDNLNN SAQLTATYRRLEDLAKQITNPHSTIYKNEKAIRTVKESLAWLHQNFYNVN KDIEGSANWWDFEIGVPRSITATLALMNNYFTDAEIKTYTDPIEHFVPDA GYFRKTLDNPFKALGGNLVDMGRVKIIEGLLRKDNTIIEKTSHSLKNLFT TATKAEGFYADGSYIDHTNVAYTGAYGNVLIDGLTQLLPIIQETDYKISN QELDMVYKWINQSFLPLIVKGELMDMSRGRSISREAASSHAAAVEVLRGF LRLANMSNEERNLDLKSTIKTIITSNKFYNVFNNLKSYSDIANMNKMLND STVATKPLKSNLSTFNSMDRLAYYNAEKDFGFALSLHSKRTLNYEGMNDE NTRGWYTGDGMFYLYNSDQSHYSNHFWPTVNPYKMAGTTEKDAKREDTTK EFMSKHSKDAKEKTGQVTGTSDFVGSVKLNDHFALAAMDFTNWDRTLTAQ KGWVILNDKIVFLGSNIKNTNGIGNVSTTIDQRKDDSKTPYTTYVNGKTI DLKQASSQQFTDTKSVFLESKEPGRNIGYIFFKNSTIDIERKEQTGTWNS INRTSKNTSIVSNPFITISQKHDNKGDSYGYMMVPNIDRTSFDKLANSKE VELLENSSKQQVIYDKNSQTWAVIKHDNQESLINNQFKMNKAGLYLVQKV GNDYQNVYYQPQTMTKTDQLAI
69	Hyaluronidase *Streptococcus agalactiae 18RS21*	MEIKKKHRIMLYSALILGTILVNNSYQAKAEELTKTTSTSQIRDTQTNNI EVLQTESTTVKETSTTTTQQDLSNPTASTATATATHSTMKQVVDNQTQNK ELVKNGDFNQTNPVSGSWSHTSAREWSAWIDKENTADKSPIIQRTEQGQV SLSSDKGFRGAVTQKVNIDPTKKYEVKFDIETSNKAGQAFLRIMEKKDNN TRLWLSEMTSGTTNKHTLTKIYNPKLNVSEVTLELYYEKGTGSATFDNIS MKAKGPKDSEHPQPVTTQIEESVNTALNKNYVFNKADYQYTLTNPSLGKI

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
		VGGILYPNATGSTTVKISDKSGKIIKEVPLSVTASTEDKFTKLLDKWNDV TIGNHVYDTNDSNMQKINQKLDETNAKNIKTIKLDSNHTFLWKDLDNLNN SAQLTATYRRLEDLAKQITNPHSTIYKNEKAIRTVKESLAWLHQNFYNVN KDIEGSANWWDFEIGVPRSITATLALMNNYFTDAEIKTYTDPIEHFVPDA GYFRKTLDNPFKALGGNLVDMGRVKIIEGLLRKDNTIIEKTSHSLKNLFT TATKAEGFYADGSYIDHTNVAYTGAYGNVLIDGLTQLLPIIQETDYKISN QELDMVYKWINQSFLPLIVKGELMDMSRGRSISREAASSHAAAVEVLRGF LRLANMSNEERNLDLISTIKTIITSNKFYNVFNNLKSYSDIANMNKMLND STVATKPLKSNLSTFNSMDRLAYYNAEKDFGFALSLHSKRTLNYEGMNDE NTRGWYTGDGMFYLYNSDQSHYSNHFWPTVNPYKMAGTTEKDAKREDTTK EFMSKHSKDAKEKTGQVTGTSDFVGSVKLNDHFALAAMDFTNWDRTLTAQ KGWVILNDKIVFLGSNIKNTNGIGNVSTTIDQRKDDSKTPYTTYVNGKTI DLKQASSQQFTDTKSVFLESKEPGRNIGYIFFKNSTIDIERKEQTGTWNS INRTSKNTSIVSNPFITISQKHDNKGDSYGYMMVPNIDRTSFDKLANSKE VELLENSSKQQVIYDKNSQTWAVIKHDNQESLINNQFKMNKAGLYLVQKV GNDYQNVYYQPQTMTKTDQLAI
70	Hyaluronidase *Streptococcus agalactiae serogroup III*	MKQVVDNQTQNKELVKNGDFNQTNPVSGSWSHTSAREWSAWIDKENTADK SPIIQRTEQGQVSLSSDKGFRGAVTQKVNIDPTKKYEVKFDIETSNKAGQ AFLRIMEKKDNNTRLWLSEMTSGTTNKHTLTKIYNPKLNVSEVTLELYYE KGTGSATFDNISMKAKGPKDSEHPQPVTTQIEESVNTALNKNYVFNKADY QYTLTNPSLGKIVGGILYPNATGSTTVKISDKSGKIIKEVPLSVTASTED KFTKLLDKWNDVTIGNHVYDTNDSNMQKINQKLDETNAKNIKTIKLDSNH TFLWKDLDNLNNSAQLTATYRRLEDLAKQITNPHSTIYKNEKAIRTVKES LAWLHQNFYNVNKDIEGSANWWDFEIGVPRSITATLALMNNYFTDAEIKT YTDPIEHFVPDAGYFRKTLDNPFKALGGNLVDMGRVKIIEGLLRKDNTII EKTSHSLKNLFTTATKAEGFYADGSYIDHTNVAYTGAYGNVLIDGLTQLL PIIQETDYKISNQELDMVYKWINQSFLPLIVKGELMDMSRGRSISREAAS SHAAAVEVLRGFLRLANMSNEERNLDLKSTIKTIITSNKFYNVFNNLKSY SDIANMNKMLNDSTVATKPLKSNLSTFNSMDRLAYYNAEKDFGFALSLHS KRTLNYEGMNDENTRDWYTGDGMFYLYNSDQSHYSNHFWPTVNPYKMAGT TEKDAKREDTTKEFMSKHSKDAKEKTGQVTGTSDFVGSVKLNDHFALAAM DFTNWDRTLTAQKGWVILNDKIVFLGSNIKNTNGIGNVSTTIDQRKDDSK TPYTTYVNGKTIDLKQASSQQFTDTKSVFLESKEPGRNIGYIFFKNSTID IERKEQTGTWNSINRTSKNTSIVSNPFITISQKHDNKGDSYGYMMVPNID RTSFDKLANSKEVELLENSSKQQVIYDKNSQTWAVIKHDNQESLINNQFK MNKAGLYLVQKVGNDYQNVYYQPQTMTKTDQLAI
71	Hyaluronidase *Staphylococcus aureus (strain COL)*	MTYRIKKWQKLSTITLLMAGVITLNGGEFRSVDKHQIAVADTNVQTPDYE KLRNTWLDVNYGYDKYDENNPDMKKKFDATEKEATNLLKEMKTESGRKYL WSGAETLETNSSHMTRTYRNIEKIAEAMRNPKTTLNTDENKKKVKDALEW LHKNAYGKEPDKKVKELSENFTKTTGKNTNLNWWDYEIGTPKSLTNTLIL LNDQFSNEEKKKFTAPIKTFAPDSDKILSSVGKAELAKGGNLVDISKVKL LECIIEEDKDMMKKSIDSFNKVFTYVQDSATGKERNGFYKDGSYIDHQDV PYTGAYGVVLLEGISQMMPMIKETPFNDKTQNDTTLKSWIDDGFMPLIYK GEMMDLSRGRAISRENETSHSASATVMKSLLRLSDAMDDSTKAKYKKIVK SSVESDSSYKQNDYLNSYSDIDKMKSLMTDNSISKNGLTQQLKIYNDMDR VTYHNKDLDFAFGLSMTSKNVARYESINGENLKGWHTGAGMSYLYNSDVK HYHDNFWVTADMKRLSGTTTLDNEILKDTDDKKSSKTFVGGTKVDDQHAS IGMDFENQDKTLTAKKSYFILNDKIVFLGTGIKSTDSSKNPVTTIENRKA NGYTLYTDDKQTTNSDNQENNSVFLESTDTKKNIGYHFLNKPKITVKKES HTGKWKEINKSQKDTQKTDEYYEVTQKHSNSDNKYGYVLYPGLSKDVFKT KKDEVTVVKQEDDFHVVKDNESVWAGVNYSNSTQTFDINNTKVEVKAKGM FILKKKDDNTYECSFYNPESTNSASDIESKISMTGYSITNKNTSTSNESG VHFELTK
72	Hyaluronidase *Streptococc*	MEIKKKHRIMLYSALILGTILVNNSYQAKAEEFTKTTSTSQIRDTQTNNV EVPQTESTTVKGTSTTTTQQDLSNSTASTATATATHSTMKQVVDNQTQNK ELVKNGDFKEKIIDKKIDKKSQWTNLYGAKDWNTYIDQTKSVNKSPIIQR

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
	us agalactiae serotype Ia	TEQGQVSLSSDKGFRGAVTQKVNIDPTKKYEVKFDIETSNKVGQAFLRIM KKKDKNTRLWLSEMTSGTTNKHTLTKIYNPKLNVSEVTLELYYEKGTGSV TFDNISMKAKGPKDSEHPQPVTTQIEESVNTALNKNYVFNKADYQYTLTN PSLGKIVGGILYPSATGSTTVKISDKSGKIIKEVPLSVTASTEDNFTKLL DKWNDVTIGNHVYDTNDSNMQKLNQKLDETNAKNIKDIKLDSNRTFLWED LKGLNNSAQLTATYRRLEDLAKQITNPHSTIYKNEKAIRTVKESLAWLHQ NFYNVNKDIEGSANWWDFEIGVPRSITATLALMNNYFTDAEIKTYTDPIE HFVPDAGYFRKTLVNPFKALGGNLVDMGRVKIIEGLLRKDNTIIKKTSHS LKNLFTTATKAEGFYADGSYIDHTNVAYTGAYGNVLIDGLTQLLPIIQET DYKISNQELDMVYKWINQSFLPLIVKGELMDMSRGRSISREAASSHAAAV EVLRGFLRLANMSNEERNLDLKSTIKTIITSNKFYNVFNNLKSYSDIANM NKLLNDSTVATKPLKSNLSTFNSMDRLAYYNAEKDFGFALSLHSKRTLNY EGMNDENTRGWYTGDGMFYLYNSDQSHYSNHFWPTVNPYKMAGTTEKDTG REDTIKKLMNRYDKTNKNSKVMTGQVTGTSDFVGSVKLNDHFALAAMDFT NWDRTLTAQKGWVILNDKIVFLGSNIKNTNGVGNVSTTIDQRKDDSKTPY TTYVNGKTVDLKQASSQQFTDTKSVFLESKEPGRNIGYIFFKNSTIDIER KEQTGTWNSINRTSKNTSIVSNPFITISQKHDNKGDSYGYMMVPNIDRTS FDKLANSKEVELLENSSKQQVIYDKNSQTWAVIKHDNQESLINNQFKMNK AGLYLVQKVGNDYQNVYYQPQTMTKTDQLAI
73	Hyaluronidase *Staphylococcus aureus (strain MRSA252)*	MTYRMKKWQKLSTITLLMAGVITLNGGEFRSIDKHQIAVADTNVQTTDYE KLRNIWLDVNYGYDKYDENNPDMKKKFEATENEAEKLLKEMKTESDRKYL WESSKDLDTKSADMTRTYRNIEKISEAMKHKNTKLKTDENKTKVKDALEW LHKNAYGKEPDKKVADLTSNFKNKTSRNTNLNWWDYEIGTPRALTNTLIL LQEDFTDEEKKKYTAPIKTFAPDSDKILSSVGKSEPAKGGNLVDISKVKL LESIIEEDKDMMKKSIDSFNTVFTYAQNSATGKERNGFYKDGSYIDHQDV PYTGAYGVVLLEGISQMMPMIKETPFNDSNQNDTTLKSWIDDGFMPLIYK GEMMDLSRGRAISRENETSHSASATVMKSLLRLSDTMDKSTKAKYKKIVK TSVESDSSYKQTDYLSSYSD
74	Hyaluronidase *Staphylococcus aureus (strain MRSA252)*	MTNKMKKWQKLSTITLLMTGVIALNNGEFRNVDKHQIAVADTNVQTPDYE KLKKTWLDVNYGYDQYDENNQDMKKKFDAKEKEAKKLLDDMKTDTNRTYL WSGAENLETNSSHMTKTYRNIEKIAESMQHKNTVLKTVENKLKIKEALDW MHKNVYGKNPSQKVEDLTKNRKGQTTPKNNSLNWWDYEIGTPRALTNTLL LMDDMLTKDEMKNYSKPISTYAPSSDKILSSVGESEDAKGGNLVDISKVK LLESVIEEDVDMLKKSIDSFNKVFTYVQDSATGKGRNGFYKDGSYIDHQD VPYTGAYGVVLLEGISQMMPMIKESPFKTTQDNATLSNWIDEGFMPLIYK GEMMDLSRGRAISRENETSHTASATVMKSLLRLNDTMDDSTKTRYKQIVK TSVNSDSSYNQNNYLNSYSDIAKMKKLMNDSTISKNDLTQQLKIYNDMDR VTYHNKDLDFAFGLSMTSKNIARYENINGENLKGWHTGAGMSYLYNSDVK HYRDNFWATADMTCLPGTTTLNDMPSTNTKNDKSFVGGTKLNNKYASIGM DFENQDKTLTAKKSYFILNDKIVFLGTGIKSTDSSKNPVTSVENRKANGY KLFKDDIEITTSDVNAQETHSVFLESNDTKKNIGYHFLDKPKITVKKESH TGKWSEINKSQKKDDKKDEYYEVTQTHNTSDSKYAYVLYPGLSKSDFKSK NNNVSIVKQDEDFHVIKDNDGVFAGVNYSDNTKSFDINGITVELKEKGMF VIKKKDDKAYKCSFYNPETTNTASNIESKIFIKGYTITNKSVINSNDAGV NFELTK
75	Hyaluronidase *Staphylococcus aureus (strain MSSA476)*	MTYRIKKWQKLSTITLLMAGVITLNGGEFRSIDKYQIAVADTNVQTPDYE KLRNTWLDVNYGYDKYDEKNDAMKKKFEATENEAKKLLSEMKTESDRKYL WENSKDLDTKSADMTRTYRNIEKIAEAMKHKDTKLKIDENKKKVKDALEW LHKNAYGKEPDKKVADLTSNFKNKTSRNTNLNWWDYEIGTPRALTNTLIL LNDQFSNDEKKKYTAPIKTFAPESDKILSSVGQPEQAKGGNLVDIAKVKL LESIIEEDKDITKNSIDAFNKVFTYVQSNATGKERNGFYKDGSYIDHQDV PYTGAYGVVLLEGISQMMPMIKETPFNDKTQNDTTLKSWIDDGFMPLIYK GEMMDLSRGRAISRENETSHTASATVMKSLLRLSDAMDDSTKAKYKQIVK TSVKSDSSYGQNDTLSSYSDISKMKSLMEDSTISTNGLTQQLKIYNDMDR VTYHNKDLDFAFGLSMTSKNVARYESINGENLKGWHTGAGMSYLYNSDVK HYRDNFWATADMKRLAGTTTLENEEPKGTDVKKSSKTFVGGTKFDDQHAS

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
		IGMDFENQDKTLTAKKSYFILNDKIVFLGTGIKSTDSSKNPVTTIENRKA NGYTLYTDDKQTTASDNQGTNSVFLESTNKPKNNIGYHFLNESKITVKKE SHTGKWSDINKSQKQDSKTNQYYEVTQKHSNTDSKYAYVLYPGLSKDDFN TKKDKVTVVKQDDDFHVVKDNESVWAGVNYSDSTQTFIINNTKVEVKAKG MFILKKKDDKTYECSFYNPESTNTASDIESKISMTGYSITNKNTSTSNES GVRFELQQTLNKDDN
76	Hyaluronidase *Staphylococcus aureus (strain NCTC 8325)*	MTYRIKKWQKLSTITLLMAGVITLNGGEFRSVDKHQIAVADTNVQTPDYE KLRNTWLDVNYGYDKYDENNPDMKKKFDATEKEATNLLKEMKTESGRKYL WSGAETLETNSSHMTRTYRNIEKIAEAMRNPKTTLNTDENKKKVKDALEW LHKNAYGKEPDKKVKELSENFTKTTGKNTNLNWWDYEIGTPKSLTNTLIL LNDQFSNEEKKKFTAPIKTFAPDSDKILSSVGKAELAKGGNLVDISKVKL LECIIEEDKDMMKKSIDSFNKVFTYVQDSATGKERNGFYKDGSYIDHQDV PYTGAYGVVLLEGISQMMPMIKETPFNDKTQNDTTLKSWIDDGFMPLIYK GEMMDLSRGRAISRENETSHSASATVMKSLLRLSDAMDDSTKAKYKKIVK SSVESDSSYKQNDYLNSYSDIDKMKSLMTDNSISKNGLTQQLKIYNDMDR VTYHNKDLDFAFGLSMTSKNVARYESINGENLKGWHTGAGMSYLYNSDVK HYHDNFWVTADMKRLSGTTTLDNEILKDTDDKKSSKTFVGGTKVDDQHAS IGMDFENQDKTLTAKKSYFILNDKIVFLGTGIKSTDSSKNPVTTIENRKA NGYTLYTDDKQTTNSDNQENNSVFLESTDTKKNIGYHFLNKPKITVKKES HTGKWKEINKSQKDTQKTDEYYEVTQKHSNSDNKYGYVLYPGLSKDVFKT KKDEVTVVKQEDDFHVVKDNESVWAGVNYSNSTQTFDINNTKVEVKAKGM FILKKKDDNTYECSFYNPESTNSASDIESKISMTGYSITNKNTSTSNESG VHFELTK
77	Hyaluronidase *Staphylococcus aureus (strain bovine RF122)*	MTYRMKKWQKLSTITLLMAGGITFNDSEFRSVDKHQIAVADTNVQTPNYE KLKNTWLDVNYGYDKYDESNPDMKKKFEATEKEARKLLSEMKTESDRKYL WENSKDLDTKSADMTRTYRNIEKIAEAMKHPKTTLKNDENKKKVKDALEW LHKNAYGKEPGKKVADLKTNFSKSAPQKNTNLNWWDYEIGTPRALTNTLI LLKEDFTDEEKKKYTAPIKTFAPKSDEILSSVGKAEPAKGGNLVDISKVK LLESIIEEDKDMMKNSIDSFNKVFTYVQDSATDKERNGFYKDGSYIDHKD VPYTGAYGVVLLEGISQMMPMIKETPFNDKTQNNTTLTSWIDDGFMPLIY KGEMMDLSRGRAISRENETSHSASATVMKSLLRLSDAMDESTKAKYKQIV KNSVKSDSSYGQNDTLSSYSDIDKMKSLMTDSTISTNGLTQQLKIYNAMD RVTYHNKDLDFAFGLSMTSKNVARYENINGENLKGWHTGAGMSYLYNSDV RHYRDNFWATADMKRLADTTTLENEEPKGTDVKKSSKTFVGGTKFDDQHA SIGMDFENQDKTLTAKKSYFILNDKIVFLGTGIKTTDSSKNPVTTIENRK AHGYTLYTDDKQTTNSNNQETNSVFLESTNSTQNNIGYHFLNKSKITVKK ESHTGKWSDINKSQKDTQKTDEYYEVTQKHSNTDDKYAYVLYPGITKDNF KSKASQVTVVKQDDDFHVVKDNESVWAGVNYSDSTQTFDINGTKVEVKAK GMFILKKKDDNTYECSFYNPESTNSASDIESKISMTGYSITNKNTSNTNE SGVRFELTK
78	Hyaluronidase *Staphylococcus aureus (strain bovine RF122)*	MTYKMKKWQKLSTITLLMAGVITLNNGEFRNVDKHQIAVADTNVQTPDYE KLKKTWLDVNYGNDQYDENNQDMKKKFDAKENEAKKLLEDMKTDTNRTYL WSGAENLETNSSHMTKTYRNIEKIAEAMRHKNTSLKTDENKLKIKDAIKW LHHNVYGKDPDKKVADLTTNRKEKDSSKKNNSLNWWDYEIGTPRALTNTL LLMDNMLTKDEMKNYSKPISIYSPSSYKILSSVGESEDAKGGNLVDIAKV KFLESVIEEDVDMMKKSIDSFNKVFTYVQDSATGKARNGFYKDGSYIDHQ DVPYTGAYGVVLLEGISQMMPMIKESPFKHTQDKATLSNWIDEGFMPLIY KGEMMDLSRGRAISRENETSHTASATVMKSLLRLSDTMDESTKTKYKQIV KTSVKSDSSYDSNDTLNSYSDIDKMKKLMNDSTISKNDLTQQLKIYNDMD RVTYHNKELDFAFGLSMTSKNIARYENINGENLKGWHTGAGMSYLYNSDV KHYRDNFWATADMTRLPGTTTLNDMPSTNTKNDKSFVGGTKLNNKYASIG MDFENQDKTLTAKKSYFILNDKIVFIGTGIKSTDSSKNPVTSVENRKANG YKLFKGDIEITTSDVNAQETHSVFLESNDTKKNIGYHFLDKPKITVKKES HTGKWSEINKSQKTDDKKDEYYEVTQTHNTSDSKYAYVLYPGLSKSDFKS KNNNVSIVKQDEDFHVIKDNDGVFAGVNYSDSTKSFDINGTIVELKEKGM FVIKKKDDNTYECSFYNPTSTNSTSNKESKISVTGYTITNQSVSNFKESD

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
		IHFELTK
79	Hyaluronidase *Staphylococcus aureus (strain USA300)*	MTYRIKKWQKLSTITLLMAGVITLNGGEFRSVDKHQIAVADTNVQTPDYE KLRNTWLDVNYGYDKYDENNPDMKKKFDATEKEATNLLKEMKTESGRKYL WSGAETLETNSSHMTRTYRNIEKIAEAMRNPKTTLNTDENKKKVKDALEW LHKNAYGKEPDKKVKELSENFTKTTGKNTNLNWWDYEIGTPKSLTNTLIL LNDQFSNEEKKKFTAPIKTFAPDSDKILSSVGKAELAKGGNLVDISKVKL LECIIEEDKDMMKKSIDSFNKVFTYVQDSATGKERNGFYKDGSYIDHQDV PYTGAYGVVLLEGISQMMPMIKETPFNDKTQNDTTLKSWIDDGFMPLIYK GEMMDLSRGRAISRENETSHSASATVMKSLLRLSDAMDDSTKAKYKKIVK SSVESDSSYKQNDYLNSYSDIDKMKSLMTDNSISKNGLTQQLKIYNDMDR VTYHNKDLDFAFGLSMTSKNVARYESINGENLKGWHTGAGMSYLYNSDVK HYHDNFWVTADMKRLSGTTTLDNEILKDTDDKKSSKTFVGGTKVDDQHAS IGMDFENQDKTLTAKKSYFILNDKIVFLGTGIKSTDSSKNPVTTIENRKA NGYTLYTDDKQTTNSDNQENNSVFLESTDTKKNIGYHFLNKPKITVKKES HTGKWKEINKSQKDTQKTDEYYEVTQKHSNSDNKYGYVLYPGLSKDVFKT KKDEVTVVKQEDDFHVVKDNESVWAGVNYSNSTQTFDINNTKVEVKAKGM FILKKKDDNTYECSFYNPESTNSASDIESKISMTGYSITNKNTSTSNESG VHFELTK
80	Hyaluronidase *Streptococcus pneumoniae*	MQTKTKKLIVSLSSLVLSGFLLNHYMTIGAEETTTNTIQQSQKEVQYQQR DTKNLVENGDFGQTEDGSSPWTGSKAQGWSAWVDQKNSADASTRVIEAKD GAITISSHEKLRAALHRMVPIEAKKKYKLRFKIKTDNKIGIAKVRIIEES GKDKRLWNSATTSGTKDWQTIEADYSPTLDVDKIKLELFYETGTGTVSFK DIELVEVADQLSEDSQTDKQLEEKIDLPIGKKHVFSLADYTYKVENPDVA SVKNGILEPLKEGTTNVIVSKDGKEVKKIPLKILASVKDAYTDRLDDWNG IIAGNQYYDSKNEQMAKLNQELEGKVADSLSSISSQADRTYLWEKFSNYK TSANLTATYRKLEEMAKQVTNPSSRYYQDETVVRTVRDSMEWMHKHVYNS EKSIVGNWWDYEIGTPRAINNTLSLMKEYFSDEEIKKYTDVIEKFVPDPE HFRKTTDNPFKALGGNLVDMGRVKVIAGLLRKDDQEISSTIRSIEQVFKL VDQGEGFYQDGSYIDHTNVAYTGAYGNVLIDGLSQLLPVIQKTKNPIDKD KMQTMYHWIDKSFAPLLVNGELMDMSRGRSISRANSEGHVAAVEVLRGIH RIADMSEGETKQCLQSLVKTIVQSDSYYDVFKNLKTYKDISLMQSLLSDA GVASVPRPSYLSAFNKMDKTAMYNAEKGFGFGLSLFSSRTLNYEHMNKEN KRGWYTSDGMFYLYNGDLSHYSDGYWPTVNPYKMPGTTETDAKRADSDTG KVLPSAFVGTSKLDDANATATMDFTNWNQTLTAHKSWFMLKDKIAFLGSN IQNTSTDTAATTIDQRKLESGNPYKVYVNDKEASLTEQEKDYPETQSVFL ESFDSKKNIGYFFFKKSSISMSKALQKGAWKDINEGQSDKEVENEFLTIS QAHKQNRDSYGYMLIPNVDRATFNQMIKELESSLIENNETLQSVYDAKQG VWGIVKYDDSVSTISNQFQVLKRGVYTIRKEGDEYKIAYYNPETQESAPD QEVFKKLEQAAQPQVQNSKEKEKSEEEKNHSDQKNLPQTGEGQSILASLG FLLLGAFYLFRRGKNN
81	Hyaluronidase *Streptococcus pneumoniae R6*	MILQYVYWSVYMQTKTKKLIVSLSSLVLSGFLLNHYMTVGAEETTTNTIQ QSQKEVQYQQRDTKNLVENGDFGQTEDGSSPWTGSKAQGWSAWVDQKNSS ADASTRVIEAKDGAITISSPEKLRAAVHRMVPIEAKKKYKLRFKIKTDNK VGIAKVRIIEESGKDKRLWNSATTSGTKDWQTIEADYSPTLDVDKIKLEL FYETGTGTVSFKDIELVEVADQPSEDSQTDKQLEEKIDLPIGKKHVFSLA DYTYKVENPDVASVKNGILEPLKEGTTNVIVSKDGKEVKKIPLKILASVK DTYTDRLDDWNGIIAGNQYYDSKNEQMAKLNQELEGKVADSLSSISSQAD RIYLWEKFSNYKTSANLTATYRKLEEMAKQVTNPSSRYYQDETVVRTVRD SMEWMHKHVYNSEKSIVGNWWDYEIGTPRAINNTLSLMKEYFSDEEIKKY TDVIEKFVPDPEHFRKTTDNPFKALGGNLVDMGRVKVIAGLLRKDDQEIS STIRSIEQVFKLVDQGEGFYQDGSYIDHTNVAYTGAYGNVLIDGLSQLLP VIQKTKNPIDKDKMQTMYHWIDKSFAPLLVNGELMDMSRGRSISRANSEG HVAAVEVLRGIHRIADMSEGETKQRLQSLVKTIVQSDSYYDVFKNLKTYK DISLMQSLLSDAGVASVPRTSYLSAFNKMDKTAMYNAEKGFGFGLSLFSS RTLNYEHMNKENKRGWYTSDGMFYLYNGDLSHYSDGYWPTVNPYKMPGTT ETDAKRADSDTGKVLPSAFVGTSKLDDANATATMDFTNWNQTLTAHKSWF

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
		MLKDKIAFLGSNIQNTSTDTAATTIDQRKLESSNPYKVYVNDKEASLTEQ EKDYPETQSVFLESSDSKKNIGYFFFKKSSISMSKALQKGAWKDINEGQS DKEVENEFLTISQAHKQNGDSYGYMLIPNVDRATFNQMIKELESSLIENN ETLQSVYDAKQGVWGIVKYDDSVSTISNQFQVLKRGVYTIRKEGDEYKIA YYNPETQESAPDQEVFKKLEQAAQPQVQNSKEKEKSEEEKNHSDQKNLPQ TGEGQSILASLGFLLLGAFYLFRRGKNN
82	Hyaluronidase *Streptococcus pneumoniae serotype 2 (strain D39/N)*	MQTKTKKLIVSLSSLVLSGFLLNHYMTVGAEETTTNTIQQSQKEVQYQQR DTKNLVENGDFGQTEDGSSPWTGSKAQGWSAWVDQKNSSADASTRVIEAK DGAITISSPEKLRAAVHRMVPIEAKKKYKLRFKIKTDNKVGIAKVRIIEE SGKDKRLWNSATTSGTKDWQTIEADYSPTLDVDKIKLELFYETGTGTVSF KDIELVEVADQPSEDSQTDKQLEEKIDLPIGKKHVFSLADYTYKVENPDV ASVKNGILEPLKEGTTNVIVSKDGKEVKKIPLKILASVKDTYTDRLDDWN GIIAGNQYYDSKNEQMAKLNQELEGKVADSLSSISSQADRIYLWEKFSNY KTSANLTATYRKLEEMAKQVTNPSSRYYQDETVVRTVRDSMEWMHKHVYN SEKSIVGNWWDYEIGTPRAINNTLSLMKEYFSDEEIKKYTDVIEKFVPDP EHFRKTTDNPFKALGGNLVDMGRVKVIAGLLRKDDQEISSTIRSIEQVFK LVDQGEGFYQDGSYIDHTNVAYTGAYGNVLIDGLSQLLPVIQKTKNPIDK DKMQTMYHWIDKSFAPLLVNGELMDMSRGRSISRANSEGHVAAVEVLRGI HRIADMSEGETKQRLQSLVKTIVQSDSYYDVFKNLKTYKDISLMQSLLSD AGVASVPRTSYLSAFNKMDKTAMYNAEKGFGFGLSLFSSRTLNYEHMNKE NKRGWYTSDGMFYLYNGDLSHYSDGYWPTVNPYKMPGTTETDAKRADSDT GKVLPSAFVGTSKLDDANATATMDFTNWNQTLTAHKSWFMLKDKIAFLGS NIQNTSTDTAATTIDQRKLESSNPYKVYVNDKEASLTEQEKDYPETQSVF LESSDSKKNIGYFFFKKSSISMSKALQKGAWKDINEGQSDKEVENEFLTI SQAHKQNGDSYGYMLIPNVDRATFNQMIKELESSLIENNETLQSVYDAKQ GVWGIVKYDDSVSTISNQFQVLKRGVYTIRKEGDEYKIAYYNPETQESAP DQEVFKKLEQAAQPQVQNSKEKEKSEEEKNHSDQKNLPQTGEGQSILASL GFLLLGAFYLFRRGKNN
83	Hyaluronidase *Streptococcus pyogenes serotype M1*	MNTYFCTHHKQLLLYSNLFLSFAMMGQGTAIYADTLTSNSEPNNTYFQTQ TLTTTDSEKKVVQPQQKDYYTELLDQWNSIIAGNDAYDKTNPDMVTFHNK AEKDAQNIIKSYQGPDHENRTYLWEHAKDYSASANITKTYRNIEKIAKQI TNPESCYYQDSKAIAIVKDGMAFMYEHAYNLDRENHQTTGKENKENWWVY EIGTPRAINNTLSLMYPYFTQEEILKYTAPIEKFVPDPTRFRVRAANFSP FEANSGNLIDMGRVKLISGILRKDDLEISDTIKAIEKVFTLVDEGNGFYQ DGSLIDHVVTNAQSPLYKKGIAYTGAYGNVLIDGLSQLIPIIQKTKSPIK ADKMATIYHWINHSFFPIIVRGEMMDMTRGRSISRFNAQSHVAGIEALRA ILRIADMSEEPHRLALKTRIKTLVTQGNAFYNVYDNLKTYHDIKLMKELL SDTSVPVQKLDSYVASFNSMDKLALYNNKHDFAFGLSMFSNRTQNYEAMN NENLHGWFTSDGMFYLYNNDLGHYSENYWATVNPYRLPGTTETEQKPLEG TPENIKTNYQQVGMTGLSDDAFVASKKLNNTSALAAMTFTNWNKSLTLNK GWFILGNKIIFVGSNIKNQSSHKAYTTIEQRKENQKYPYCSYVNNQPVDL NNQLVDFTNTKSIFLESDDPAQNIGYYFFKPTTLSISKALQTGKWQNIKA DDKSPEAIKEVSNTFITIMQNHTQDGDRYAYMMLPNMTRQEFETYISKLD IDLLENNDKLAAVYDHDSQQMHVIHYGKKATMFSNHNLSHQGFYSFPHPV RQNQQ
84	Hyaluronidase *Streptococcus pyogenes serotype M2 (strain MGAS10)*	MVYFYLVNQSTFIISFLYWRNVSVNTYFCTHHKQLLLYSNLFLSFAMIGQ GTAIYADTLTSNSEPNNTYFQTQTLTTTDSEKKVVQPQQKDYYTELLDQW NSIIAGNDAYDKTNPDMVTFHNKAEKDAQNIIKSYQGPDHENRTYLWEHA KDYSASTNITKTYRNIEKIAKQITNPESCYYQDSKAIAIVKDGMAFMYEH AYNLNRENHQTTGKENKENWWVYEIGTPRAINNTLSLMYPYFTQEEILKY TAPIEKFVPDPTRFRVRAANFSPFEANSGNLIDMGRVKLISGILRKDDLE ISDTIKAIEKVFTLVDEGNGFYQDGSLIDHVVTNTQSPLYKKGIAYTGAY GNVLIDGLSQLIPIIQKTKSPIEADKMATIYHWINHSFFPIIVRGEMMDM TRGRSISRFNAQSHVAGIEALRAILRIADMSEEPHRLELKTRIKTLVTQG NAFYNVYDNLKTYHDIKLMKELLSDTSVPVQKLDSYVASFNSMDKLALYN NKHDFAFGLSMFSNRTQNYEAMNNENLHGWFTSDGMFYLYNNDLGHYSEN

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
		YWATVNPYRLPGTTETEQKPLEGTPENIKTNYQQVGMTSLSDDAFVASKK LNNTSALAAMTFTNWNKSLTLNKGWFILGNKIIFVGSNIKNQSSHKAYTT IEQRKENQKHPYCSYVNNQPVDLNNQLVDFTNTKSIFLESDDPAQNIGYY FFKPRTLSISKALQTGKWQNIKADDKSPEAIKEVSNTFITIMQNHTQEGD RYAYMMLPNMTRQEFETYISKLDIDLLENN
85	Hyaluronidase *Streptococcus pyogenes serotype M4 (strain MGAS10)*	MNTYFCTHHKQLLLYSNLFLSFAMMGQGTAIYADTLTSNSKPNNTYFQTQ TLTTTDSEKKVVQPQQKDYYTELLDQWNSIIAGNDAYDKTNPDMVTFHNK AEKDAQNIIKSYQEPDHENRTYLWEHAKDYSASANITKTYRNIEKIAKQI TNPESCYYQDSKAIAIVKDGMAFMYEHAYNLDRENHQTTGKENKENWWDY EIGTPRAINNTLSLMYPYFTQEEILKYTAPIEKFVPDPTRFRVRAANFPP FEANSGNLIDMGRVKLISGILRKDDLEISDTIKAIEKVFTLVDEGNGFYQ DGSLIDHVVTNAQSPLYKKGIAYTGAYGNVLIDGLSQLIPIIQKTKSPIE ADKMATIYHWINHSFFPIIVRGEMMDMTRGRSISRFNAQSHVAGIEALRA ILRIADMSEEPHRLALKTRIKTLVTQGNVFYNVYDNLKTYHDIKLMKELL SDTSVPVQKLDSYVASFNSMDKLALYNNKHDFAFGLSMFSNRTQNYEAMN NENLHGWFTSDGMFYLYNNDLGHYSENYWATVNPYRLPGTTETEQKPLEG TPENIKTNYQQVGMTSLSDDAFVASKKLNNTSALAAMTFTNWNKSLTLNK GWFILGNKIIFVGSNIKNQSSHKAYTTIEQRKENQKHPYCSYVNNQPVDL NNQLVDFTNTKSIFLESDDPAQNIGYYFFKPTTLSISKALQTGKWQNIKA DDKSPEAIKEVSNTFITIMQNHTQDGDRYAYMMLPNMTRQEFETYISKLD IDLLENNDKLAAVYDHDSQQMHVIHYEKKATTFSNHNLSHQGFYSFPHPV KQNQQQKLAHQGIAAKNNALNSHKIPHKRQRRLPRTGYQSSSLEFLGGAL VASFNHITKPFRKKDLRI
86	Hyaluronidase *Streptococcus pyogenes serotype M6*	MVYFYLVDQFTFIISFLYWRNLSVNTYFCTHHKQLLLYSNLFLSFAMMGQ GTAIYADTLTSNSEPNNTYFQTQTLTTTDSEKKVVQPQQKDYYTELLDQW NSIIAGNDAYDKTNPDMVTFHNKAEKDAQNIIKSYQGPDHENRTYLWEHA KDYSASTNITKTYRNIEKIAKQITNPESCYYQDSKAIAIVKDGMAFMYEH AYNLDRENHQTTGKENKENWWVYEIGTPRAINNTLSLMYPYFTQEEILKY TAPIEKFVPDPTRFRVRAANFSPFEANSGNLIDMGRVKLISGILRKDDLE ISDTIKAIEKVFTLVDEGNGFYQDGSLIDHVVTNAQSPLYKKGIAYTGAY GNVLIDGLSQLIPIIQKTKSPIEADKMATIYHWINHSFFPIIVRGEMMDM TRGRSISRFNAQSHVAGIEALRAILRIADMSEEPHRLALKTRIKTLVTQG NAFYNVYDNLKTYHDIKLMKELLSDTFVPVQKLDSYVASFNSMDKLALYN NKHDFAFGLSMFSNRTQNYEAMNNENLHGWFTSDGMFYLYNNDLGHYSEN YWATVNPYRLPGTTETEQKPLEGTPENIKTDYQQVGMTSLSDDAFVASKK LNNTSALAAMTFTNWNKSLTLNKGWFILGNKIIFVGSNIKNQSSHKAYTT IEQRKENQKHPYCSYVNNQPVDLNNQLVDFTNTKSIFLESDDPAQNIGYY FFKPTTLSISKALQTGKWQNIKADDKSPEAIKEVSNTFITIMQNHTQDGD RYAYMMLPNMTRQEFETYISKLDIDLLENNDKLAAVYDHDSQQMHVIHYE KKATMFSNHNLSHQGFYSFPHPVKQNQQ
87	Hyaluronidase *Streptococcus pyogenes serotype M12 (strain MGAS2)*	MVYFYLVNQSTFIISFLYWRNLSVNTYFCTHHKQLLLYSNLFLSFAMMGQ GTAIYADTLTSNSEPNNTYFQTQTLTTTDSEKKVVQPQQKDYYTELLDQW NSIIAGNDAYDKTNPDMVTFHNKAEKDAQNIIKSYQGPDHENRTYLGNMQ RIIPLLLISRKLTAILKKISKMKSLMEDSTISTNGLTQQLKIYNDMDRVT YHNKGLDFAFGLSMTSKNVARYESINGENLKGWHTGAGMSYLYNSDVKHY RDNFWATADMKRLAGTTTLDNEEPKSTDVKKSSKTFVGGTKFDDQHASIG MDFENQDKTLTAKKSYFILNDKIVFLGTGIKSTDSSKNPVTTIENRKAND YKLYKDDTQTTNSDNQETNSLFLESTNSTQNNIGYHFLNESKITVKKESH TGKWSDINKSQKDIQKTDEYYEVTQKHSNTDSKYAYVLYPGLSKDVFKSK ASKVTVVKQEDDFHVVKDNESVWAGINYSDSAKTFEINNTKVEVKAKGMF ILTKKDDNTYECSFYNPESTNSVSDIESKISMTGYSIINKNTSTSNESGV RFELTK
88	Hyaluronidase *Streptococc*	MAFMYEHAYNLNRENHQTTGKENKENWWVYEIGTPRAINNTLSLMYPYFT QEEILKYTAPIEKFVPDPTRFRVRAANFSPFEASSGNLIDMGRVKLISGI LRKDDLEISDTIKAIEKVFTLVDEGNGFYQDGSLIDHVVTNAQSPLYKKG

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
	us pyogenes serotype M12 (strain MGAS2)	IAYTGAYGNVLIDGLSQLIPIIQKTKSPIEADKMATIYHWINHSFFPIIV RGEMMDMTRGRSISRFNAQSHVAGIEALRAILRIADMSEEPHRLALKTRI KTLVTQGNAFYNVYDNLKTYHDIKLMKELLSDTSVPVQKLDSYVASFNSM DKLALYNNKHDFAFGLSMFSNRTQNYEAMNNENLHGWFTSDGMFYLYNND LGHYSENYWATVNPYRLPGTTETEQKPLEGTPENIKTNYQQVGMTSLSDD AFVASKKLNNTSALAAMTFTNWNKSLTLNKGWFILGNKIIFVGSNIKNQS SHKAYTTIEQRKENQKHPYCSYVNNQPVDLNNQLVDFTNTKSIFLESDDP AQNIGYYFFKPTTLSISKALQTGKWQNIKADDKSPEAIKEVSNTFITIMQ NHTQDGDRYAYMMLPNMTRQEFETYISKLDIDLLENNDKLAAVYDHDSQQ MHVIHYEKKATMFSNHNLSHQGFYSFPHPVKQNQQ
89	Hyaluronidase *Streptococcus pyogenes serotype M12 (strain MGAS9)*	MVYFYLVNQSTFIISFLYWRNLSVNTYFCTHHKQLLLYSNLFLSFAMMGQ GTAIYADTLTSNSEPNNTYFQTQTLTTTDSEKKVVQPQQKDYYTELLDQW NSIIAGNDAYVKTNPDMVTFHNKAEKDAQNIIKSYQGPDHENRTYLWEHA KDYSASTNITKTYRNIEKIAKQITNPESCYYQDSKAIAIVKDGMAFMYEH AYNLNRENHQTTGKENKENWWVYEIGTPRAINNTLSLMYPYFTQEEILKY TAPIEKFVPDPTRFRVRAANFSPFEASSGNLIDMGRVKLISGILRKDDLE ISDTIKAIEKVFTLVDEGNGFYQDGSLIDHVVTNAQSPLYKKGIAYTGAY GNVLIDGLSQLIPIIQKTKSPIEADKMATIYHWINHSFFPIIVRGEMMDM TRGRSISRFNAQSHVAGIEALRAILRIADMSEEPHRLALKTRIKTLVTQG NAFYNVYDNLKTYHDIKLMKELLSDTSVPVQKLDSYVASFNSMDKLALYN NKHDFAFGLSMFSNRTQNYEAMNNENLHGWFTSDGMFYLYNNDLGHYSEN YWATVNPYRLPGTTETEQKPLEGTPENIKTNYQQVGMTSLSDDAFVASKK LNNTSALAAMTFTNWNKSLTLNKGWFILGNKIIFVGSNIKNQSSHKAYTT IEQRKENQKHPYCSYVNNQPVDLNNQLVDFTNTKSIFLESDDPAQNIGYY FFKPTTLSISKALQTGKWQNIKADDKSPEAIKEVSNTFITIMQNHTQDGD RYAYMMLPNMTRQEFETYISKLDIDLLENNDKLAAVYDHDSQQMHVIHYE KKATMFSNHNLSHQGFYSFPHPVKQNQQ
90	Hyaluronidase *Streptococcus pyogenes serotype M28*	MVYFYLVNQFTFIISFLYRRNLSVNTYFCTHHKQLLLYSNLFLSFAMMGQ GTAIYADTLTSNSEPNNTYFQTQMLTTTDSEKKVVQPQQKDYYTELLDQW NSIIAGNDAYDKTNPDMVTFHNKAEKDAQNIIKSYQGPDHENRTYLWEHA KDYSASANITKTYRNIEKIAKQITNPESCYYQDSKAIAIVKDGMAFMYEH AYNLDRENHQTTGKENKENWWVYEIGTPRAINNTLSLMYPYFTQEEILKY TAPIEKFVPDPTRFRVRAANFSPFEANSGNLIDMGRVKLISGILRKDDLE ISDTIKAIEKVFTLVDEGNGFYQDGSLIDHVVTNAQSPLYKKGIAYTGAY GNVLIDGLSQLIPIIQKTKSSIEADKMATIYHWINHSFFPIIVRGEMMDM TRGRSISRFNAQSHVAGIEALRAILRIADMSEEPHRLALKTRIKTLVTQG NAFYNVYDNLKTYHDIKLMKELLSDTSVPVQKLDSYVASFNSMDKLALYN NKHDFAFGLSMFSNRTQNYEAMNNENLHGWFTSDGMFYLYNNDLGHYSEN YWATVNPYRLPGTTETEQKPLEGTPENIKTNYQQVGMTSLSDDAFVASKK LNNTSALAAMTFTNWNKSLTLNKGWFILGNKIIFVGSNIKNQSSHKAYTT IEQRKENQKHPYHAYVNNQPVDLNNQLVDFTNTKSIFLESDDSAQNIGYY FFKPTTLSISKALQTGKWQNIKADDKSPEAIKEVSNTFITIMQNHTQDGD RYAYMMLPNMTRQEFETYISKLDIDLLENNDKLAAVYDHDSQQMHVIHYE KKATMFSNHNLSHQGFYSFPHPVKQNQQ
91	Hyaluronidase *Streptococcus suis*	MGFFISQSKQHYGIRKYKVGVCSALIALSILGTRVAANQLPSTETASPQS SQLVETTPETTEAVNLTTEAVMTSEVSSEVSPVTSTETQPSSTAAETLAS PQAVQATKEEEKNLVANGEFASTTAASGNWADPAATNWETWIPANVKKEN GQVRIDEGRLHISSTASYRVAVHQTVDVDPNKRYLFSYNVETKDLKGSGV RVRLRSLTAEGKDLSPQEFAYTPYKNGSQAEHIEQILTVSPETRKLKVEL FFENSVGQAWLDNISLVEYVEKTPETPEPSLELVQPETGQISLASNKVYL PVRPDLTYRIADAAVAIVEKNMIRPLAAGKTQVDVYDKDTKLSSFELTVT EHQATVFDTLRNNWEDISLANKRYQSNDTQMKAFLGRLDAGVASSLKKWV EPTNQGKTIFNDIDFSKSSHLTTVYRRLEQMAQVVENPDSAYYHDRSLID LVRKGMNWLYTNVYNENKSIDGNWWDYEIGTPRAVVNTLIYMHPYFSQEE ILTYTKPISKFVPDPTTISVKH

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
92	Hyaluronidase *Streptococcus suis*	MGFFISQSKQHYGIRKYKVGVCSALIALSILGTRVAANQLPSTETASPQS SQLVETTPETTEAVMTSEVSSEVSPVTSTETQPSSTTAETLASPQAVQAT KEEKNLVANGEFISTTAPSGNWKELAATNWETWIPANVKKENGQVRIDEG RLHISSTASYRVAVHQTVDVDPNKRYLFSYDVETKDLKGSGVRVRLRSLT AEGKDLSPQEFAYTPYKNGSQAEHIEQILTVSPETRKLKVELFFENSVGQ AWLDNISLVEYVEKTPETPEQSPELVQPETGQISLASNKVYLPVRPDLTY RIADAAVATVEKNMIRPLAAGKTQVDVYDKDTKLSSFELIVTEHQATVFD TLRNNWEDISLANKRYQSNDAQMKAFLGRLDAGVASSLEKWVEPTEQSKT IFNDIDFSKSSHLTTVYRRLEQMAQVVENPDSAYYHDRSLIDLVRKGMNW LYANVYNENKSIDGNWWDYEIGTSRAVVNTLIYMHPYFSQEEILTYTKPI SKFVPDPTTIRKTLTNPVPAVGGNQTDLSKVAILEGALREDADRVRAGAQ GLTTIMKFVDKGEGFYRDGSFIDHTNVAYTGAYGNVLIEGFSQLLPVIQP TEFALKEEQTNILYEWIEKAFMPILVRGELMDMTRGRSISRATGESHVQA MEILRSLVRIAESAQPEQKTKLLSFVKAQLTSDTFYDSYRSLKSYKDIDL VNKLLADNQIPAEVDKDYIAAFNNMDKFVYRSAQEGFTFALSMYSSRTQN YEDMNNENRKGWYTADGMVYLYNDDLSHYSNHYWATVDPYRLPGTTTTKD KREDGSGEVTLASDFVGASQLGNRLATIAMDFNNWNNSLTARKAWIVLGN KIVFLGTDIQHQSAQGAETTIENRKLLTGEKYSYYINGQPVDLSKEVVTD KTQSFYMTNGKDNQSIGYVFLNQLPTHAKLDQRTGKWSDINYNQSKEEVS NSFVSLWHEHAQTSSNYAYVLVPNQSMEKVNQAAASVKLLHQDRDLQVVY DQEQNVWGVVKYTDTAYKLTDDITLTDAGLYTIQKVEGGYRIAFYNPSTR TVKNGIELTKAGSSLTVEMEPTAAYPSTVWKVTMPEGSDKQTGSVEKTEK EEKQLKENQPSSEVKQVVHHAAEKTKPSKPRLPQTGEEASLGLGFLGLLT LGAVVDFKCRRSHS
93	Hyaluronidase (*Streptococcus suis)*	MGFFISQSKQHYGIRKYKVGVCSALIALSILGTRVAANQLPSTETASPQS SQLVETTPETTEAVMTSEVSSEVSPVTSTETQPSSTTAETLASPQAVQAT KEEKNLVANGEFISTTAPSGNWKELAATNWETWIPANVKKENGQVRIDEG RLHISSTASYRVAVHQTVDVDPNKRYLFSYDVETKDLKGSGVRVRLRSLT AEGKDLSPQEFAYTPYKNGSQAEHIEQILTVSPETRKLKVELFFENSVGQ AWLDNISLVEYVEKTPETPEQSPELVQPETGQISLASNKVYLPVRPDLTY RIADAAVATVEKNMIRPLAAGKTQVDVYDKDTKLSSFELIVTEHQATVFD TLRNNWEDISLANKRYQSNDAQMKAFLGRLDAGVASSLEKWVEPTEQSKT IFNDIDFSKSSHLTTVYRRLEQMAQVVENPDSAYYHDRSLIDLVRKGMNW LYTNVYNENKSIDGNWWDYEIGTPRAVVNTLIYMHPYFSQEEILTYTKPI SKFVPDPTTIRKTLTNPVPAVGGNQTDLSKVAILEGALREDADRVRAGAQ GLTTIMKFVDKGEGFYRDGSFIDHTNVAYTGAYGNVLIEGFSQLLPVIQP TEFALKEEQTNILYEWIEKAFMPILVRGELMDMTRGRSISRATGESHVQA MEILRSLVRIAESAQPEQKTKLLSFVKAQLTSDTFYDSYRSLKSYKDIDL VNKLLADNQIPAEVDKDYIAAFNNMDKFVYRSAQEGFTFALSMYSSRTQN YEDMNNENRKGWYTADGMVYLYNDDLSHYSNHYWATVDPYRLPGTTTTKD KREDGSGEVTLASDFVGASQLGNRLATIAMDFNNWNNSLTARKAWIVLGN KIVFLGTDIQHQSAQGAETTIENRKLLTGEKYSYYINGQPVDLSKEVVTD KTQSFYMTNGKDNQSIGYVFLNQLPTHAKLDQRTGKWSDINYNQSKEEVS NSFVSLWHEHAQTSSNYAYVLVPNQSMEKVNQAAASVKLLHQDRDLQVVY DQEQNVWGVVKYTDTAYKLTDDITLTDAGLYTIQKVEGGYRIAFYNPSTR TVKNGIELTKAGSSLTVEMEPTAAYPSTVWKVTMPEGSDKQTGSVEKTEK EEKQLKENQPSSEVKQVVHHAAEKTKPSKPRLPQTGEEASLGLGFLGLLT LGAVVDFKCRRSHS
94	Hyaluronidase *Vibrio fischeri (strain ATCC 700601 / ES114)*	MYMIKKHRLNTIALSMLFLFTGNAYAAKNTQTPQYLPSDFEQVRENWAEN YLGDPAITFDQTLKNMVTSTNSSAQKHWDSMTPQPNASGIWDDLPLIDKD TTLGPNIRNSYQRLFTMAKAYRLRDGNLENNQLMLNDIMTAMNYINQNFY FVNQLEYGNWWQWELAIPKDIHNILVLLFDDIKDNYQTIITNHLNATRYF TPDPTHLGVSPGAAESTNPNYRESTGGNRTDNAQVVLIRGMLENNSEEIS QAIAALPAVIEYVSEGDGYYTDGSFLQHSDIAYNGTYGNVLLGGLGIQMN AVAGSPWSMDNQTISNVYNIINQSYEPLLYKGAMMDMVNGRSISRSAEQN HDVGLNIVNSMLFYTNGPDSDKNKQLSSLIKTQITDDTYQNFFDKIYYVS TYQAAQHIVNDPTVSLKDPLIGNFSYPSMDRIVHRRTDWAFALAMHSYRI

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
		GNYECMNGENLKGWFTGDGMIYLYNDQLDHYTGYWPTVNASRMPGTTVDS QIMADCSGERVGGNVNTNMQWVGSTSLNNYGIAGMQFYNWSDTLSAYKSW FMFDNEVVMLGSNIKDQSNANNITTIENRKRLAETKLFIDGTEQAALPYQ GAPATFSIRNKTLANSDLSYVMLTPKTISISQNDVDGNWSDIGNSKGDVS DSYLQATLTQVDQADYQYALLPNQNNDTVQNYAQHPDVTVLRQDEQAHAV QENTLNIIAANNWKNNPVNITDTITLNSMMGFMIKEESSNTFTVAVSEPI QTIDSVNFTFDKQGIVIKEDIENRVVLNGTTLTINTSGLQGQSYSFQVTI QD
95	Hyaluronidase (*Hiduro nipponia*)	>sp|X4Y2L4|LHYAL_HIRNI Hyaluronoglucuronidase OS=Hirudo nipponia PE=1 SV=1 MKEIAVTIDDKNVIASVSESFHGVAFDASLFSPKGLWSFVDITSPKLFKLLE GLSPGYFRVGGTFANWLFFDLDENNKWKDYWAFKDKTPETATITRRWLFRKQ NNLKKETFDDLVKLTKGSKMRLLFDLNAEVRTGYEIGKKMTSTWDSSEAEKL FKYCVSKGYGDNIDWELGNEPDHTSAHNLTEKQVGEDFKALHKVLEKYPTLN KGSLVGPDVGWMGVSYVKGLADGAGDHVTAFTLHQYYFDGNTSDVSTYLDAT YFKKLQQLFDKVKDVLKNSPHKDKPLWLGETSSGYNSGTKDVSDRYVSGFLT LDKLGLSAANNVKVVIRQTIYNGYYGLLDKNTLEPNPDYWLMHVHNSLVGNT VFKVDVSDPTNKARVYAQCTKTNSKHTQSRYYKGSLTIFALNVGDEDVTLKI DQYSGKKIYSYILTPEGGQLTSQKVLLNGKELKLVSDQLPELNADESKTSFT LSPKTFGFFVVSDANVEACKK
96	Hyaluronidase (*Staphylococcus aureus*)	>WP_070029084.1 hyaluronate lyase [Staphylococcus aureus] MTYRMKKWQKLSTITLLMAGAITLNGGEFRSIDKHQIAVADTNVQTPDYEKL RNTWLNVNYGYDQYDEKNDAMKKKFDATEKEAEKLLSSMKTESGRTYLWDSA KDLDNKSADMTRTYRNIEKIAEAMKHKDTKLNTPDNKNKVKDALEWLHKNAY GKEPVKKLEELKTNFSKSAPQKNTNLNWWDYEIGTPRALTNTLILLKEDFTD EEKKKYTAPIKTFAPKSDEILSSVGKAEPAKGGNLVDISKVKLLESIIEEDA TMMKESIEAFNKVFTYVQSNATGKERNGFYKDGSYIDHQDVPYTGAYGVVLL EGISQMMPMIKETPFKDSNQNDTTLKSWIDEGFMPLIYKGEMMDLSRGRAIS RENETSHSTSATVMKSLLRLSDAMDESTKAKYKQIVKTSVKSDSSYKQNDYL SSYSDISKMKSLIEDSTISTNGLTQQLKIYNDMNRVTYHNKDLDFAFGLSMT SKNVAHYESINGENLKGWHTGAGMSYLYNSDVKHYRDNFWATADMKRLAGTT TLDNEEPKENKNSDKTFVGGTKFDDQHASIGMDFENQDKTLTAKKSYFILND KIVFLGTGIKSTDSSKNPVTTIENRKSNGYTLFTDDKQTTASNINDQETNSV FLESTDTKKNIGYHFLNESKITVKKESHTGKWSDINKSQKSDDKTDEYYEVT QKHSNTDDKYAYVLYPGLSKDNFKSKASQVTIVKQDDDFHIVKDNESVWAGV NYSNSTQTFDINNTKVEVKAKGMFILKNKDDNTYECSFYNPESTNTASDIES KISMTGYSITNKNTSTSNESGVRFELQQTLNKDDN
97	Hyaluronidase (*Loxosceles intermedia*)	>sp|R4J7Z9|HYAL_LOXIN Hyaluronidase OS=Loxosceles intermedia PE=2 SV=1 MQTILVLTTFLSAWFLAVGFDVFWNVPSQQCKKYGMKFVPLLEQYSILVNKE DNFKGDKITIFYESQLGLYPHIGANDESFNGGIPQLGDLKAHLEKSAVDIRR DILDKSATGLRIIDWEAWRPIWEFNWSSLRKYQDKMKKVVRQFNPTAHESTV AKLAHNEWENSSKSWMLSTLQLGKQLRPNSVWCYYLFPDCYNYDGNSVQEFQ CSEAIRKGNDRLKWLWEESTAVCPSIYIKEGQLTNYTLQKRIWFTNGRLQEA LRVAQPKARIYPYINYSIKPGMMVPEVEFWRLIAQIASLGMDGAVIWGSSAS VGSKNHCAQLMKYIADVLGPATLRIKENVARCSKQACSGRGRCTWPKDTSVI AWKFLVEKEDYDFYLGDIECKCVEGYEGRYCEQKTK
99	Hyaluronidase (*Streptomyces koganeiensis*)	>AKQ62598.1 hyaluronidase [Streptomyces koganeiensis] MPVARRLFLGSFTAGAVTVATAAATGTASAAGENGATTTFDGPVAAERFSAD TTLEAAFLKTTSETNHAATIYQAGTSGDGAALNVISDNPGTSAMYLSGTETA RGTLKITHRGYADGSDKDAAALSLDLRVAGTAAQGIYVTATNGPTKGNLIAL RNNTGLDDFVVKGTGRIGVGIDRAATPRAQVHIVQRGDALAALLVEGSVRIG NAATVPTSVDSSGGGALYASGGALLWRGSNGTVTTIAPA

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
100	Hyaluronidase (*Mycobacterium tuberculosis*)	>WP_055373619.1 hypothetical protein [Mycobacterium tuberculosis] MTESRPVFAVVISAGLSAIPMVGGPLQTVFDAIEERTRHRAETTTREICESV GGADTVLSRIDKNPELEPLLSQAIEAATRTSMEAKRRLLAQAAAAALEDDQK VEPASLIVATLSQLEPVHIHALVRLAKAAKSSPDQDEIQRREVMRAASKVEP VPVLAALIQTGVAIATTTVWHGNGTGTPAEESGHILIHDVSDFGHRLLAYLR AADAGAELLILPSGGSAPTGDHPTPHPSTSR
101	Hyaluronidase (*Hiduronipponia*)	MDWTWILFLVAAATRVHSGRKEIAVTIDDKNVIASVSESFHGVAFDASLFSP KGLWSFVDITSPKLFKLLEGLSPGYFRVGGTFANWLFFDLDENNKWKDYWAF KDKTPETATITRRWLFRKQNNLKKETFDDLVKLTKGSKMRLLFDLNAEVRTG YEIGKKMTSTWDSSEAEKLFKYCVSKGYGDNIDWELGNEPDHTSAHNLTEKQ VGEDFKALHKVLEKYPTLNKGSLVGPDVGWMGVSYVKGLADGAGDHVTAFTL HQYYFDGNTSDVSTYLDATYFKKLQQLFDKVKDVLKNSPHKDKPLWLGETSS GYNSGTKDVSDRYVSGFLTLDKLGLSAANNVKVVIRQTIYNGYYGLLDKNTL EPNPDYWMHVHNSLVGNTVFKVDVSDPTNKARVYAQCTKTNSKHTQSRYYKG SLTIFALNVGDEDVTLKIDQYSGKKIYSYILTPEGGQLTSQKVLLNGKELKL VSDQLPELNADESKTSFTLSPKTFGFFVVSD ANVEACKK
102	Hyaluronidase (*Staphylococcus aureus*)	MDWTWILFLVAAATRVHSGRDTNVQTPDYEKLRNTWLNVNYGYDQYDEKNDA MKKKFDATEKEAEKLLSSMKTESGRTYLWDSAKDLDNKSADMTRTYRNIEKI AEAMKHKDTKLNTPDNKNKVKDALEWLHKNAYGKEPVKKLEELKTNFSKSAP QKNTNLNWWDYEIGTPRALTNTLILLKEDFTDEEKKKYTAPIKTFAPKSDEI LSSVGKAEPAKGGNLVDISKVKLLESIIEEDATMMKESIEAFNKVFTYVQSN ATGKERNGFYKDGSYIDHQDVPYTGAYGVVLLEGISQMMPMIKETPFKDSNQ NDTTLKSWIDEGFMPLIYKGEMMDLSRGRAISRENETSHSTSATVMKSLLRL SDAMDESTKAKYKQIVKTSVKSDSSYKQNDYLSSYSDISMKSLIEDSTISTN GLTQQLKIYNDMNRVTYHNKDLDFAFGLSMTSKNVAHYESINGENLKGWHTG AGMSYLYNSDVKHYRDNFWATADMKRLAGTTTLDNEEPKENKNSDKTFVGGT KFDDQHASIGMDFENQDKTLTAKKSYFILNDKIVFLGTGIKSTDSSKNPVTT IENRKSNGYTLFTDDKQTTASNINDQETNSVFLESTDTKKNIGYHFLNESKI TVKKESHTGKWSDINKSQKSDDKTDEYYEVTQKHSNTDDKYAYVLYPGLSKD NFKSKASQVTIVKQDDDFHIVKDNESVWAGVNYSNSTQTFDINNTKVEVKAK GMFILKNKDDNTYECSFYNPESTNTASDIESKISMTGYSITNKNTSTSNESG VHFELTK
103	Hyaluronidase (*Loxosceles intermedia*)	MDWTWILFLVAAATRVHSGRDVFWNVPSQQCKKYGMKFVPLLEQYSILVNKE DNFKGDKITIFYESQLGLYPHIGANDESFNGGIPQLGDLKAHLEKSAVDIRR DILDKSATGLRIIDWEAWRPIWEFNWSSLRKYQDKMKKVVRQFNPTAHESTV AKLAHNEWENSSKSWMLSTLQLGKQLRPNSVWCYYLFPDCYNYDGNSVQEFQ CSEAIRKGNDRLKWLWEESTAVCPSIYIKEGQLTNYTLQKRIWFTNGRLQEA LRVAQPKARIYPYINYSIKPGMMVPEVEFWRLIAQIASLGMDGAVIWGSSAS VGSKNHCAQLMKYIADVLGPATLRIKENVARCSKQACSGRGRCTWPKDTSVI AWKFLVEKEDYDFYLGDIECKCVEGYEGRYCEQKTK
104	Hyaluronidase (*Streptomyces koganeiensi*	MDWTWILFLVAAATRVHSGRATGTASAAGENGATTTFDGPVAAERFSADTTL EAAFLKTTSETNHAATIYQAGTSGDGAALNVISDNPGTSAMYLSGTETARGT LKITHRGYADGSDKDAAALSLDLRVAGTAAQGIYVTATNGPTKGNLIALRNN TGLDDFVVKGTGRIGVGIDRAATPRAQVHIVQRGDALAALLVEGSVRIGNAA TVPTSVDSSGGGALYASGGALLWRGSNGTVTTIAPA

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
	s)	
105	Hyaluronidase (*Mycobacterium tuberculosis*)	MDWTWILFLVAAATRVHSGRGSAIEERTRHRAETTTREICESVGGADTVLSR IDKNPELEPLLSQAIEAATRTSMEAKRRLLAQAAAAALEDDQKVEPASLIVA TLSQLEPVHIHALVRLAKAAKSSPDQDEIQRREVMRAASKVEPVPVLAALIQ TGVAIATTTVWHGNGTGTPAEESGHILIHDVSDFGHRLLAYLRAADAGAELL ILPSGGSAPTGDHPTPHPSTSR
106	HYAL1	MAAHLLPICALFLTLLDMAQGFRGPLLPNRPFTTVWNANTQWCLERHGVD VDVSVFDVVANPGQTFRGPDMTIFYSSQLGTYPYYTPTGEPVFGGLPQNA SLIAHLARTFQDILAAIPAPDFSGLAVIDWEAWRPRWAFNWDTKDIYRQR SRALVQAQHPDWPAPQVEAVAQDQFQGAARAWMAGTLQLGRALRPRGLWG FYGFPDCYNYDFLSPNYTGQCPSGIRAQNDQLGWLWGQSRALYPSIYMPA VLEGTGKSQMYVQHRVAEAFRVAVAAGDPNLPVLPYVQIFYDTTNHFLPL DELEHSLGESAAQGAAGVVLWVSWENTRTKESCQAIKEYMDTTLGPFILN VTSGALLCSQALCSGHGRCVRRTSHPKALLLLNPASFSIQLTPGGGPLSL RGALSLEDQAQMAVEFKCRCYPGWQAPWCERKSMW
107	HYAL2	MRAGPGPTVTLALVLAVAWAMELKPTAPPIFTGRPFVVAWDVPTQDCGPR LKVPLDLNAFDVQASPNEGFVNQNITIFYRDRLGLYPRFDSAGRSVHGGV PQNVSLWAHRKMLQKRVEHYIRTQESAGLAVIDWEDWRPVWVRNWQDKDV YRRLSRQLVASRHPDWPPDRIVKQAQYEFEFAAQQFMLETLRYVKAVRPR HLWGFYLFPDCYNHDYVQNWESYTGRCPDVEVARNDQLAWLWAESTALFP SVYLDETLASSRHGRNFVSFRVQEALRVARTHHANHALPVYVFTRPTYSR RLTGLSEMDLISTIGESAALGAAGVILWGDAGYTTSTETCQYLKDYLTRL LVPYVVNVSWATQYCSRAQCHGHGRCVRRNPSASTFLHLSTNSFRLVPGH APGEPQLRPVGELSWADIDHLQTHFRCQCYLGWSGEQCQWDHRQAAGGAS EAWAGSHLTSLLALAALAFTWTL
108	HYAL3	MTTQLGPALVLGVALCLGCGQPLPQVPERPFSVLWNVPSAHCEARFGVHL PLNALGIIANRGQHFHGQNMTIFYKNQLGLYPYFGPRGTAHNGGIPQALP LDRHLALAAYQIHHSLRPGFAGPAVLDWEEWCPLWAGNWGRRRAYQAASW AWAQQVFPDLDPQEQLYKAYTGFEQAARALMEDTLRVAQALRPHGLWGFY HYPACGNGWHSMASNYTGRCHAATLARNTQLHWLWAASSALFPSIYLPPR LPPAHHQAFVRHRLEEAFRVALVGHRHPLPVLAYVRLTHRRSGRFLSQDD LVQSIGVSAALGAAGVVLWGDLSLSSSEEECWHLHDYLVDTLGPYVINVT RAAMACSHQRCHGHGRCARRDPGQMEAFLHLWPDGSLGDWKSFSCHCYWG WAGPTCQEPRPGPKEAV
109	HYAL4	MKVLSEGQLKLCVVQPVHLTSWLLIFFILKSISCLKPARLPIYQRKPFIA AWNAPTDQCLIKYNLRLNLKMFPVIGSPLAKARGQNVTIFYVNRLGYYPW YTSQGVPINGGLPQNISLQVHLEKADQDINYYIPAEDFSGLAVIDWEYWR PQWARNWNSKDVYRQKSRKLISDMGKNVSATDIEYLAKVTFEESAKAFMK ETIKLGIKSRPKGLWGYYLYPDCHNYNVYAPNYSGSCPEDEVLRNNELSW LWNSSAALYPSIGVWKSLGDSENILRFSKFRVHESMRISTMTSHDYALPV FVYTRLGYRDEPLFFLSKQDLVSTIGESAALGAAGIVIWGDMNLTASKAN CTKVKQFVSSDLGSYIANVTRAAEVCSLHLCRNNGRCIRKMWNAPSYLHL NPASYHIEASEDGEFTVKGKASDTDLAVMADTFSCHCYQGYEGADCREIK TADGCSGVSPSPGSLMTLCLLLLASYRSIQL
110	CXCR4	>sp|P25025|CXCR2_HUMAN C-X-C chemokine receptor type 2 OS=Homo sapiens GN=CXCR2 PE=1 SV=2 MEDFNMESDSFEDFWKGEDLSNYSYSSTLPPFLLDAAPCEPESLEINKYFVV IIYALVFLLSLLGNSLVMLVILYSRVGRSVTDVYLLNLALADLLFALTLPIW AASKVNGWIFGTFLCKVVSLLKEVNFYSGILLLACISVDRYLAIVHATRTLT QKRYLVKFICLSIWGLSLLLALPVLLFRRTVYSSNVSPACYEDMGNNTANWR MLLRILPQSFGFIVPLLIMLFCYGFTLRTLFKAHMGQKHRAMRVIFAVVLIF LLCWLPYNLVLLADTLMRTQVIQETCERRNHIDRALDATEILGILHSCLNPL

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
		IYAFIGQKFRHGLLKILAIHGLISKDSLPKDSRPSFVGSSSGHTSTTL
111	CCR2	>sp|P41597|CCR2_HUMAN C-C chemokine receptor type 2 OS=Homo sapiens GN=CCR2 PE=1 SV=1 MLSTSRSRFIRNTNESGEEVTTFFDYDYGAPCHKFDVKQIGAQLLPPLYSLV FIFGFVGNMLVVLILINCKKLKCLTDIYLLNLAISDLLFLITLPLWAHSAAN EWVFGNAMCKLFTGLYHIGYFGGIFFIILLTIDRYLAIVHAVFALKARTVTF GVVTSVITWLVAVFASVPGIIFTKCQKEDSVYVCGPYFPRGWNNFHTIMRNI LGLVLPLLIMVICYSGILKTLLRCRNEKKRHRAVRVIFTIMIVYFLFWTPYN IVILLNTFQEFFGLSNCESTSQLDQATQVTETLGMTHCCINPIIYAFVGEKF RSLFHIALGCRIAPLQKPVCGGPGVRPGKNVKVTTQGLLDGRGKGKSIGRAP EASLQDKEGA
112	CCL5	>sp|P13501|CCL5_HUMAN C-C motif chemokine 5 OS=Homo sapiens GN=CCL5 PE=1 SV=3 MKVSAAALAVILIATALCAPASASPYSSDTTPCCFAYIARPLPRAHIKEYFY TSGKCSNPAVVFVTRKNRQVCANPEKKWVREYINSLEMS
113	CCR5	>sp|P51681|CCR5_HUMAN C-C chemokine receptor type 5 OS=Homo sapiens GN=CCR5 PE=1 SV=1 MDYQVSSPIYDINYYTSEPCQKINVKQIAARLLPPLYSLVFIFGFVGNMLVI LILINCKRLKSMTDIYLLNLAISDLFFLLTVPFWAHYAAAQWDFGNTMCQLL TGLYFIGFFSGIFFIILLTIDRYLAVVHAVFALKARTVTFGVVTSVITWVVA VFASLPGIIFTRSQKEGLHYTCSSHFPYSQYQFWKNFQTLKIVILGLVLPLL VMVICYSGILKTLLRCRNEKKRHRAVRLIFTIMIVYFLFWAPYNIVLLLNTF QEFFGLNNCSSSNRLDQAMQVTETLGMTHCCINPIIYAFVGEKFRNYLLVFF QKHIAKRFCKCCSIFQQEAPERASSVYTRSTGEQEISVGL
114	HMGB1	>sp|P09429|HMGB1_HUMAN High mobility group protein B1 OS=Homo sapiens GN=HMGB1 PE=1 SV=3 MGKGDPKKPRGKMSSYAFFVQTCREEHKKKHPDASVNFSEFSKKCSERWKTM SAKEKGKFEDMAKADKARYEREMKTYIPPKGETKKKFKDPNAPKRPPSAFFL FCSEYRPKIKGEHPGLSIGDVAKKLGEMWNNTAADDKQPYEKKAAKLKEKYE KDIAAYRAKGKPDAAKKGVVKAEKSKKKKEEEEDEEDEEDEEEEEDEEDEDE EEDDDDE
115	PIAS3	>sp|Q9Y6X2|PIAS3_HUMAN E3 SUMO-protein ligase PIAS3 OS=Homo sapiens GN=PIAS3 PE=1 SV=2 MAELGELKHMVMSFRVSELQVLLGFAGRNKSGRKHELLAKALHLLKSSCAPS VQMKIKELYRRRFPRKTLGPSDLSLLSLPPGTSPVGSPGPLAPIPPTLLAPG TLLGPKREVDMHPPLPQPVHPDVTMKPLPFYEVYGELIRPTTLASTSSQRFE EAHFTFALTPQQVQQILTSREVLPGAKCDYTIQVQLRFCLCETSCPQEDYFP PNLFVKVNGKLCPLPGYLPPTKNGAEPKRPSRPINITPLARLSATVPNTIVV NWSSEFGRNYSLSVYLVRQLTAGTLLQKLRAKGIRNPDHSRALIKEKLTADP DSEVATTSLRVSLMCPLGKMRLTVPCRALTCAHLQSFDAALYLQMNEKKPTW TCPVCDKKAPYESLIIDGLFMEILSSCSDCDEIQFMEDGSWCPMKPKKEASE VCPPPGYGLDGLQYSPVQGGDPSENKKKVEVIDLTIESSSDEEDLPPTKKHC SVTSAAIPALPGSKGVLTSGHQPSSVLRSPAMGTLGGDFLSSLPLHEYPPAF PLGADIQGLDLFSFLQTESQHYGPSVITSLDEQDALGHFFQYRGTPSHFLGP LAPTLGSSHCSATPAPPPGRVSSIVAPGGALREGHGGPLPSGPSLTGCRSDI ISLD
116	IL15	>sp|P40933|IL15_HUMAN Interleukin-15 OS=Homo sapiens GN=IL15 PE=1 SV=1 MRISKPHLRSISIQCYLCLLLNSHFLTEAGIHVFILGCFSAGLPKTEANWVN VISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGD ASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ MFINTS

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
117	HysA	>sp|Q59801|HYSA_STAA8 Hyaluronate lyase OS=Staphylococcus aureus (strain NCTC 8325) OX=93061 GN=hysA PE=3 SV=1 MTYRIKKWQKLSTITLLMAGVITLNGGEFRSVDKHQIAVADTNVQTPDYEKL RNTWLDVNYGYDKYDENNPDMKKKFDATEKEATNLLKEMKTESGRKYLWSGA ETLETNSSHMTRTYRNIEKIAEAMRNPKTTLNTDENKKKVKDALEWLHKNAY GKEPDKKVKELSENFTKTTGKNTNLNWWDYEIGTPKSLTNTLILLNDQFSNE EKKKFTAPIKTFAPDSDKILSSVGKAELAKGGNLVDISKVKLLECIIEEDKD MMKKSIDSFNKVFTYVQDSATGKERNGFYKDGSYIDHQDVPYTGAYGVVLLE GISQMMPMIKETPFNDKTQNDTTLKSWIDDGFMPLIYKGEMMDLSRGRAISR ENETSHSASATVMKSLLRLSDAMDDSTKAKYKKIVKSSVESDSSYKQNDYLN SYSDIDKMKSLMTDNSISKNGLTQQLKIYNDMDRVTYHNKDLDFAFGLSMTS KNVARYESINGENLKGWHTGAGMSYLYNSDVKHYHDNFWVTADMKRLSGTTT LDNEILKDTDDKKSSKTFVGGTKVDDQHASIGMDFENQDKTLTAKKSYFILN DKIVFLGTGIKSTDSSKNPVTTIENRKANGYTLYTDDKQTTNSDNQENNSVF LESTDTKKNIGYHFLNKPKITVKKESHTGKWKEINKSQKDTQKTDEYYEVTQ KHSNSDNKYGYVLYPGLSKDVFKTKKDEVTVVKQEDDFHVVKDNESVWAGVN YSNSTQTFDINNTKVEVKAKGMFILKKKDDNTYECSFYNPESTNSASDIESK ISMTGYSITNKNTSTSNESGVHFELTK
118	SPAM1 (*Sus scrofa*)	>tr|Q8MI02|Q8MI02_PIG Hyaluronidase OS=Sus scrofa OX=9823 GN=SPAM-1 PE=1 SV=1 MGVQRLQHISFRSFFVPSGAPQVVFTFLLIPCCLALDFRASPIIPNTTFLWV WNAPTESCAKKFYMPPDLSLFSFVTSPRASVTGQFLTLFYANRLGYYPHVDE NTGKNVNGGIPQLGSLQRHLDKAEKDILHYMQIDKVGLSVIDWENWRPTWER NWKEKAIYRRQSIELVQQKNIKLTPAAATKLAKREFEKAGKTFMQETLKLGK LLRPNHLWGYYLFPDCYNHNYHKPGYNGSCLDIEKRRNDALDWLWKESTALF PSIYLNTRLKPSQVALFVRNRVQEAIRVSKVANAQSPLPVFVYTRPVFSGAS SRYLSQDDLVNTIGETVALGASGIVMWGSLNLSLTMQSCMNLGSYLKTTLNP YLINVTLAAKMCSQVLCQEQGVCTRKHWNSSDYLHLNPANFAIRTGKGNKYI VHGKPTLEDLKEFSKNFYCSCFANFHCKERADIENIHAINVCITEDVCVEAF LNSEPELPDEVQQDNQPPCGGSGRC
119	LIGHT	>sp|O43557|TNF14_HUMAN Tumor necrosis factor ligand superfamily member 14 OS=Homo sapiens OX=9606 GN=TNFSF14 PE=1 SV=2 MEESVVRPSVFVVDGQTDIPFTRLGRSHRRQSCSVARVGLGLLLLLMGAGLA VQGWFLLQLHWRLGEMVTRLPDGPAGSWEQLIQERRSHEVNPAAHLTGANSS LTGSGGPLLWETQLGLAFLRGLSYHDGALVVTKAGYYYIYSKVQLGGVGCPL GLASTITHGLYKRTPRYPEELELLVSQQSPCGRATSSSRVWWDSSFLGGVVH LEAGEKVVVRVLDERLVRLRDGTRSYFGAFMV
120	ITAC	>sp|O14625|CXL11_HUMAN C-X-C motif chemokine 11 OS=Homo sapiens OX=9606 GN=CXCL11 PE=1 SV=1 MSVKGMAIALAVILCATVVQGFPMFKRGRCLCIGPGVKAVKVADIEKASIMY PSNNCDKIEVIITLKENKGQRCLNPKSKQARLIIKKVERKNF
121	fractalkine	>sp|P78423|X3CL1_HUMAN Fractalkine OS=Homo sapiens OX=9606 GN=CX3CL1 PE=1 SV=1 MAPISLSWLLRLATFCHLTVLLAGQHHGVTKCNITCSKMTSKIPVALLIHYQ QNQASCGKRAIILETRQHRLFCADPKEQWVKDAMQHLDRQAAALTRNGGTFE KQIGEVKPRTTPAAGGMDESVVLEPEATGESSSLEPTPSSQEAQRALGTSPE LPTGVTGSSGTRLPPTPKAQDGGPVGTELFRVPPVSTAATWQSSAPHQPGPS LWAEAKTSEAPSTQDPSTQASTASSPAPEENAPSEGQRVWGQGQSPRPENSL EREEMGPVPAHTDAFQDWGPGSMAHVSVVPVSSEGTPSREPVASGSWTPKAE EPIHATMDPQRLGVLITPVPDAQAATRRQAVGLLAFLGLLFCLGVAMFTYQS LQGCPRKMAGEMAEGLRYIPRSCGSNSYVLVPV
122	HysA	MDWTWILFLVAAATRVHSGRDTNVQTPDYEKLRNTWLNVNYGYDQYDEKNDA MKKKFDATEKEAEKLLSSMKTESGRTYLWDSAKDLDNKSADMTRTYRNIEKI

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
		AEAMKHKDTKLNTPDNKNKVKDALEWLHKNAYGKEPVKKLEELKTNFSKSAP QKNTNLNWWDYEIGTPRALTNTLILLKEDFTDEEKKKYTAPIKTFAPKSDEI LSSVGKAEPAKGGNLVDISKVKLLESIIEEDATMMKESIEAFNKVFTYVQSN ATGKERNGFYKDGSYIDHQDVPYTGAYGVVLLEGISQMMPMIKETPFKDSNQ NDTTLKSWIDEGFMPLIYKGEMMDLSRGRAISRENETSHSTSATVMKSLLRL SDAMDESTKAKYKQIVKTSVKSDSSYKQNDYLSSYSDISKMKSLIEDSTIST NGLTQQLKIYNDMNRVTYHNKDLDFAFGLSMTSKNVAHYESINGENLKGWHT GAGMSYLYNSDVKHYRDNFWATADMKRLAGTTTLDNEEPKENKNSDKTFVGG TKFDDQHASIGMDFENQDKTLTAKKSYFILNDKIVFLGTGIKSTDSSKNPVT TIENRKSNGYTLFTDDKQTTASNINDQETNSVFLESTDTKKNIGYHFLNESK ITVKKESHTGKWSDINKSQKSDDKTDEYYEVTQKHSNTDDKYAYVLYPGLSK DNFKSKASQVTIVKQDDDFHIVKDNESVWAGVNYSNSTQTFDINNTKVEVKA KGMFILKNKDDNTYECSFYNPESTNTASDIESKISMTGYSITNKNTSTSNES GVHFELTK
123	HysA(Q59801)	>sp|Q59801|HYSA_STAA8 Hyaluronate lyase OS=Staphylococcus aureus (strain NCTC 8325) OX=93061 GN=hysA PE=3 SV=1 MTYRIKKWQKLSTITLLMAGVITLNGGEFRSVDKHQIAVADTNVQTPDYEKL RNTWLDVNYGYDKYDENNPDMKKKFDATEKEATNLLKEMKTESGRKYLWSGA ETLETNSSHMTRTYRNIEKIAEAMRNPKTTLNTDENKKKVKDALEWLHKNAY GKEPDKKVKELSENFTKTTGKNTNLNWWDYEIGTPKSLTNTLILLNDQFSNE EKKKFTAPIKTFAPDSDKILSSVGKAELAKGG NLVDISKVKLLECIIEEDKDMMKKSIDSFNKVFTYVQDSATGKERNGFYKDG SYIDHQDVPYTGAYGVVLLEGISQMMPMIKETPFNDKTQNDTTLKSWIDDGF MPLIYKGEMMDLSRGRAISRENETSHSASATVMKSLLRLSDAMDDSTKAKYK KIVKSSVESDSSYKQNDYLNSYSDIDKMKSLMTDNSISKNGLTQQLKIYNDM DRVTYHNKDLDFAFGLSMTSKNVARYESINGE NLKGWHTGAGMSYLYNSDVKHYHDNFWVTADMKRLSGTTTLDNEILKDTDDK KSSKTFVGGTKVDDQHASIGMDFENQDKTLTAKKSYFILNDKIVFLGTGIKS TDSSKNPVTTIENRKANGYTLYTDDKQTTNSDNQENNSVFLESTDTKKNIGY HFLNKPKITVKKESHTGKWKEINKSQKDTQKTDEYYEVTQKHSNSDNKYGYV LYPGLSKDVFKTKKDEVTVVKQEDDFHVVKDNESVWAGVNYSNSTQTFDINN TKVEVKAKGMFILKKKDDNTYECSFYNPESTNSASDIESKISMTGYSITNKN TSTSNESGVHFELTK
124	lin	MDWTWILFLVAAATRVHSGRDVFWNVPSQQCKKYGMKFVPLLEQYSILVNKE DNFKGDKITIFYESQLGLYPHIGANDESFNGGIPQLGDLKAHLEKSAVDIRR DILDKSATGLRIIDWEAWRPIWEFNWSSLRKYQDKMKKVVRQFNPTAHESTV AKLAHNEWENSSKSWMLSTLQLGKQLRPNSVWCYYLFPDCYNYDGNSVQEFQ CSEAIRKGNDRLKWLWEESTAVCPSIYIKEGQLTNYTLQKRIWFTNGRLQEA LRVAQPKARIYPYINYSIKPGMMVPEVEFWRLIAQIASLGMDGAVIWGSSAS VGSKNHCAQLMKYIADVLGPATLRIKENVARCSKQACSGRGRCTWPKDTSVI AWKFLVEKEDYDFYLGDIECKCVEGYEGRYCEQKTK
125	rv	MDWTWILFLVAAATRVHSGRGSAIEERTRHRAETTTREICESVGGADTVLSR IDKNPELEPLLSQAIEAATRTSMEAKRRLLAQAAAAALEDDQKVEPASLIVA TLSQLEPVHIHALVRLAKAAKSSPDQDEIQRREVMRAASKVEPVPVLAALIQ TGVAIATTTVWHGNGTGTPAEESGHILIHDVSDFGHRLLAYLRAADAGAELL ILPSGGSAPTGDHPTPHPSTSR
126	sko	MDWTWILFLVAAATRVHSGRATGTASAAGENGATTTFDGPVAAERFSADTTL EAAFLKTTSETNHAATIYQAGTSGDGAALNVISDNPGTSAMYLSGTETARGT LKITHRGYADGSDKDAAALSLDLRVAGTAAQGIYVTATNGPTKGNLIALRNN TGLDDFVVKGTGRIGVGIDRAATPRAQVHIVQRGDALAALLVEGSVRIGNAA TVPTSVDSSGGGALYASGGALLWRGSNGTVTTIAPA
127	UniProtKB - R4J7Z9 (HYAL_LOXIN)	>sp|R4J7Z9|HYAL_LOXIN Hyaluronidase OS=Loxosceles intermedia OX=58218 PE=2 SV=1 MQTILVLTTFLSAWFLAVGFDVFWNVPSQQCKKYGMKFVPLLEQYSILVNKE DNFKGDKITIFYESQLGLYPHIGANDESFNGGIPQLGDLKAHLEKSAVDIRR DILDKSATGLRIIDWEAWRPIWEFNWSSLRKYQDKMKKVVRQFNPTAHESTV AKLAHNEWENSSKSWMLSTLQLGKQLRPNSVWCYYLFPDCYNYDGNSVQEFQ

No.	PROTEIN/NUCLIEC ACID	EXEMPLARY AMINO ACID/NUCLEIC ACID SEQUENCES
		CSEAIRKGNDRLKWLWEESTAVCPSIYIKEGQ LTNYTLQKRIWFTNGRLQEALRVAQPKARIYPYINYSIKPGMMVPEVEFWRL IAQIASLGMDGAVIWGSSASVGSKNHCAQLMKYIADVLGPATLRIKENVARC SKQACSGRGRCTWPKDTSVIAWKFLVEKEDYDFYLGDIECKCVEGYEGRYCE QKTK
128	UniProtKB - P95202 (P95202_MYCTU)	>tr|P95202|P95202_MYCTU Possible secreted protein OS=Mycobacterium tuberculosis (strain ATCC 25618 / H37Rv) OX=83332 GN=Rv0394c PE=1 SV=1 MTEPRPVFAVVISAGLSAIPMVGGPLQTVFDAIEERTRHRAETTTREICESV GGADTVLSRIDKNPELEPLLSQAIEAATRTSMEAKRRLLAQAAAAALEDDQK VEPASLIVATLSQLEPVHIHALVRLAKAAKSSPDQDEIQRREVMRAASKVEP VPVLAALIQTGVAIATTTVWHGNGTGTPAEESGHILIHDVSDFGHRLLAYLR AADAGAELLILPSGGSAPTGDHPTPHPSTSR
129	UniProtKB - A0A0U2E2J7 (A0A0U2E2J7_9ACTN)	>tr|A0A0U2E2J7|A0A0U2E2J7_9ACTN Hyaluronidase OS=Streptomyces koganeiensis OX=1684313 PE=1 SV=1 MPVARRLFLGSFTAGAVTVATAAATGTASAAGENGATTTFDGPVAAERFSAD TTLEAAFLKTTSETNHAATIYQAGTSGDGAALNVISDNPGTSAMYLSGTETA RGTLKITHRGYADGSDKDAAALSLDLRVAGTAAQGIYVTATNGPTKGNLIAL RNNTGLDDFVVKGTGRIGVGIDRAATPRAQVHIVQRGDALAALLVEGSVRIG NAATVPTSVDSSGGGALYASGGALLWRGSNGT VTTIAPA
130	PH-20 (truncated)	MGVLKFKHIFFRSFVKSSGVSQIVFTFLLIPCCLTLNFRAPPVIPNVPFLWA WNAPSEFCLGKFDEPLDMSLFSFIGSPRINATGQGVTIFYVDRLGYYPYIDS ITGVTVNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWAR NWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGK LLRPNHLWGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALY PSIYLNTQQSPVAATLYVRNRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQV LKFLSQDELVYTFGETVALGASGIVIWGTLSIMRSMKSCLLLDNYMETILNP YIINVTLAAKMCSQVLCQEQGVCIRKNWNSSDYLHLNPDNFAIQLEKGGKFT VRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAVDVCIADGVCIDAF LKPPMETEEPQIFYNASPSTL

CLAIMS

1. A modified oncolytic virus, comprising an exogenous nucleic acid that codes for a chemokine receptor, a membrane associated protein that is capable of degrading hyaluronan, a microbial protein that is capable of degrading hyaluronan, or any combinations thereof.

2. The modified oncolytic virus of claim 1, comprising the exogenous nucleic acid that codes for a chemokine receptor, wherein the chemokine receptor comprises at least one of CXCR4 and CCR2.

3. The modified oncolytic virus of claim 1 or 2, comprising the exogenous nucleic acid that codes for the membrane associated protein.

4. The modified oncolytic virus of claim 3, wherein the membrane associated protein comprises a membraned associated hyaluronidase.

5. The modified oncolytic virus of claim 4, wherein the membrane associated hyaluronidase comprises PH-20.

6. The modified oncolytic virus of claim 5, wherein the PH-20 is GPI-anchored.

7. The modified oncolytic virus of claim 1, comprising the exogenous nucleic acid that codes for the microbial protein, wherein the microbial protein comprises a secreted hyaluronidase.

8. The modified oncolytic virus of claim 7, wherein the secreted hyaluronidase comprises at least one of HysA, lin, sko, and rv, or any combinations thereof.

9. The modified oncolytic virus of claim 1 or 2, comprising the exogenous nucleic acid that codes for the microbial protein.

10. The modified oncolytic virus of claim 9, wherein the microbial protein comprises HysA.

11. The modified oncolytic virus of any one of claims 1-10, further comprising a modification in the genome of the virus, wherein the modification enhances production of enveloped extracellular form (EEV) of the virus.

12. The modified oncolytic virus of claim9, comprising the modification in the genome of the virus, wherein the modification comprises a mutation or a deletion of the B5R gene.

13. The modified oncolytic virus of claim 9, comprising the modification in the genome of the virus, wherein the modification comprises a mutation or a deletion in a SCR region of the B5R gene, wherein said SCR region comprises SCR1, SCR3, SCR4, or any combinations thereof, and wherein the SCR region does not comprise SCR2.

14. The modified oncolytic virus of claim 12, comprising the deletion of the B5R gene, wherein the deletion is a partial deletion of the B5R gene.

15. The modified oncolytic virus of any one of claims 1-14, further comprising a modification in the genome of the virus, wherein the modification enhances replication of the virus, increases tumor cell killing potential of the virus, or any combinations thereof.

16. The modified oncolytic virus of claim 15, comprising the modification in the genome of the virus, wherein the modification comprises a mutation or a deletion of the A52R gene.

17. The modified oncolytic virus of claim 16, comprising the deletion of the A52R gene.

18. The modified oncolytic virus of any one of claims 1-17, further comprising at least one additional modification in the genome of the virus, wherein the additional modification comprises a mutation or a deletion of a further viral gene.

19. The modified oncolytic virus of claim 18, comprising the mutation or the deletion of the further viral gene, wherein the further viral gene comprises at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, and N1L, and a functional domain or fragment or variant thereof, or any combinations thereof.

20. The modified oncolytic virus of any one of claims 1-19, further comprising at least one additional exogenous nucleic acid.

21. The modified oncolytic virus of claim 20, wherein the at least one additional exogenous nucleic acid comprises a nucleic acid sequence that codes for a protein or a fragment thereof that: modulates NFκB signaling, promotes reduction of interstitial fluid pressure (IFP) in a tumor, modulates STAT3-mediated gene activation, promotes T cell activation, promotes attraction of NK cells to virus-infected cells, modulates metabolic program of virus-infected cells, modulates fatty acid uptake by virus-infected cells, promotes therapeutic targeting of MDSCs, or any combinations thereof.

22. The modified oncolytic virus of claim 20 or 21, wherein the at least one additional exogenous nucleic acid comprises a nucleic acid coding for at least one of HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, and a functional domain or fragment or variant thereof, or any combinations thereof.

23. The modified oncolytic virus of any one of claims 2-22, comprising:
    (a)the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; and
    (b)the exogenous nucleic acid that codes for a hyaluronidase.

24. The modified oncolytic virus of any one of claims 12-22, comprising:
    (a)the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; and
    (b)the mutation or the deletion of B5R gene.

25. The modified oncolytic virus of any one of claims 12-22, comprising:
(a)the exogenous nucleic acid that codes for a hyaluronidase; and
(b)the mutation or the deletion of B5R gene.
26. The modified oncolytic virus of any one of claims 12-22, comprising:
(a)the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2;
(b)the exogenous nucleic acid that codes for a hyaluronidase; and
(c)the mutation or the deletion of B5R gene.
27. The modified oncolytic virus of any one of claims 19-22, comprising:
(a)the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; and
(b)the mutation or the deletion of the further viral gene comprising at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, and a functional domain or fragment or variant thereof, or any combinations thereof.
28. The modified oncolytic virus of any one of claims 19-22, comprising:
(a)the exogenous nucleic acid that codes for a hyaluronidase; and
(b)the mutation or the deletion of the further viral gene comprising at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, and a functional domain or fragment or variant thereof, or any combinations thereof.
29. The modified oncolytic virus of claims 22, comprising:
(a)the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2;
(b)the exogenous nucleic acid that codes for a hyaluronidase; and
(c)the mutation or the deletion of the further viral gene comprising at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, or N1L, a functional domain thereof, or any combinations thereof.
30. The modified oncolytic virus of any one of claims 19-22, comprising:
(a)the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2;
(b)the mutation or the deletion of B5R gene; and
(c)the mutation or the deletion of the further viral gene comprising at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, and a functional domain or fragment or variant thereof, or any combinations thereof.
31. The modified oncolytic virus of any one of claims 19-22, comprising:
(a)the exogenous nucleic acid that codes for a hyaluronidase;

(b)the mutation or the deletion of B5R gene; and

(c)the mutation or the deletion of the further viral gene comprising at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, or N1L, a functional domain thereof, or any combinations thereof.

32. The modified oncolytic virus of any one of claims 19-22, comprising:

(a)the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2 ;

(b)the exogenous nucleic acid that codes for a hyaluronidase ;

(c)the mutation or the deletion of B5R gene; and

(d)the mutation or the deletion of the further viral gene comprising at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, and a functional domain or fragment or variant thereof, or any combinations thereof.

33. The modified oncolytic virus of claim 22, comprising:

(a)the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; and

(b)the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, and a functional domain or fragment or variant thereof, or any combinations thereof.

34. The modified oncolytic virus of claim 22, comprising:

(a)the exogenous nucleic acid that codes for a hyaluronidase; and

(b)the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, and a functional domain or fragment or variant thereof, or any combinations thereof.

35. The modified oncolytic virus of claim 22, comprising:

(a)the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2;

(b)the exogenous nucleic acid that codes for a hyaluronidase; and

(c)the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, and a functional domain or fragment or variant thereof, or any combinations thereof.

36. The modified oncolytic virus of claim 22, comprising:

(a)the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2;

(b)the mutation or the deletion of B5R gene; and

(c)the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, and a functional domain or fragment or variant thereof, or any combinations thereof.

37. The modified oncolytic virus of claim 22, comprising:
(a)the exogenous nucleic acid that codes for a hyaluronidase;
(b)the mutation or the deletion of B5R gene; and
(c)the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, and a functional domain or fragment or variant thereof, or any combinations thereof.
38. The modified oncolytic virus of claim 22, comprising:
(a)the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2;
(b)the exogenous nucleic acid that codes for a hyaluronidase;
(c)the mutation or the deletion of B5R gene; and
(d)the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, a functional domain or fragment or variant thereof, or any combinations thereof.
39. The modified oncolytic virus of claim 22, comprising:
(a)the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2;
(b)the exogenous nucleic acid that codes for a hyaluronidase;
(c)the mutation or the deletion of B5R gene;
(d)the mutation or the deletion of the further viral gene comprising at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, or N1L, and a functional domain or fragment or variant thereof, or any combinations thereof; and
(e)the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, a functional domain or fragment or variant thereof, or any combinations thereof.
40. The modified oncolytic virus of any one of claims 23-39, wherein the hyaluronidase comprises PH-20.
41. The modified oncolytic virus of any one of claims 23-39, wherein the hyaluronidase comprises HysA.
42. The modified oncolytic virus of any one of claims 23-41, comprising the mutation or deletion of A52R.
43. The modified oncolytic virus of any one of claims 1-42, further comprising an exogenous nucleic acid that codes for a viral VH1 protein.

44. The modified oncolytic virus claim 43, comprising the exogenous nucleic acid coding for the viral VH1 protein, wherein the exogenous nucleic acid is from a genome of a poxvirus, wherein the poxvirus is not vaccinia virus.

45. The modified oncolytic virus of claim 44, wherein the poxvirus comprises a measles virus, a poliovirus, a poxvirus, a vaccinia virus, an adenovirus, an adeno associated virus, a herpes simplex virus, a vesicular stomatitis virus, a reovirus, a Newcastle disease virus, a senecavirus, a lentivirus, a mengovirus, or a myxomavir.

46. The modified oncolytic virus of any one of claims 1-45, wherein the viral genome comprises a thymidine kinase gene.

47. The modified oncolytic virus of any one of claims 1-45, wherein a thymidine kinase gene is deleted from the viral genome.

48. The modified oncolytic virus of any one of claims 1-47, wherein the viral genome comprises a thymidine kinase gene from a herpes simplex virus.

49. The modified oncolytic virus of any one of claims 1-48, wherein the virus exhibits enhanced intratumoral and intertumoral spreading, enhanced immune evasion, enhanced tumor-specific replication, enhanced tumor-targeted delivery, compared to an otherwise identical oncolytic virus that does not comprise the modifications of any one of claims 1-48.

50. The modified oncolytic virus of any one of claims 1-49, wherein the oncolytic virus comprises a poxvirus, an adeno associated virus, an adenovirus, a reovirus, a lentivirus, a herpes simplex virus, a vesicular stomatitis virus, a mengovirus, or a myxomavir.

51. The modified oncolytic virus of claim 50, wherein the oncolytic virus is the poxvirus.

52. The modified oncolytic virus of claim 50, wherein the poxvirus is a vaccinia virus.

53. An oncolytic vaccinia virus comprising at least two of the following:

(a) a modification that enhances intratumoral and intertumoral spreading of the virus;

(b) a modification that enhances systemic delivery of the virus;

(c) a modification that enhances tumor-specific replication of the virus; and

(d) a modification that enhances immune evasion of the virus.

54. An oncolytic vaccinia virus comprising an exogenous nucleic acid that codes for a protein, or a fragment thereof, that enhances degradation of an extracellular matrix (ECM) of a tumor.

55. The oncolytic vaccinia virus of claim 54, wherein the protein or the fragment thereof that enhances degradation of the ECM is a hyaluronidase.

56. The oncolytic vaccinia virus of claim 55, wherein the hyaluronidase comprises a membrane associated hyaluronidase or a microbial hyaluronidase, and wherein the membrane associated hyaluronidase is PH-20.

57. The oncolytic vaccinia virus of claim 56, wherein the PH-20 is GPI-anchored.

58. The oncolytic vaccinia virus of claim 55, wherein the hyaluronidase comprises a microbial hyaluronidase, and wherein the microbial hyaluronidase is HysA.

59. The oncolytic vaccinia virus of any one of claims 54-58, further comprising at least one of the following:

(a) a modification that enhances intratumoral and intertumoral spreading of the virus;

(b) a modification that enhances systemic delivery of the virus;

(c) a modification that enhances tumor-specific replication of the virus; and

(d) a modification that enhances immune evasion of the virus.

60. An oncolytic vaccinia virus comprising an exogenous nucleic acid that codes for a chemokine receptor, wherein expression of the chemokine receptor from the virus enhances systemic delivery of the virus.

61. The oncolytic vaccinia virus of claim 60, wherein the chemokine receptor comprises at least one of CXCR4 and CCR2.

62. The oncolytic vaccinia virus of claim 60 or 61, comprising the exogenous nucleic acid that codes for the chemokine receptor, further comprising at least one of the following:

(a) a modification that enhances degradation of an ECM of a tumor; and

(b) a modification that enhances production of EEV form of the virus.

63. An oncolytic vaccinia virus, comprising a first modification in the genome of the virus that enhances production of EEV form of the virus, and at least one of the following further modifications:

(a) a modification that enhances intratumoral and intertumoral spreading of the virus;

(b) a modification that enhances systemic delivery of the virus;

(c) a modification that enhances tumor-specific replication of the virus; and

(d) a modification that enhances immune evasion of the virus.

64. The oncolytic vaccinia virus of claim 63, wherein the first modification comprises a mutation or a deletion of the B5R gene, wherein the deletion comprises a complete or partial deletion, wherein the mutation or a deletion in a SCR region of the B5R gene, wherein said SCR region comprises SCR1, SCR3, SCR4, or any combinations thereof, and wherein the SCR region does not comprise SCR2.

65. The oncolytic vaccinia virus of any one of claims 53-64, comprising a mutation or a deletion of a further viral gene, wherein the further viral gene comprises at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, and A52R, and a functional domain or fragment or variant thereof, or any combinations thereof.

66. The oncolytic vaccinia virus of any one of claims 53-65, further comprising a nucleic acid sequence that codes for a protein or a fragment thereof that: modulates NFκB signaling, promotes reduction of interstitial fluid pressure (IFP), modulates STAT3-mediated gene activation, promotes T cell activation, promotes attraction of NK cells to virus-infected cells, modulates metabolic program of virus-infected cells, modulates fatty acid uptake by virus-infected cells, promotes therapeutic targeting of MDSCs, or any combinations thereof.

67. The oncolytic vaccinia virus of claim 66, wherein the at least one additional exogenous nucleic acid comprises a nucleic acid coding for at least one of HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, and a functional domain or fragment or variant thereof, or any combinations thereof.

68. The oncolytic vaccinia virus of any one of claims 53-67, comprising:
(a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; and
(b) the exogenous nucleic acid that codes for a hyaluronidase.

69. The oncolytic vaccinia virus of any one of claims 53-67, comprising:
(a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; and
(b) the mutation or the deletion of B5R gene.

70. The oncolytic vaccinia virus of any one of claims 53-67, comprising:
(a) the exogenous nucleic acid that codes for a hyaluronidase; and
(b) the mutation or the deletion of B5R gene.

71. The oncolytic vaccinia virus of any one of claims 53-67, comprising:
(a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2;
(b) the exogenous nucleic acid that codes for a hyaluronidase; and
(c) the mutation or the deletion of B5R gene.

72. The oncolytic vaccinia virus of any one of claims 53-67, comprising:
(a) the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; and
(b) the mutation or the deletion of the further viral gene comprising at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L,

K7R, N1L, A52R, and a functional domain or fragment or variant thereof, or any combinations thereof.

73. The oncolytic vaccinia virus of any one of claims 53-67, comprising:
(a)the exogenous nucleic acid that codes for a hyaluronidase; and
(b)the mutation or the deletion of the further viral gene comprising at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, A52R, and a functional domain or fragment or variant thereof, or any combinations thereof.

74. The oncolytic vaccinia virus of any one of claims 53-67, comprising:
(a)the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2;
(b)the exogenous nucleic acid that codes for a hyaluronidase; and
(c)the mutation or the deletion of the further viral gene comprising at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, A52R, and a functional domain or fragment or variant thereof, or any combinations thereof.

75. The oncolytic vaccinia virus of any one of claims 53-67, comprising:
(a)the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2;
(b)the mutation or the deletion of B5R gene; and
(c)the mutation or the deletion of the further viral gene comprising at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, A52R, and a functional domain or fragment or variant thereof, or any combinations thereof.

76. The oncolytic vaccinia virus of any one of claims 53-67, comprising:
(a)the exogenous nucleic acid that codes for a hyaluronidase;
(b)the mutation or the deletion of B5R gene; and
(c)the mutation or the deletion of the further viral gene comprising at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, A52R, and a functional domain or fragment or variant thereof, or any combinations thereof.

77. The oncolytic vaccinia virus of any one of claims 53-67, comprising:
(a)the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2 ;
(b)the exogenous nucleic acid that codes for a hyaluronidase ;
(c)the mutation or the deletion of B5R gene; and

(d)the mutation or the deletion of the further viral gene comprising at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, A52R, and a functional domain or fragment or variant thereof, or any combinations thereof.

78. The oncolytic vaccinia virus of any one of claims 53-67, comprising:
(a)the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2; and
(b)the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-R, LIGHT, ITAC, fractalkine, CCL5, and a functional domain or fragment or variant thereof, or any combinations thereof.
79. The oncolytic vaccinia virus of any one of claims 53-67, comprising:
(a)the exogenous nucleic acid that codes for a hyaluronidase; and
(b)the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, and a functional fragment or domain or variant thereof, or any combinations thereof.
80. The oncolytic vaccinia virus of any one of claims 53-67, comprising:
(a)the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2;
(b)the exogenous nucleic acid that codes for a hyaluronidase; and
(c)the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, a functional domain or fragment or variant thereof, or any combinations thereof.
81. The oncolytic vaccinia virus of any one of claims 53-67, comprising:
(a)the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2;
(b)the mutation or the deletion of B5R gene; and
(c)the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, and a functional domain or fragment or variant thereof, or any combinations thereof.
82. The oncolytic vaccinia virus of any one of claims 53-67, comprising:
(a)the exogenous nucleic acid that codes for a hyaluronidase;
(b)the mutation or the deletion of B5R gene; and
(c)the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, a functional domain or fragment or variant thereof, or any combinations thereof.
83. The oncolytic vaccinia virus of any one of claims 53-67, comprising:
(a)the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2;

(b)the exogenous nucleic acid that codes for a hyaluronidase;

(c)the mutation or the deletion of B5R gene; and

(d)the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, Fractalkine, CCL5, and a functional domain or fragment or variant thereof, or any combinations thereof.

84. The oncolytic vaccinia virus of any one of claims 53-67, comprising:

(a)the exogenous nucleic acid that codes for at least one of CXCR4 and CCR2;

(b)the exogenous nucleic acid that codes for a hyaluronidase;

(c)the mutation or the deletion of B5R gene;

(d)the mutation or the deletion of the further viral gene comprising at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, A52R, and a functional domain or fragment or variant thereof, or any combinations thereof; and

(e)the at least one additional exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, and a functional domain or fragment or variant thereof, or any combinations thereof.

85. An oncolytic vaccinia virus, comprising an exogenous nucleic acid that codes for a chemokine receptor, a protein that is capable of degrading hyaluronan, or any combinations thereof.

86. The modified oncolytic vaccinia virus of claim 85, comprising the protein that is capable of degrading hyaluronan, wherein the protein comprises PH-20 or HysA.

87. A modified oncolytic virus comprising a mutation or a deletion of A52R gene.

88. The modified oncolytic virus of claim 87, comprising the deletion of A52R gene.

89. The modified oncolytic virus of claim 87 or 88, further comprising an exogenous nucleic acid that codes for a chemokine receptor, a protein that is capable of degrading hyaluronan, or any combinations thereof.

90. The modified oncolytic virus of claim 89, comprising the exogenous nucleic acid that codes for the chemokine receptor, wherein the chemokine receptor comprises CXCR4 or CCR2.

91. The modified oncolytic virus of claim 89 or 90 comprising the exogenous nucleic acid that codes for the protein that is capable of degrading hyaluronan, wherein the protein comprises a membrane associated hyaluronidase or a secreted hyaluronidase.

92. The modified oncolytic virus of claim 91, comprising the exogenous nucleic acid that codes for the membrane associated hyaluronidase, wherein the membrane associated hyaluronidase is PH-20.

93. The modified oncolytic virus of claim 92, wherein the PH-20 is GPI-anchored.

94. The modified oncolytic virus of claim 91, comprising the exogenous nucleic acid that codes for the secreted hyaluronidase, wherein the secreted hyaluronidase is HysA.

95. A modified oncolytic virus comprising a mutation or a deletion of K7R gene.

96. The modified oncolytic virus of claim 95, comprising the deletion of the K7R gene.

97. The modified oncolytic virus of claim 95 or 96, further comprising at least one of an exogenous nucleic acid that codes for a cytokine, an exogenous nucleic acid that codes for a cytokine receptor, and an exogenous nucleic acid that codes for a chemokine.

98. The modified oncolytic virus of claim 97, comprising the exogenous nucleic acid that codes for the cytokine, wherein the cytokine comprises IL15.

99. The modified oncolytic virus of claim 97 or 98, comprising the exogenous nucleic acid that codes for the cytokine receptor, wherein the cytokine receptor comprises IL15-Rα.

100. The modified oncolytic virus of any one of claims 97-99, comprising the exogenous nucleic acid that codes for the chemokine, wherein the chemokine comprises CCL5.

101. The modified oncolytic virus of any one of claims 97-100, further comprising an exogenous nucleic acid that codes for at least one of HMGB1, PIAS3, LIGHT, ITAC, a fractalkine, and a functional domain or fragment or variant thereof, or any combinations thereof.

102. The modified oncolytic virus of any one of claims 97-101, further comprising a mutation or a deletion of a further viral gene comprising at least one of F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, N1L, A52R, and a functional domain or fragment or a variant thereof, or any combinations thereof.

103. A modified oncolytic virus comprising an exogenous nucleic acid that codes for IL15 and IL15-Rα.

104. A modified oncolytic virus comprising an exogenous nucleic acid that codes for IL15 and CCL5.

105. A modified oncolytic virus comprising an exogenous nucleic acid that codes for at least one of: IL15, IL15-Rα, ITAC, fractalkine, and a functional domain or fragment or variant thereof, or any combinations thereof.

106. The modified oncolytic virus of any one of claims 95-105, wherein the virus exhibits enhanced activation and attraction of natural killer cells.

107. The modified oncolytic virus of any one of claims 1-52 and 87-106, wherein the virus exhibits at least one of: enhanced intratumoral and intertumoral spreading, enhanced immune evasion, enhanced tumor-specific replication, enhanced tumor-targeted delivery, compared to an otherwise identical vaccinia virus that does not comprise the modifications of any one of claims 1-52 and 87-106.

108. The modified oncolytic virus of any one of claims 1-52 and 87-107, wherein the oncolytic virus comprises a poxvirus, an adeno associated virus, an adenovirus, a reovirus, a lentivirus, a herpes simplex virus, a vesicular stomatitis virus, a mengovirus, or a myxomavir.

109. The modified oncolytic virus of claim108, wherein the oncolytic virus is the poxvirus.

110. The modified oncolytic virus of claim 109, wherein the poxvirus is a vaccinia virus.

111. The modified oncolytic virus of any one of claims 108-110, wherein the virus exhibits at least one of: enhanced intratumoral and intertumoral spreading, enhanced immune evasion, enhanced tumor-specific replication, enhanced tumor-targeted delivery, compared to an otherwise identical vaccinia virus that does not comprise the modifications of any one of claims 108-110.

112. The modified oncolytic virus of any one of claims 1-52 and 87-111, wherein the virus is suitable for systemic delivery.

113. The oncolytic vaccinia virus of any one of claims 53-84, wherein the virus exhibits at least one of: enhanced intratumoral and intertumoral spreading, enhanced immune evasion, enhanced tumor-specific replication, enhanced tumor-targeted delivery, compared to an otherwise identical vaccinia virus that does not comprise the modifications of any one of claims 53-86.

114. The oncolytic vaccinia virus of any one of claims 53-85, wherein the virus is suitable for systemic delivery.

115. The oncolytic vaccinia virus of any one of claims 53-85, wherein the virus is capable of immune evasion.

116. The oncolytic vaccinia virus of claim 114 or 115, wherein the systemic delivery comprises oral administration, parenteral administration, intranasal administration, sublingual administration, rectal administration, transdermal administration, or any combinations thereof.

117. The oncolytic vaccinia virus of claim 116, wherein the parenteral administration comprises an intravenous injection.

118. The oncolytic vaccinia virus of any one of claims 53-85, wherein the virus is suitable for intratumoral delivery.

119. The oncolytic vaccinia virus of any one of claims 53-85 and 113-118, further comprising an exogenous nucleic acid that codes for a viral VH1 protein.

120. The oncolytic vaccinia virus of any one of claims 119, comprising the exogenous nucleic acid coding for the viral VH1 protein, wherein the exogenous nucleic acid is from a genome of a poxvirus, wherein the poxvirus is not vaccinia virus.

121. The oncolytic vaccinia virus of claim 120, wherein the poxvirus comprises a betaentomopoxvirus, a yatapoxvirus, a cervidpoxvirus, a gammaentomopoxvirus, a leporipoxvirus, a suipoxvirus, a molluscipoxvirus, a crocodylidpoxvirus, a alphaentomopoxvirus, a capripoxvirus, a avipoxvirus, a parapoxvirus.

122. The oncolytic vaccinia virus of any one of claims 53-85 and 113-121, wherein the viral genome comprises a thymidine kinase gene.

123. The oncolytic vaccinia virus of claim 122, wherein a thymidine kinase gene is deleted from the viral genome.

124. The oncolytic vaccinia virus of any one of claims 53-85 and 113-123, further comprising a thymidine kinase gene from a herpes simplex virus.

125. A pharmaceutical composition comprising a modified oncolytic virus as defined in any one of claims 1-52 and 87-112 or an oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124.

126. The pharmaceutical composition of claim 125, further comprising a solubilizing agent and an excipient.

127. The pharmaceutical composition of claim 126, wherein the excipient comprises one or more of a buffering agent, a stabilizer, an antioxidant, a binder, a diluent, a dispersing agent, a rate controlling agent, a lubricant, a glidant, a disintegrant, a plasticizer, a preservative, or any combinations thereof.

128. The pharmaceutical composition of claim 127, wherein the excipient comprises di-sodium hydrogen phosphate dihydrate, sodium dihydrogen phosphate dihydrate, sodium chloride, myo-inositol, sorbitol, or any combinations thereof.

129. The pharmaceutical composition of any one of claims 125-128, that does not comprise a preservative.

130. The pharmaceutical composition of any one of claims 125-129, further comprising one or more of a preservative, a diluent, and a carrier.

131. The pharmaceutical composition of any one of claims 125-130, further comprising an additional active ingredient or a salt thereof.

132. The pharmaceutical composition of any one of claims 125-131, wherein the solubilizing agent is sterile water.

133. The pharmaceutical composition of any one of claims 125-133, comprising an additional active ingredient, wherein the additional active ingredient is a further oncolytic virus.

134. A method of enhancing therapeutic effect of an oncolytic virus upon systemic delivery of the virus to a subject, comprising a systemic administration of a modified oncolytic virus as defined in any one of claims 1-52 and 87-112, an oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition as defined in any one of claims 125-133.

135. The method of claim 134, wherein the systemic administration comprises oral administration, parenteral administration, intranasal administration, sublingual administration, rectal administration, transdermal administration, or any combinations thereof.

136. The method of claim 135, wherein the parenteral administration comprises intravenous injection.

137. A process for engineering a modified oncolytic virus comprising: (i) obtaining a modified oncolytic virus DNA backbone vector, the modified oncolytic virus DNA backbone vector comprising one or more modifications as defined in any one of claims 1-52 and 87-112; (ii) further modifying the modified oncolytic virus DNA vector to produce an engineered DNA vector; (iii) transfecting mammalian cells with the engineered DNA vector; (iv) culturing the mammalian cells under conditions suitable for viral replication; and (v) harvesting the viral particles.

138. A process for engineering an oncolytic vaccinia virus comprising: (i) obtaining an oncolytic vaccinia virus DNA backbone vector, the oncolytic vaccinia virus DNA backbone vector comprising one or more modifications as defined in any one of claims 53-86 and 113-124; (ii) further modifying the oncolytic vaccinia virus DNA vector to produce an engineered DNA vector; (iii) transfecting mammalian cells with the engineered DNA vector; (iv) culturing the mammalian cells under conditions suitable for viral replication; and (v) harvesting the viral particles.

139. A process for producing a modified oncolytic virus as defined in any one of claims 1-52 and 87-112, comprising: (i) generating a modified oncolytic virus DNA vector, the

modified oncolytic virus DNA vector comprising the modifications according to any one of claims 1-52 and 87-112; (ii) transfecting mammalian cells with the modified oncolytic virus DNA vector; (iii) culturing the mammalian cells under conditions suitable for viral replication; and (iv) harvesting the viral particles.

140. A process for producing an oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, comprising: (i) generating an oncolytic vaccinia virus DNA vector, the oncolytic vaccinia virus DNA vector comprising the modifications according to any one of claims 53-86 and 113-124; (ii) transfecting mammalian cells with the oncolytic vaccinia virus DNA vector; (iii) culturing the mammalian cells under conditions suitable for viral replication; and (iv) harvesting the viral particles.

141. The process of any one of claims 137-140, wherein the mammalian cells comprise HeLa cells, 293 cells, or Vero cells.

142. A kit, comprising: a modified oncolytic virus according to any one of claims 1-52 and 87-112, an oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition as defined in any one of claims 125-133, and instruction for administering the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition; and instructions for administering said pharmaceutical composition to a subject to treat a disorder associated with pathological angiogenesis.

143. A kit, comprising: a modified oncolytic virus according to any one of claims 1-52 and 87-112, an oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition as defined in any one of claims 125-133, and instruction for administering the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition; a container; and instructions for administering said pharmaceutical composition to a subject to treat a disorder associated with pathological angiogenesis.

144. A kit for treating a cancer, comprising: a modified oncolytic virus according to any one of claims 1-52 and 87-112, an oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition as defined in any one of claims 125-133, and instruction for administering the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition; and instructions for administering said pharmaceutical composition to a subject to treat a disorder associated with pathological angiogenesis.

145. A kit for treating a cancer , comprising: a modified oncolytic virus according to any one of claims 1-52 and 87-112, an oncolytic vaccinia virus as defined in any one of claims

53-86 and 113-124, or a pharmaceutical composition as defined in any one of claims 125-133, and instruction for administering the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition; a container; and instructions for administering said pharmaceutical composition to a subject to treat a disorder associated with pathological angiogenesis.

146. The kit of any one of claims 142-145, wherein the cancer is a solid tumor, a leukemia, or a lymphoma.

147. A kit for treating a tumor, comprising: a modified oncolytic virus according to any one of claims 1-52 and 87-112, an oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition as defined in any one of claims 125-133, and instruction for administering the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition; and instructions for administering said pharmaceutical composition to a subject to treat a disorder associated with pathological angiogenesis.

148. A kit for treating a tumor, comprising: a modified oncolytic virus according to any one of claims 1-52 and 87-112, an oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition as defined in any one of claims 125-133, and instruction for administering the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition; a container; and instructions for administering said pharmaceutical composition to a subject to treat a disorder associated with pathological angiogenesis.

149. The kit of claim 147 or 148, wherein the tumor is a solid tumor, a leukemia, or a lymphoma.

150. The kit of any one of claims 142-149, wherein the subject is in need of the treatment.

151. The kit of any one of claims 142-150, wherein the subject is human.

152. The kit of any one of claims 142-151, wherein the instructions for administering comprise instructions for a systemic administration.

153. The kit of claim 152, wherein the systemic administration comprises oral administration, parenteral administration, intranasal administration, sublingual administration, rectal administration, transdermal administration, or any combinations thereof.

154. The kit of claim 153, wherein the parenteral administration comprises intravenous injection.

155. A method of treating a tumor, the method comprising administering to a subject a therapeutically effective amount of a modified oncolytic virus according to any one of claims 1-52 and 87-112, an oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition as defined in any one of claims 125-133.

156. The method of claim 155, comprising administering to a subject a therapeutically effective amount of a modified oncolytic virus according to any one of claims 1-52 and 87-112, an oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition as defined in claim 125-133, wherein the tumor is a solid tumor, a leukemia, or a lymphoma.

157. A method of treating a cancer, comprising administering to a subject a therapeutically effective amount of a modified oncolytic virus according to any one of claims 1-52 and 87-112, an oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition as defined in any one of claims 125-133.

158. The method of claim 157, comprising administering to a subject a therapeutically effective amount of a modified oncolytic virus according to any one of claims 1-52 and 87-112, an oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition as defined in any one of claims 125-133, wherein the cancer is a solid tumor, a leukemia, or a lymphoma.

159. The method of any one of claims 155-158, comprising administering to a subject a therapeutically effective amount of a modified oncolytic virus according to any one of claims 1-52 and 87-112, an oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition as defined in any one of claims 125-133, wherein the method further comprises administration of a further therapy.

160. The method of claim 159, wherein the further therapy comprises chemotherapy, radiation, oncolytic viral therapy with an additional virus, treatment with immunomodulatory proteins, a CAR T cellular therapy, an anti-cancer agent, or any combinations thereof.

161. The method of claim 159, wherein the further therapy comprises administration of an immunomodulatory agent comprising anti-CD33 antibody and variable region thereof, an anti-CD11b antibody and variable region thereof, a COX2 inhibitor, a cytokine, a chemokine, an anti-CTLA4 antibody or an antigen binding fragment thereof, an anti-PD-1 antibody or an antigen binding fragment thereof, an anti-PD-L1 antibody or an antigen binding fragment thereof, or a TLR agonist.

162. The method of claim 159, comprising administration of the further therapy, wherein the further therapy comprises administration of the anti-cancer agent, wherein the anti-cancer agent is a chemotherapeutic agent.

163. The method of claim 162, wherein the chemotherapeutic agent is a prodrug.

164. The method of claim 163, wherein upon administration of the prodrug in combination with the oncolytic vaccinia virus, the modified vaccinia virus, or the pharmaceutical composition comprising the same, the prodrug is converted to an active form.

165. The method of claim 164, wherein the prodrug comprises ganciclovir.

166. The method of any one of claims 159-165, comprising administration of the further therapy, wherein the further therapy is administered concurrently or sequentially.

167. The method of claim 166, comprising sequential administration of the further therapy, wherein the further therapy is administered prior to administering the modified oncolytic virus according to any one of claims 1-52 and 87-112, the oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or the pharmaceutical composition as defined in any one of claims 125-133.

168. The method of claim 167, comprising sequential administration of the further therapy, wherein the further therapy is administered after administering the modified oncolytic virus according to any one of claims 1-52 and 87-112, the oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or the pharmaceutical composition as defined in any one of claims 125-133.

169. A method of producing a toxic effect in a cancer cell, the method comprising administering to a cancer cell a therapeutically effective amount of a modified oncolytic virus according to any one of claims 1-52 and 87-112, an oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition as defined in any one of claims 125-133.

170. The method of claim 169, wherein the cancer cell is present in a subject.

171. The method of claim 170, wherein the subject is in need of the method that producing the toxic effect in the cancer cell.

172. A method of treating a subject, the method comprising producing a toxic effect in a cancer cell that is present in the subject by administering to the cancer cell a therapeutically effective amount of a modified oncolytic virus according to any one of claims 1-52 and 87-112, an oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition as defined in any one of claims 125-133.

173. The method of claim 172, wherein the subject is in need of the treatment.

174. A method of treating cancer in a subject, comprising, infecting a cancer cell of the subject by administration of a therapeutically effective amount of a modified oncolytic virus according to any one of claims 1-52 and 87-112, an oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition as defined in any one of claims 125-133, wherein the administration is a systemic administration.

175. The method of claim 174, wherein the systemic administration comprises oral administration, parenteral administration, intranasal administration, sublingual administration, rectal administration, transdermal administration, or any combinations thereof.

176. The method of claim 175, wherein the parenteral administration comprises intravenous injection.

177. A method of treating cancer in a subject, comprising administering cells infected with a modified oncolytic virus according to any one of claims 1-52 and 87-112or an oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124.

178. The method of any one of claims 155-177, wherein the cancer comprises melanoma, hepatocellular carcinoma, breast cancer, lung cancer, peritoneal cancer, prostate cancer, bladder cancer, ovarian cancer, leukemia, lymphoma, renal carcinoma, pancreatic cancer, epithelial carcinoma, gastric cancer, colon carcinoma, duodenal cancer, pancreatic adenocarcinoma, mesotheliorna, glioblastoma multiforrne, astrocytoma, multiple myeloma, prostate carcinoma, hepatocellular carcinoma, cholangiosarcoma, pancreatic adenocarcinoma, head and neck squamous cell carcinoma, colorectal cancer, intestinal-type gastric adenocarcinoma, cervical squamous-cell carcinoma, osteosarcoma, epithelial ovarian carcinoma, acute lymphoblastic lymphoma, myeloproliferative neoplasms, or sarcoma.

179. The method of any one of claims 155-178, wherein the cancer cell is present in an organ of the subject selected from the group consisting of: the bladder, blood, bone, bone marrow, brain, breast, colon, esophagus, gastrointestine, gum, head, kidney, liver, lung, nasopharynx, neck, ovary, prostate, skin, stomach, testis, tongue, or uterus.

180. The method of any one of claims 155-179, wherein the cancer is metastatic.

181. A method of treating a cancer in a subject comprising administering a modified oncolytic virus according to claim 48 or a pharmaceutical composition comprising the same in combination with a chemotherapeutic prodrug.

182. A method of treating a cancer in a subject comprising administering an oncolytic vaccinia virus according to claim 124 or a pharmaceutical composition comprising the same in combination with a chemotherapeutic prodrug.

183. A method of treating a cancer in a subject comprising, (i) administering a modified oncolytic virus according to claim 48 or a pharmaceutical composition comprising the same; (ii) assaying a viral titer in a first and a second biological sample isolated from the subject, wherein the first biological sample comprises a cancer cell and the second biological sample comprises a non-cancer cell; and (iii) administering a chemotherapeutic prodrug if the viral titer is equal to or higher in the second sample than the first sample, wherein administration of the chemotherapeutic prodrug results in inhibition of replication of the modified oncolytic virus in the subject.

184. A method of treating a cancer in a subject comprising, (i) administering an oncolytic vaccinia virus according to claim 124 or a pharmaceutical composition comprising the same; (ii) assaying a viral titer in a first and a second biological sample isolated from the subject, wherein the first biological sample comprises a cancer cell and the second biological sample comprises a non-cancer cell; and (iii) administering a chemotherapeutic prodrug if the viral titer is equal to or higher in the second sample than the first sample, wherein administration of the chemotherapeutic prodrug results in inhibition of replication of the oncolytic vaccinia virus in the subject.

185. The method of any one of claims 155-184, wherein the method increases efficacy of oncolytic virus based cancer therapy.

186. The method of any one of claims 155-185, comprising the administration of the modified oncolytic virus according to any one of claims 1-52 and 87-112, the oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or the pharmaceutical composition as defined in any one of claims 125-133, wherein the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition is administered at a dosage that comprises about 10^6 PFU/mL to about 10^{10} PFU/mL of the oncolytic vaccinia virus.

187. The method of any one of claims 155-186, comprising the administration of the modified oncolytic virus according to any one of claims 1-52 and 87-112, the oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition as defined in any one of claims 125-133, wherein the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition is administered at a dosage that comprises about 5×10^9 PFU/mL of the oncolytic vaccinia virus.

188. The method of any one of claims 155-187, comprising the administration of the modified oncolytic virus according to any one of claims 1-52 and 87-112, the oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition as defined in any one of claims 125-133, wherein the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition is administered, independently, in an initial dose for a first period of time, an intermediate dose for a second period of time, and a high dose for a third period of time.

189. The method of claim 188, comprising administration of the initial, the intermediate, and the high dose, independently, wherein the initial dose is lower than the intermediate dose and the intermediate dose is lower than the high dose.

190. The method of claim 188 or 189, wherein the first, second, and third periods of time are each from about 1 week to about 3 weeks.

191. The method of any one of claims 155-190, comprising administering the modified oncolytic virus according to any one of claims 1-52 and 87-112, the oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition as defined in any one of claims 125-133, wherein the modified oncolytic virus, the oncolytic vaccinia virus, and the pharmaceutical composition independently comprises a liquid dosage form that is administered at a volume of about 1 mL to about 5 mL, about 5 mL to 10 mL, about 15 mL to about 20 mL, about 25 mL to about 30 mL, about 30 mL to about 50 mL, about 50 mL to about 100 mL, about 100 mL to 150 mL, about 150 mL to about 200 mL, about 200 mL to about 250 mL, about 250 mL to about 300 mL, about 300 mL to about 350 mL, about 350 mL to about 400 mL, about 400 mL to about 450 mL, about 450 mL to 500 mL, about 500 mL to 750 mL, or about 750 mL to 1000 mL.

192. The method of any one of claims 155-191, comprising administering the modified oncolytic virus according to any one of claims 1-52 and 87-112, the oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or the pharmaceutical composition as defined in any one of claims 125-133, wherein the modified oncolytic virus, the oncolytic vaccinia virus, or a pharmaceutical composition is administered in a liquid dosage form, a solid dosage form, an inhalable dosage form, an intranasal dosage form, a liposomal formulation, a dosage form comprising nanoparticles, a dosage form comprising microparticles, a polymeric dosage form, or any combinations thereof .

193. The method of any one of claims 155-192, comprising administering the modified oncolytic virus according to any one of claims 1-52 and 87-112, the oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition

as defined in any one of claims 125-133, wherein the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition is administered for a duration of about 1 week, about 2 week, about 3 weeks, about 4 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 9 weeks, about 10, weeks, or about 12 weeks.

194. The method of any one of claims 155-193, comprising administering the modified oncolytic virus according to any one of claims 1-52 and 87-112, the oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition as defined in any one of claims 125-133, wherein the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition is administered once daily, twice daily, once every week, once every two weeks, or once every three weeks.

195. The method of any one of claims 155-194, comprising administering the modified oncolytic virus according to any one of claims 1-52 and 87-112, the oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition as defined in any one of claims 125-133, wherein the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition is administered intravenously, intraperitoneally, or by an intratumoral injection.

196. The method of any one of claims 155-195, comprising administering the modified oncolytic virus according to any one of claims 1-52 and 87-112, the oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition as defined in any one of claims 125-133, wherein the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition is administered as a bolus injection or a slow infusion.

197. The method of any one of claims 155-196, comprising administering the modified oncolytic virus according to any one of claims 1-52 and 87-112, the oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition as defined in any one of claims 125-133, wherein the administration of the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition results in a first peak viral load after about 1 hour to about 3 days and a second peak viral load after about 3 days to about 10 days from administration of a first dose.

198. The method of any one of claims 155-197, comprising administration of the further therapy, wherein the further therapy is administered for a duration of about 1 week, about 2 week, about 3 weeks, about 4 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 9 weeks, about 10, weeks, or about 12 weeks.

199. The method of any one of claims 155-198, comprising administration of the further therapy, wherein the further therapy is administered once daily, once every week, once every two weeks, or once every three weeks.

200. The method of any one of claims 155-199, comprising administration of the further therapy, wherein the further therapy is administered in a liquid dosage form, a solid dosage form, an inhalable dosage form, an intranasal dosage form, a liposomal formulation, a dosage form comprising nanoparticles, a dosage form comprising microparticles, a polymeric dosage form, or any combinations thereof.

201. The method of any one of claims 155-200, comprising administration of the further therapy, wherein the further therapy is administered orally, intravenously, by an intratumoral injection, or by radiation.

202. The method of any one of claims 155-201, comprising the administration of the modified oncolytic virus according to any one of claims 1-52 and 87-112, the oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition as defined in any one of claims 125-133to a subject in need thereof, wherein the subject is human.

203. The method of any one of claims 155-202, comprising the administration of the modified oncolytic virus according to any one of claims 1-52 and 87-112, the oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition as defined in any one of claims 125-133 to the subject in need thereof, wherein prior to administration of the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition the subject has been diagnosed with a cancer.

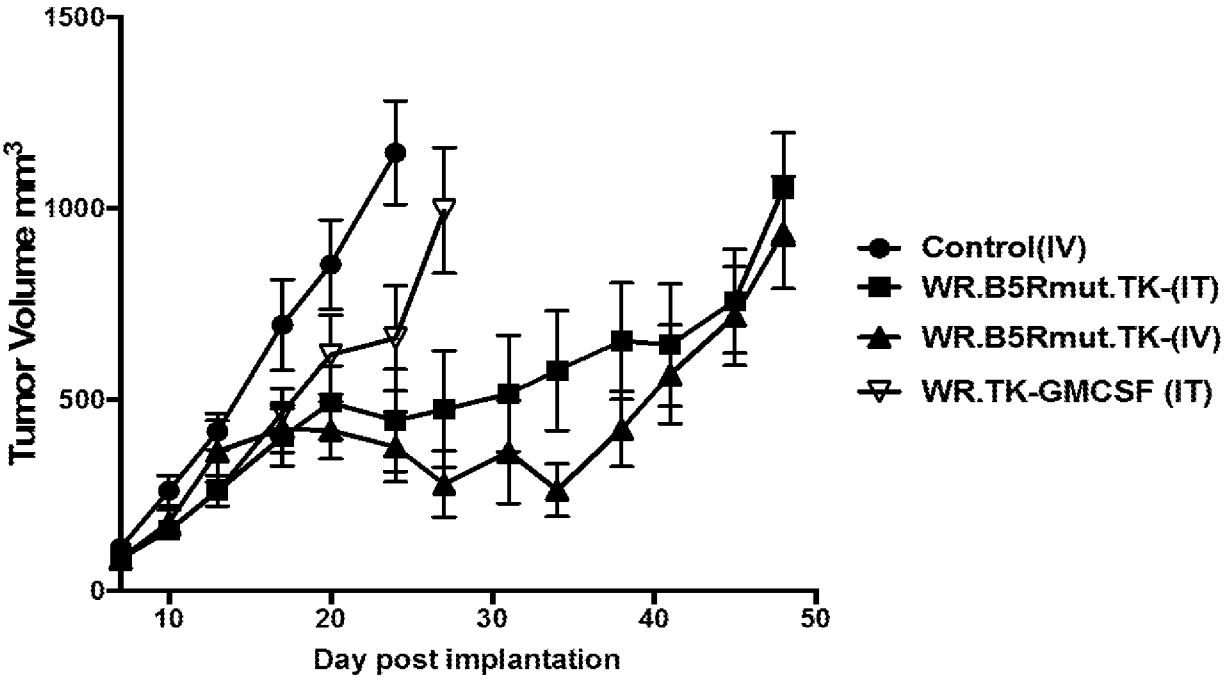
204. The method of any one of claims 155-203, comprising the administration of the modified oncolytic virus according to any one of claims 1-52 and 87-112, the oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition as defined in any one of claims 125-133 to the subject in need thereof in combination with the further therapy, wherein prior to administration of the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition or the further therapy the subject has been diagnosed with a cancer.

205. The method of any one of claims 155-202, comprising the administration of the modified oncolytic virus according to any one of claims 1-52 and 87-112, the oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition as defined in any one of claims 125-133 to the subject in need thereof, wherein

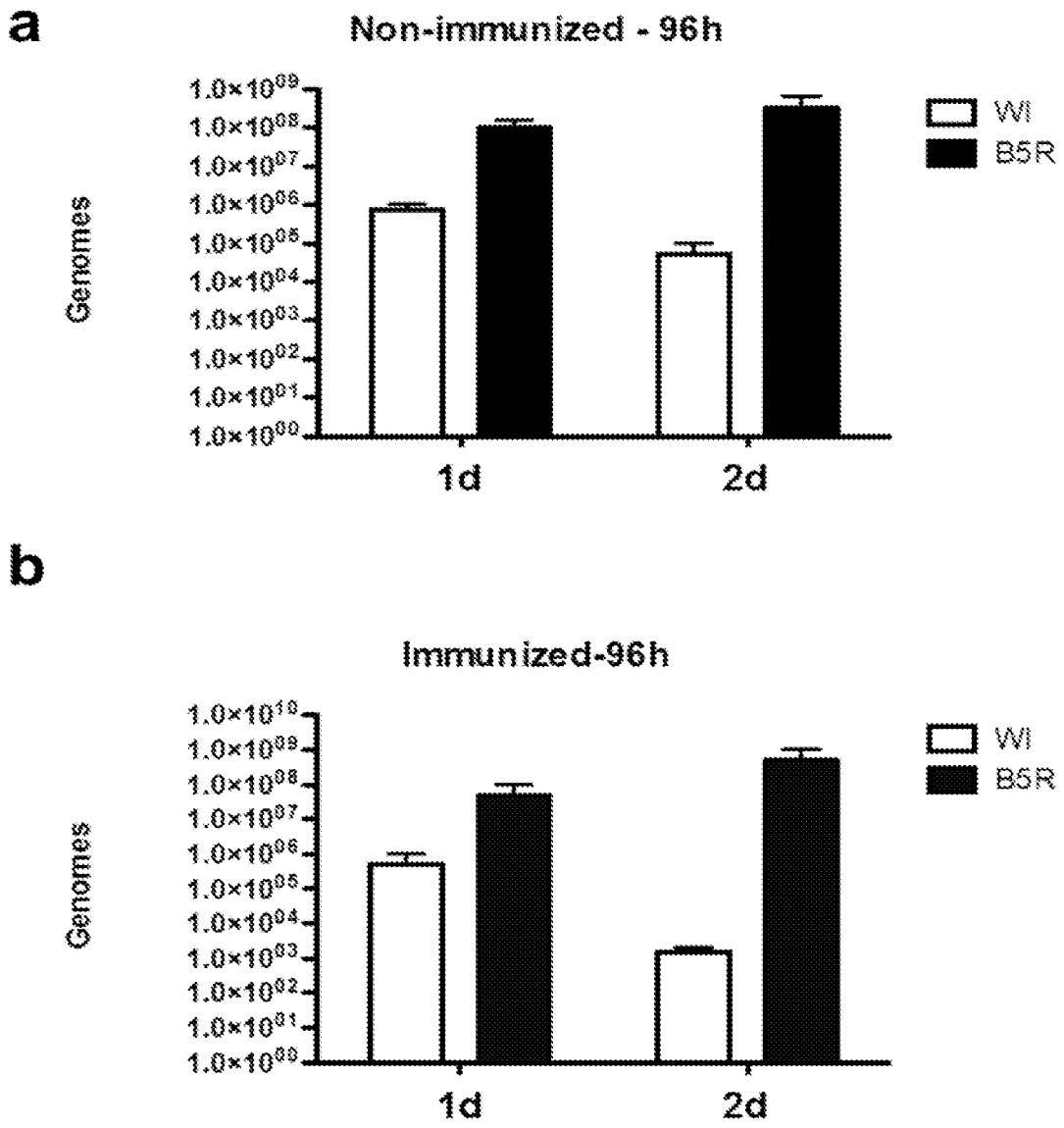
prior to administration of the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition the subject has been diagnosed with a tumor.

206. The method of any one of claims 155-202 and 205, comprising the administration of the modified oncolytic virus according to any one of claims 1-52 and 87-112, the oncolytic vaccinia virus as defined in any one of claims 53-86 and 113-124, or a pharmaceutical composition as defined in any one of claims 125-133 to the subject in need thereof in combination with the further therapy, wherein prior to administration of the modified oncolytic virus, the oncolytic vaccinia virus, or the pharmaceutical composition or the further therapy the subject has been diagnosed with a cancer.

FIG. 1


1500
1000
500
0
Tumor Volume mm3
10
20
30
40
50
Day post implantation
Control(IV)
WR.B5Rmut.TK-(IT)
WR.B5Rmut.TK-(IV)
WR.TK-GMCSF (IT)

FIGS. 2A-2B


a
Non-immunized - 96h
Genomes
1.0×10^09
1.0×10^08
1.0×10^07
1.0×10^06
1.0×10^05
1.0×10^04
1.0×10^03
1.0×10^02
1.0×10^01
1.0×10^00
1d
2d
WI
B5R
b
Immunized-96h
Genomes
1.0×10^10
1.0×10^09
1.0×10^08
1.0×10^07
1.0×10^06
1.0×10^05
1.0×10^04
1.0×10^03
1.0×10^02
1.0×10^01
1.0×10^00
1d
2d
WI
B5R

FIGS. 3A-3B

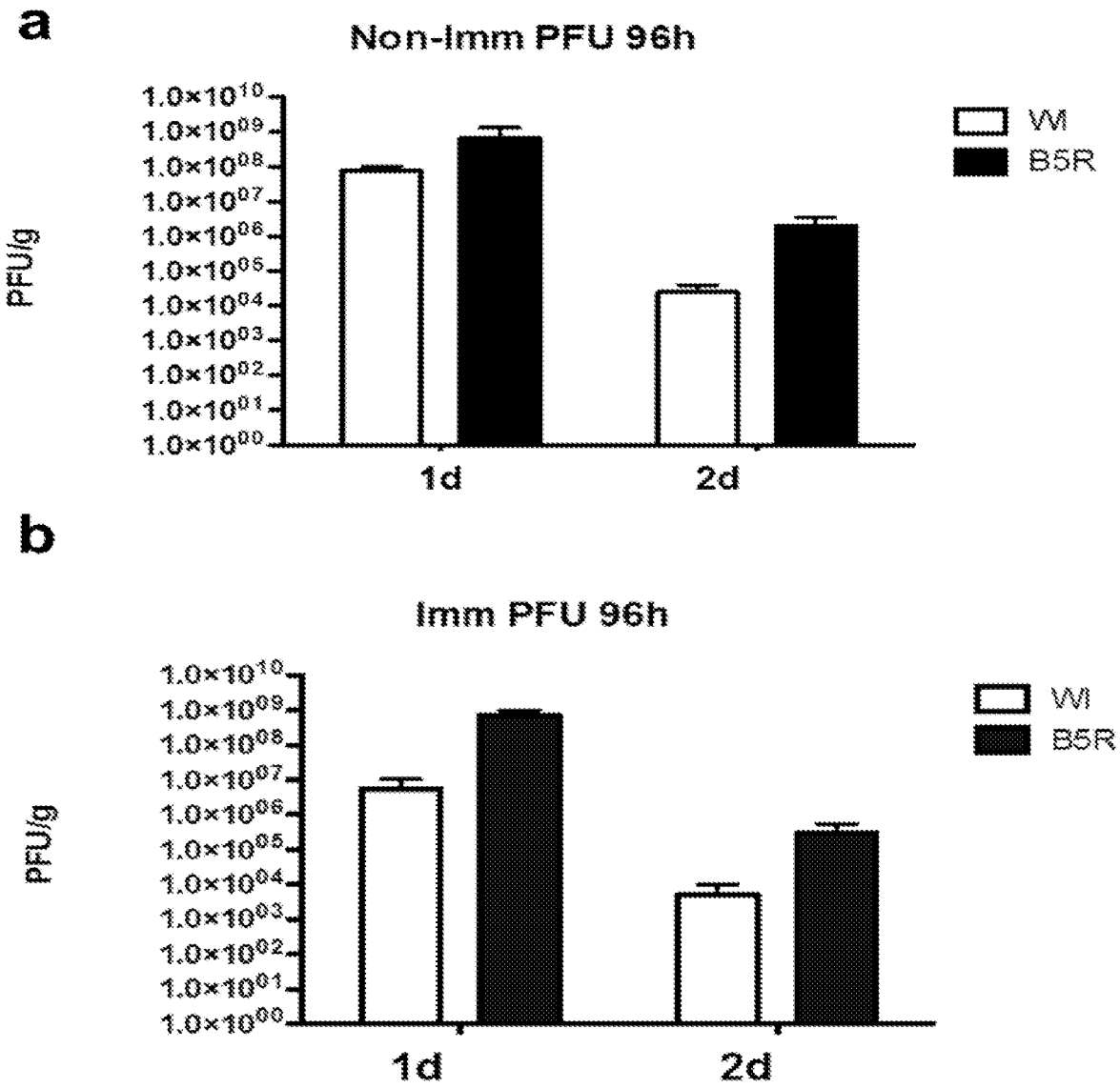

a
Non-Imm PFU 96h
PFU/g
1.0×10^10
1.0×10^09
1.0×10^08
1.0×10^07
1.0×10^06
1.0×10^05
1.0×10^04
1.0×10^03
1.0×10^02
1.0×10^01
1.0×10^00
1d
2d
WI
B5R
b
Imm PFU 96h
PFU/g
1.0×10^10
1.0×10^09
1.0×10^08
1.0×10^07
1.0×10^06
1.0×10^05
1.0×10^04
1.0×10^03
1.0×10^02
1.0×10^01
1.0×10^00
1d
2d
WI
B5R

FIG. 4

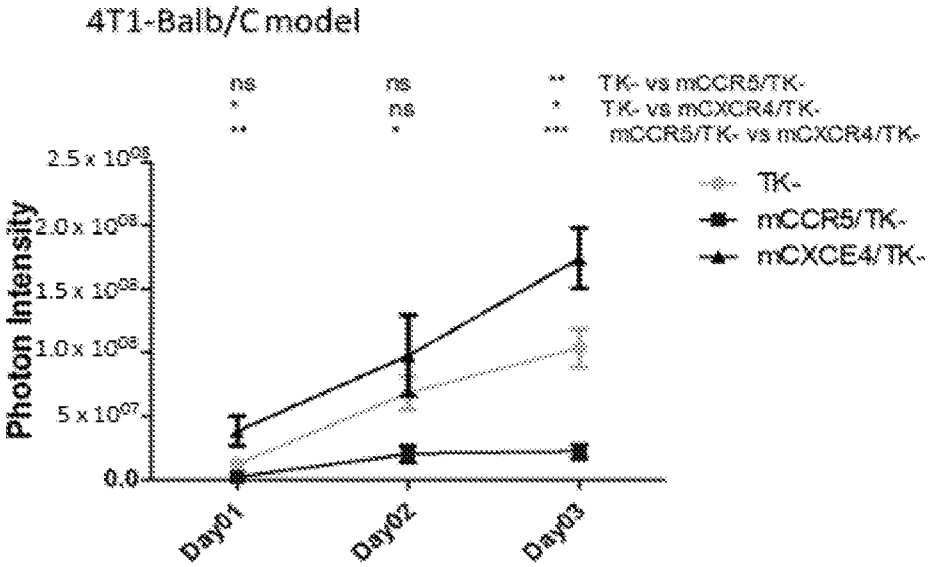
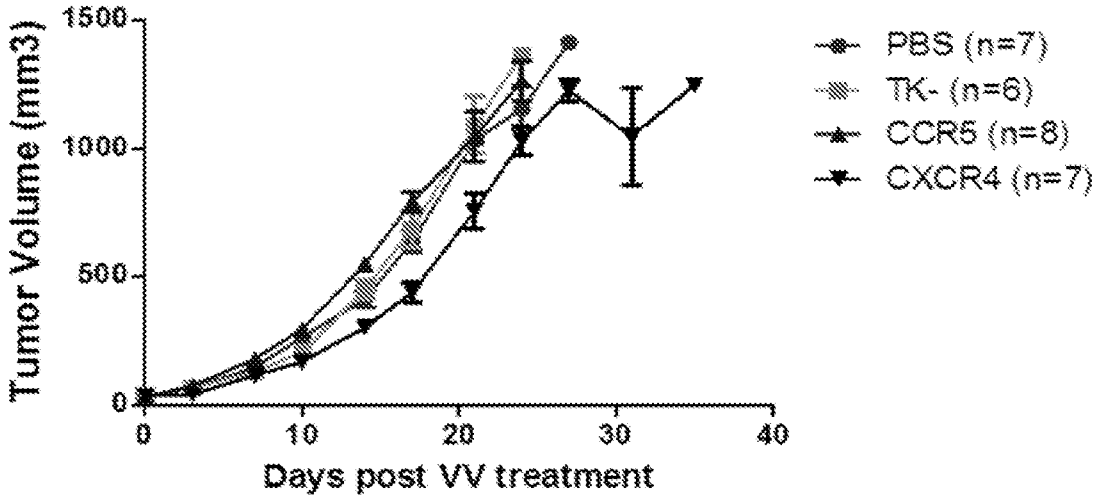

4T1-Balb/C model
ns ns ** TK- vs mCCR5/TK-
* ns * TK- vs mCXCR4/TK-
** * *** mCCR5/TK- vs mCXCR4/TK-
Photon Intensity
2.5 x 10^08
2.0 x 10^08
1.5 x 10^08
1.0 x 10^08
5 x 10^07
0.0
Day01
Day02
Day03
TK-
mCCR5/TK-
mCXCE4/TK-

FIG. 5A

a



	PBS vs TK-	PBS vs CCR5	PBS vs CXCR4	TK- vs CCR5	TK- vs CXCR4	CCR5 vs CXCR4
Day07	ns	ns	**	*	ns	***
Day10	ns	**	*	ns	**	***
Day14	ns	ns	*	ns	**	***
Day17	*	*	*	ns	ns	ns

FIG. 5B

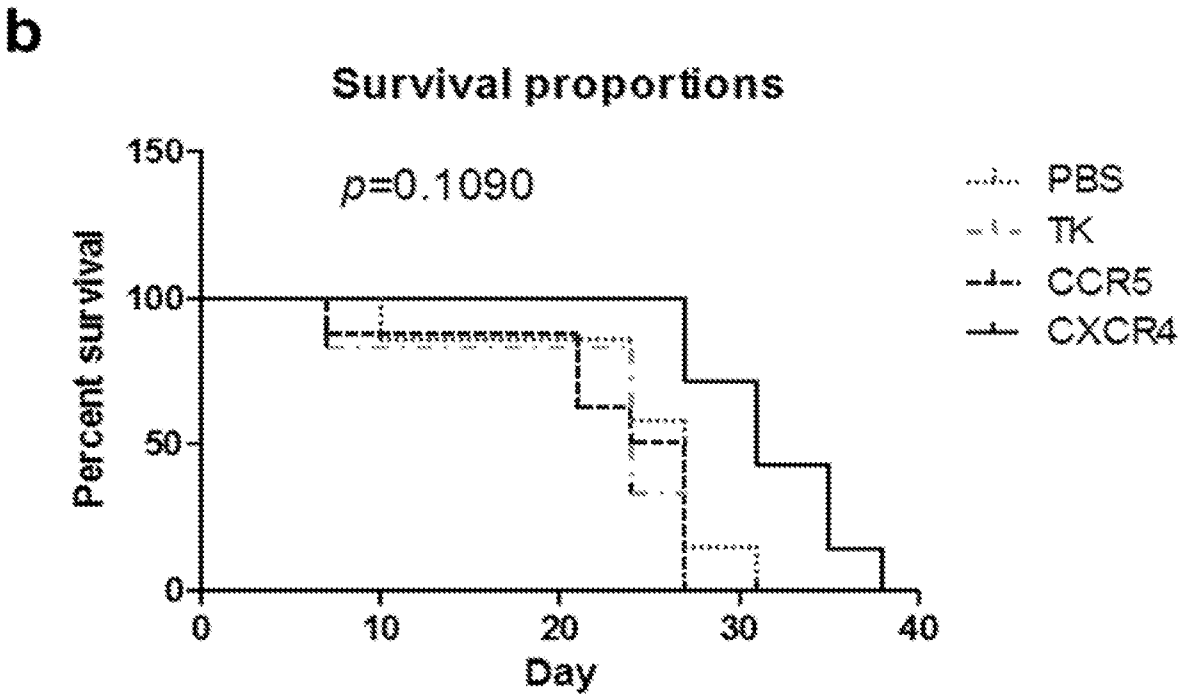

b
Survival proportions
p=0.1090
Percent survival
150
100
50
0
0
10
20
30
40
Day
PBS
TK
CCR5
CXCR4

FIG. 6A

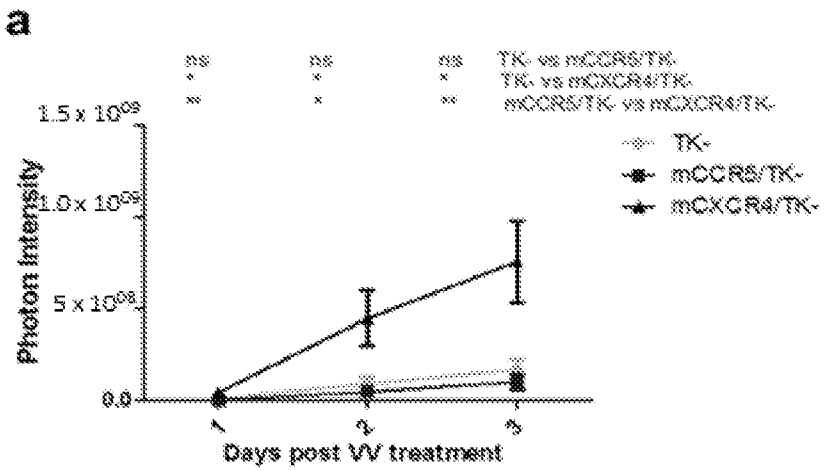

a
ns ns ns TK- vs mCCR5/TK-
* * * TK- vs mCXCR4/TK-
** * ** mCCR5/TK- vs mCXCR4/TK-
1.5 x 10^09
1.0 x 10^09
5 x 10^08
0.0
Photon Intensity
TK-
mCCR5/TK-
mCXCR4/TK-
1
2
3
Days post VV treatment

FIG. 6B

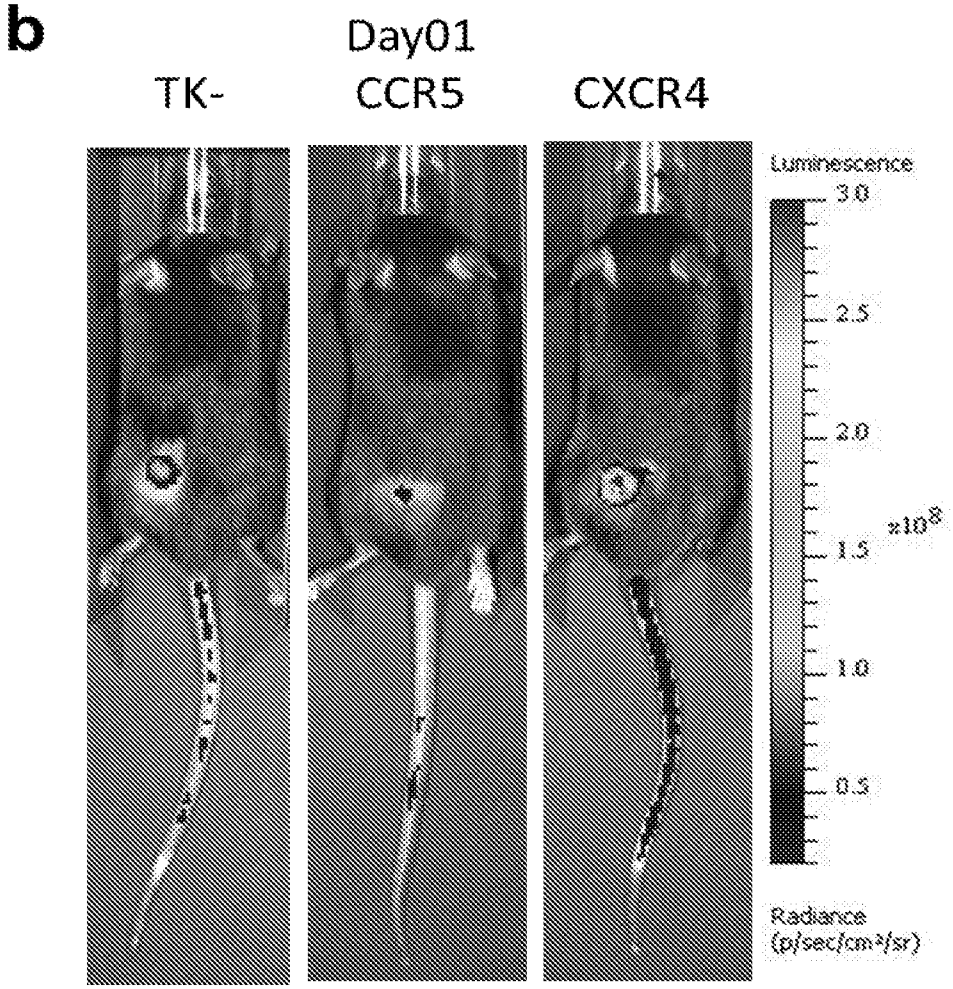
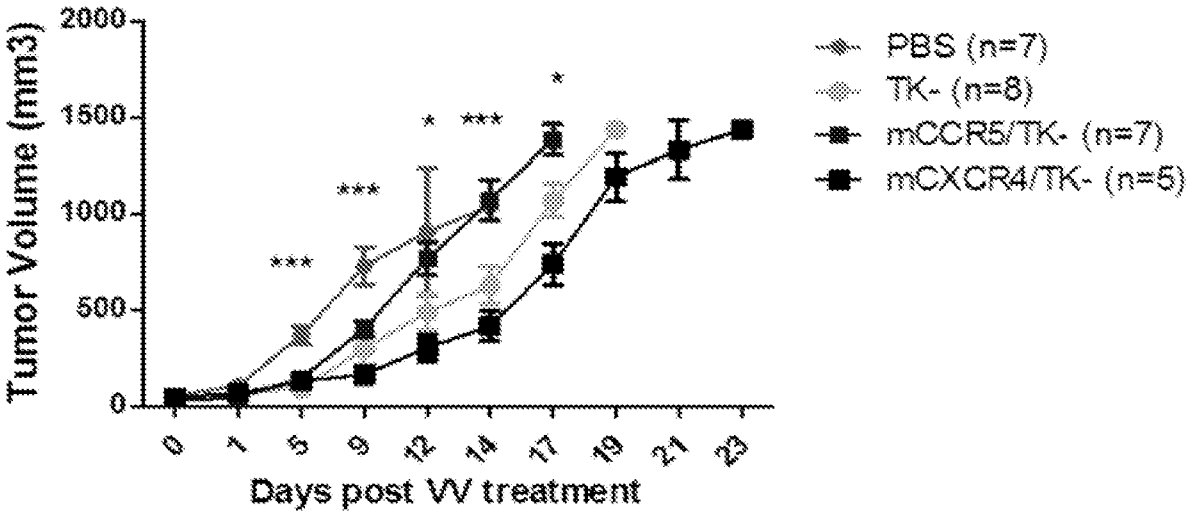
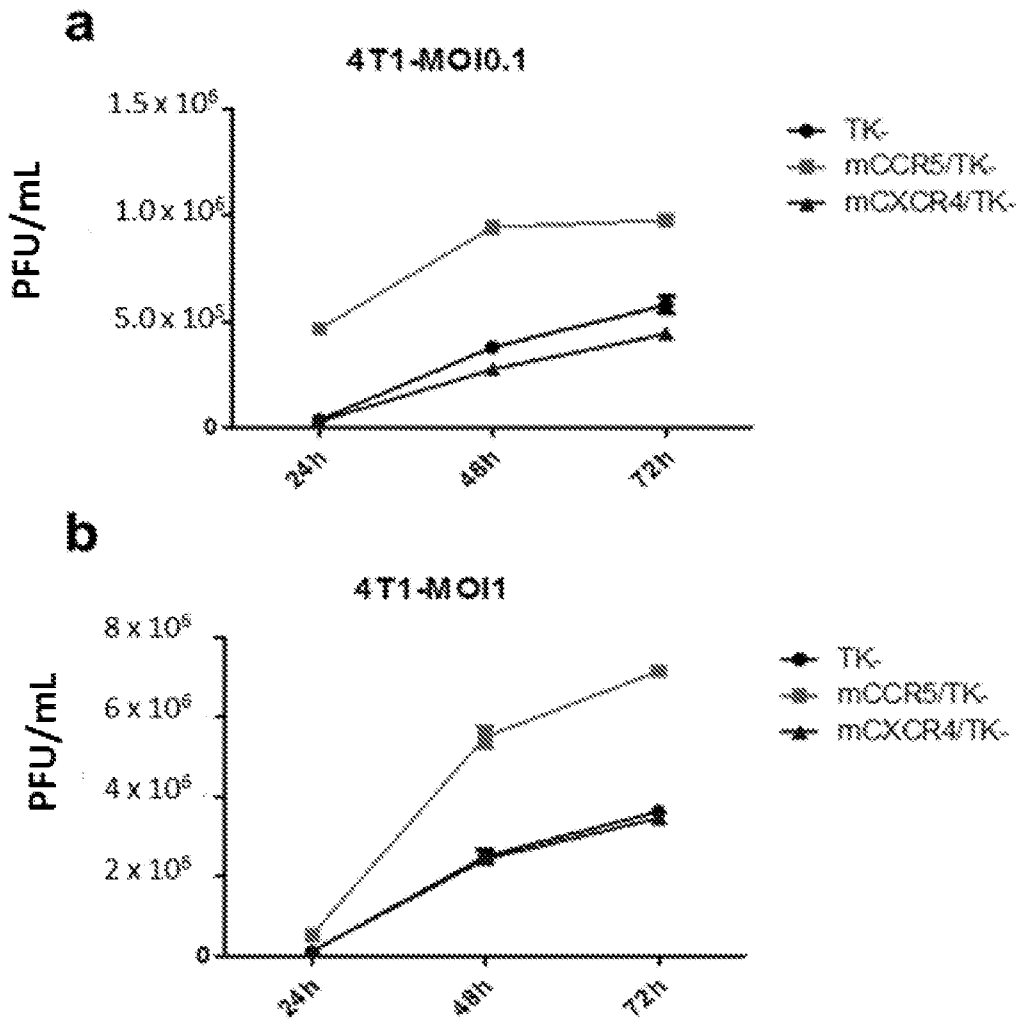

b
Day01
TK-
CCR5
CXCR4
Luminescence
3.0
2.5
2.0
1.5
1.0
0.5
x10^8
Radiance
(p/sec/cm²/sr)

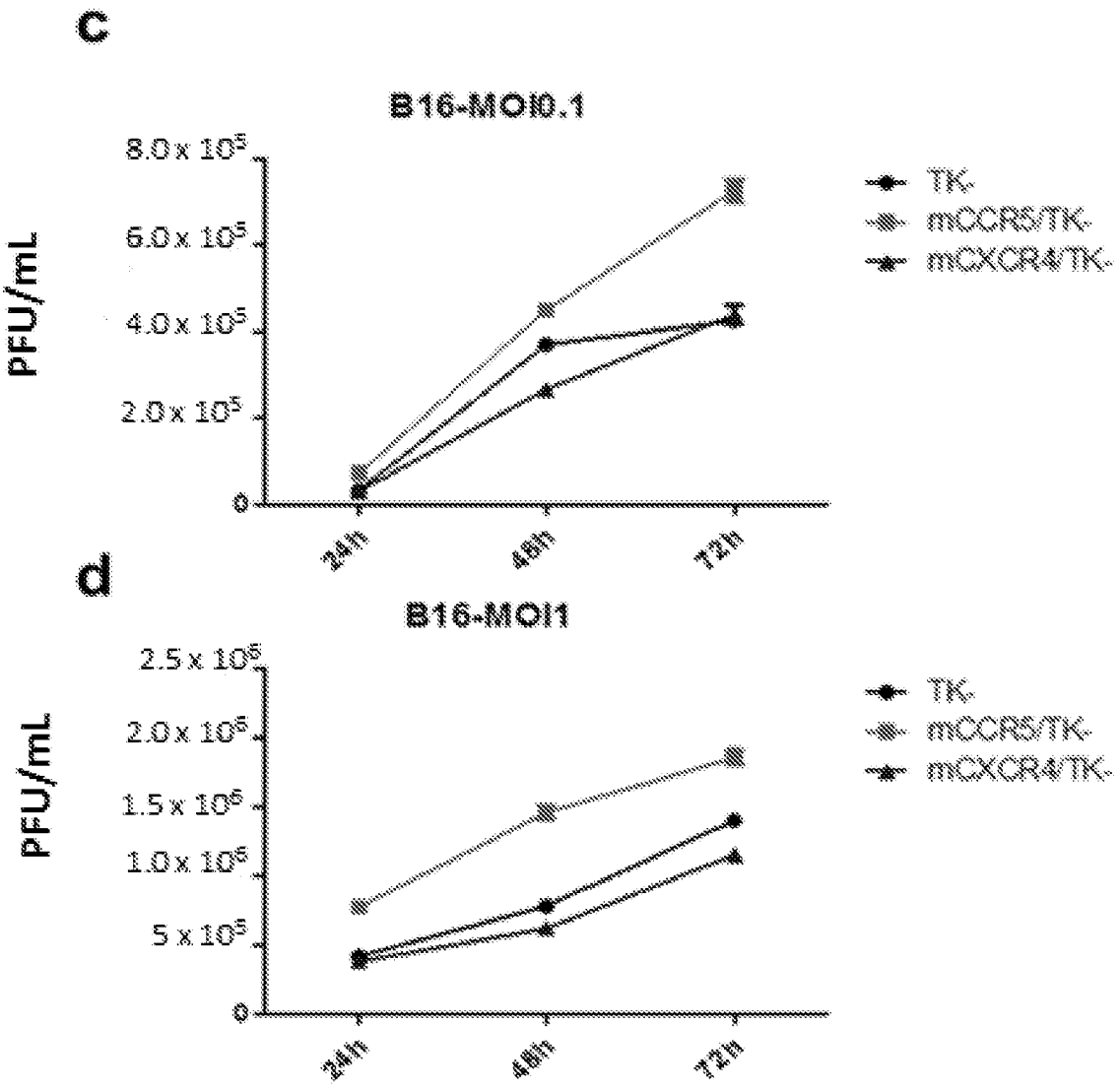
FIG. 7


Tumor Volume (mm3)
2000
1500
1000
500
0
***
***
*
***
*
0
1
5
9
12
14
17
19
21
23
Days post VV treatment
PBS (n=7)
TK- (n=8)
mCCR5/TK- (n=7)
mCXCR4/TK- (n=5)

FIGS. 8A-8B


a
4T1-MOI0.1
PFU/mL
1.5 x 10^6
1.0 x 10^6
5.0 x 10^5
0
24h
48h
72h
TK-
mCCR5/TK-
mCXCR4/TK-
b
4T1-MOI1
PFU/mL
8 x 10^6
6 x 10^6
4 x 10^6
2 x 10^6
0
24h
48h
72h
TK-
mCCR5/TK-
mCXCR4/TK-

FIGS. 8C-8D


c
B16-MOI0.1
PFU/mL
8.0 x 10^5
6.0 x 10^5
4.0 x 10^5
2.0 x 10^5
0
24h
48h
72h
TK-
mCCR5/TK-
mCXCR4/TK-
d
B16-MOI1
PFU/mL
2.5 x 10^6
2.0 x 10^6
1.5 x 10^6
1.0 x 10^6
5 x 10^5
0
24h
48h
72h
TK-
mCCR5/TK-
mCXCR4/TK-

FIGS. 9A-9C

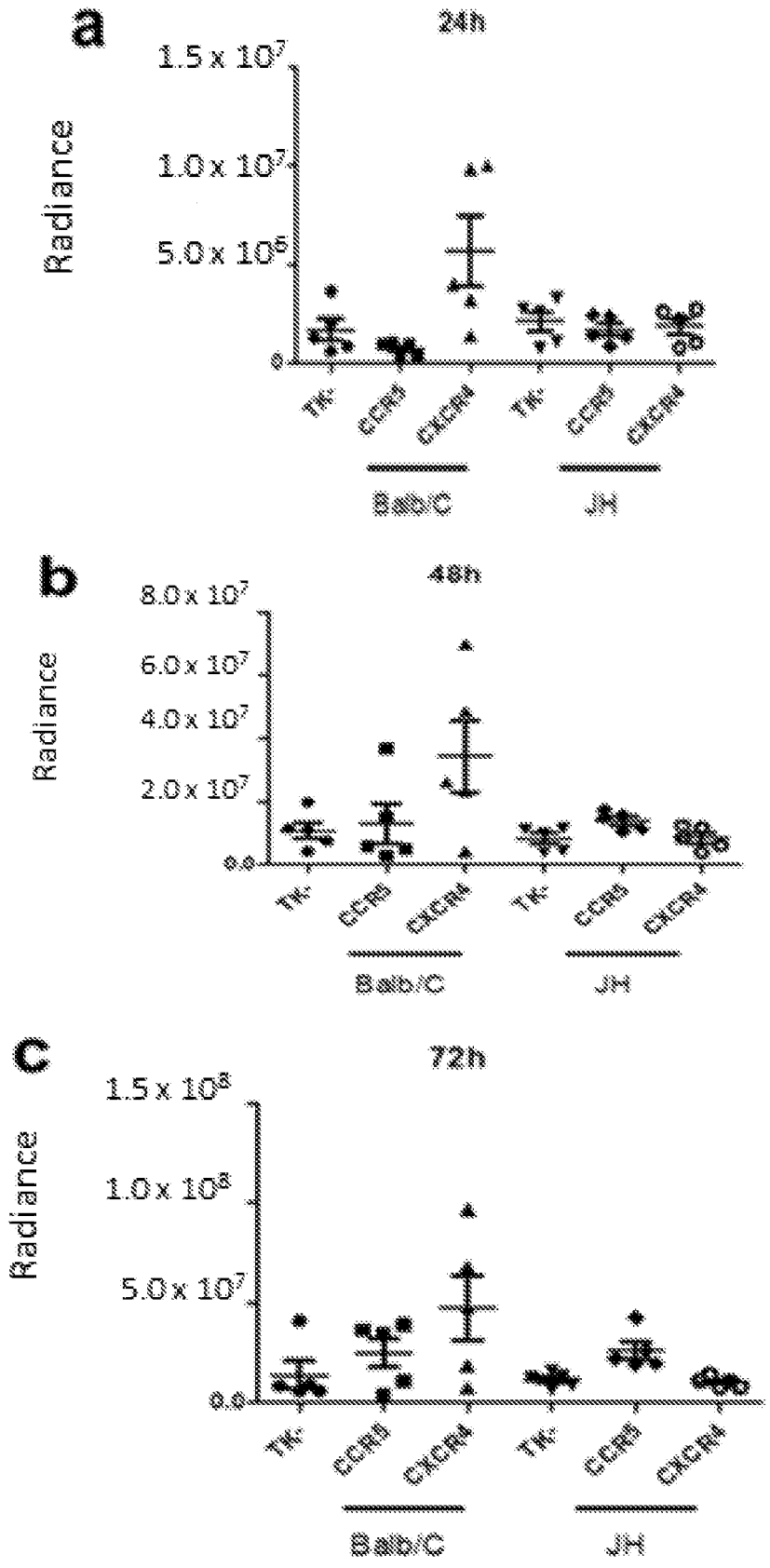

a
24h
Radiance
1.5 x 10^7
1.0 x 10^7
5.0 x 10^6
0
TK-
CCR5
CXCR4
TK-
CCR5
CXCR4
Balb/C
JH
b
48h
Radiance
8.0 x 10^7
6.0 x 10^7
4.0 x 10^7
2.0 x 10^7
0.0
TK-
CCR5
CXCR4
TK-
CCR5
CXCR4
Balb/C
JH
c
72h
Radiance
1.5 x 10^8
1.0 x 10^8
5.0 x 10^7
0.0
TK-
CCR5
CXCR4
TK-
CCR5
CXCR4
Balb/C
JH

FIG. 10A

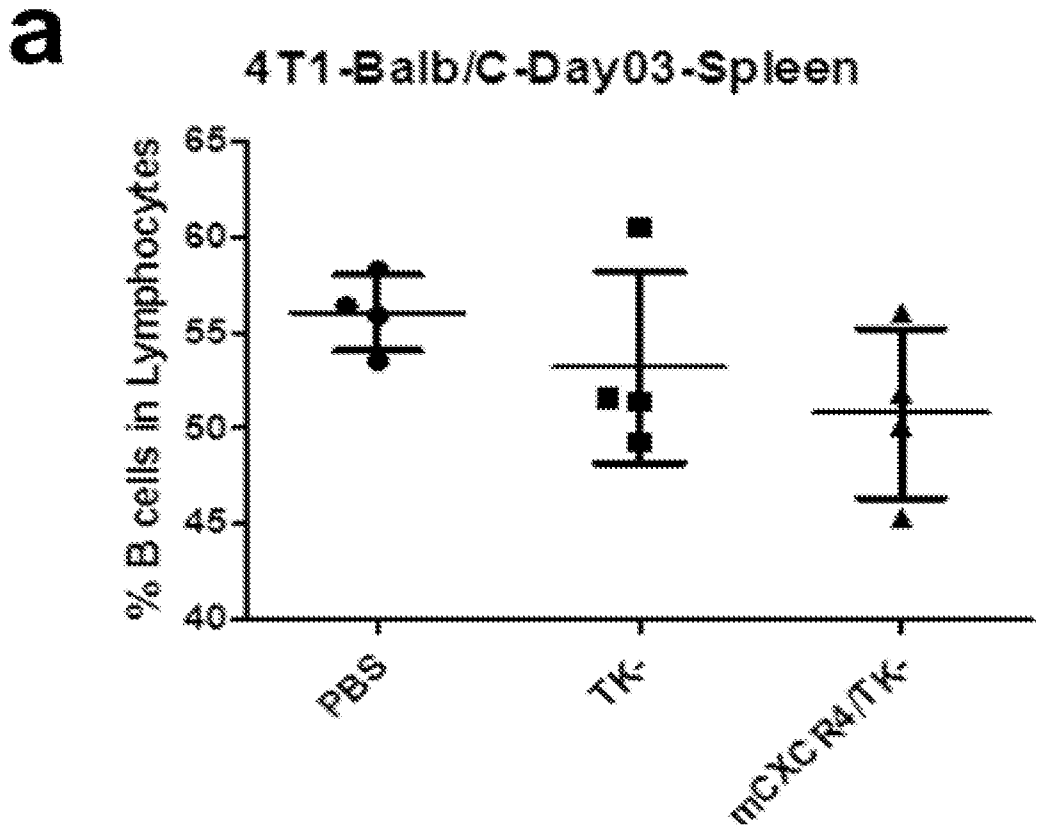

a
4T1-Balb/C-Day03-Spleen
% B cells in Lymphocytes
65
60
55
50
45
40
PBS
TK-
mCXCR4/TK-

FIG. 10B

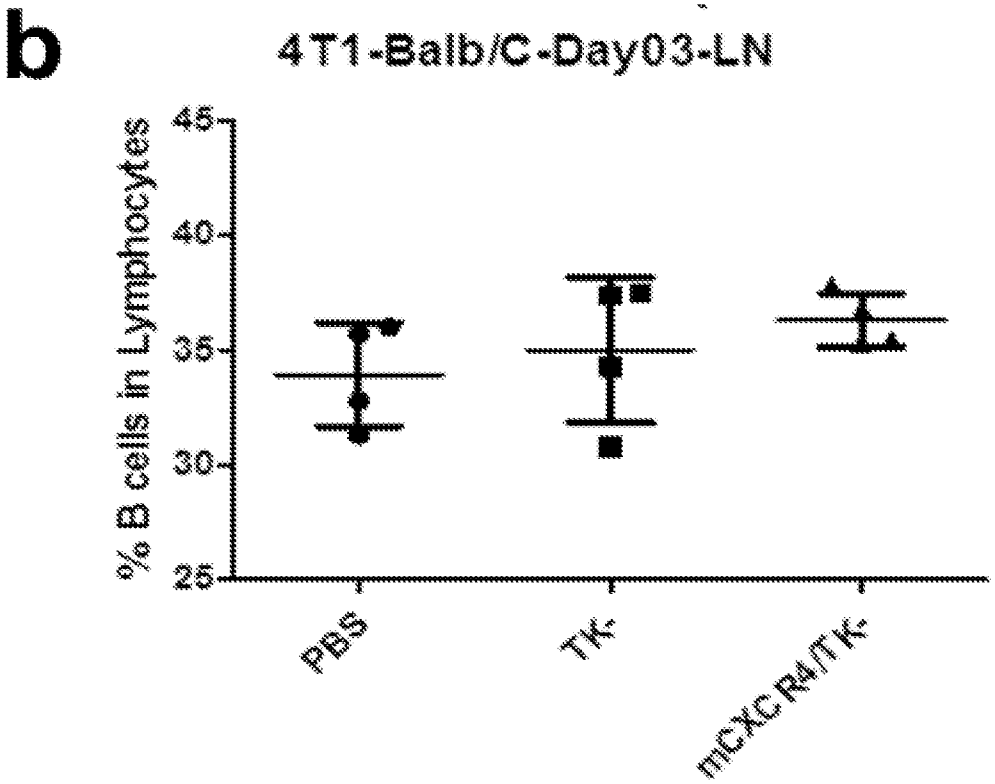
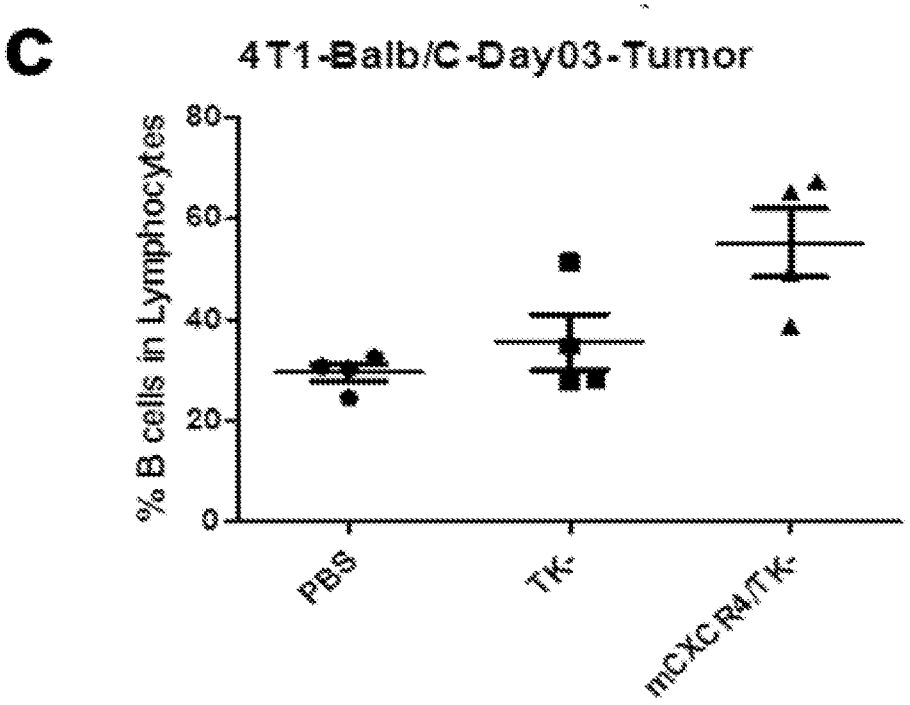
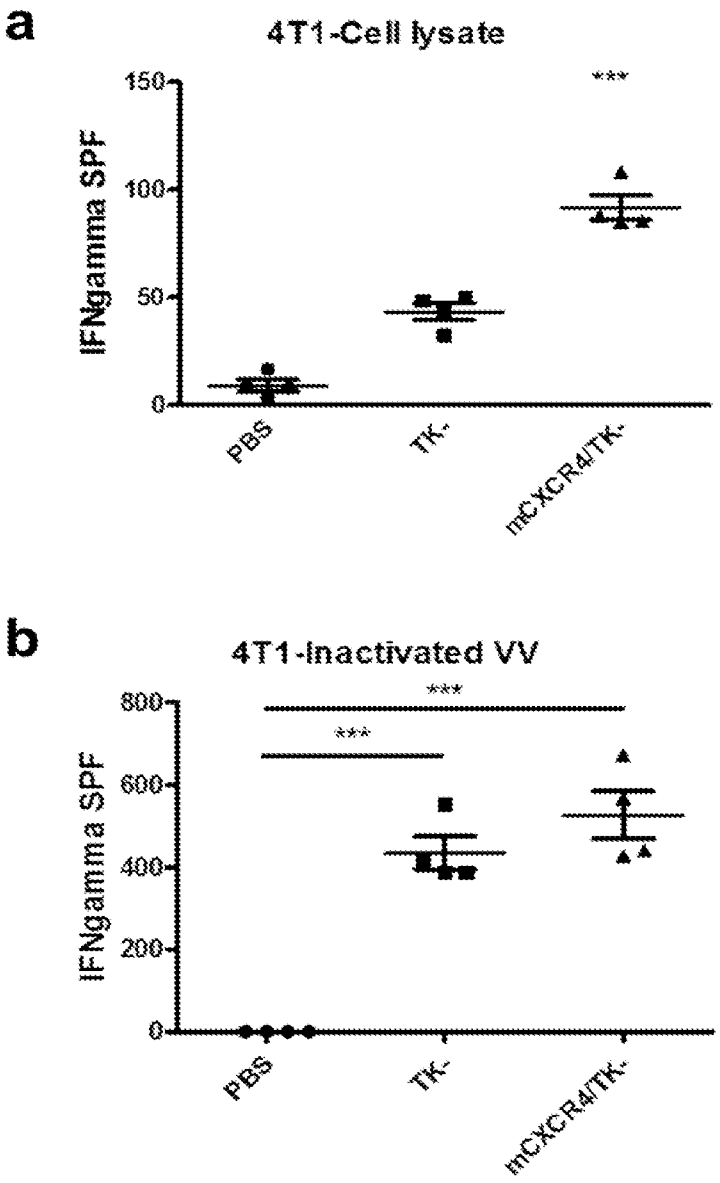

b
4T1-Balb/C-Day03-LN
% B cells in Lymphocytes
45
40
35
30
25
PBS
TK-
mCXCR4/TK-

FIG. 10C


C
4T1-Balb/C-Day03-Tumor
% B cells in Lymphocytes
80
60
40
20
0
PBS
TK-
mCXCR4/TK-

FIGS. 11A-11B


a
4T1-Cell lysate
IFNgamma SPF
150
100
50
0
***
PBS
TK-
mCXCR4/TK-
b
4T1-Inactivated VV
IFNgamma SPF
800
600
400
200
0
***
***
PBS
TK-
mCXCR4/TK-

FIGS. 12A-12B

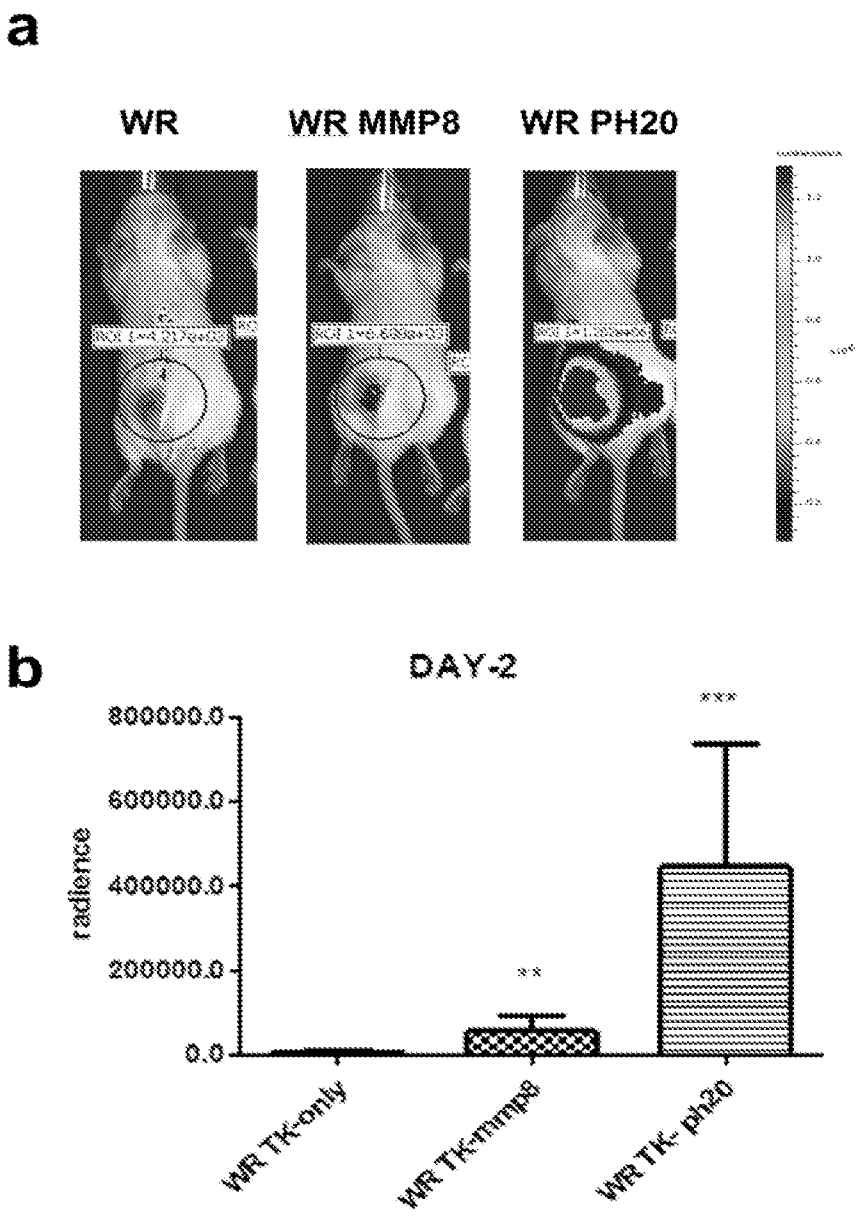

a
WR
WR MMP8
WR PH20
b
DAY-2
radience
800000.0
600000.0
400000.0
200000.0
0.0
***
**
WR TK-only
WR TK-mmp8
WR TK- ph20

FIG. 13A

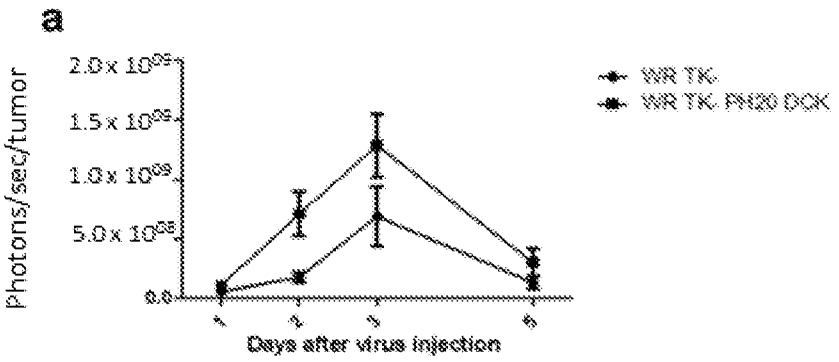

a
Photons/sec/tumor
2.0 x 10^09
1.5 x 10^09
1.0 x 10^09
5.0 x 10^08
0.0
Days after virus injection
WR TK-
WR TK- PH20 DCK

FIG. 13B

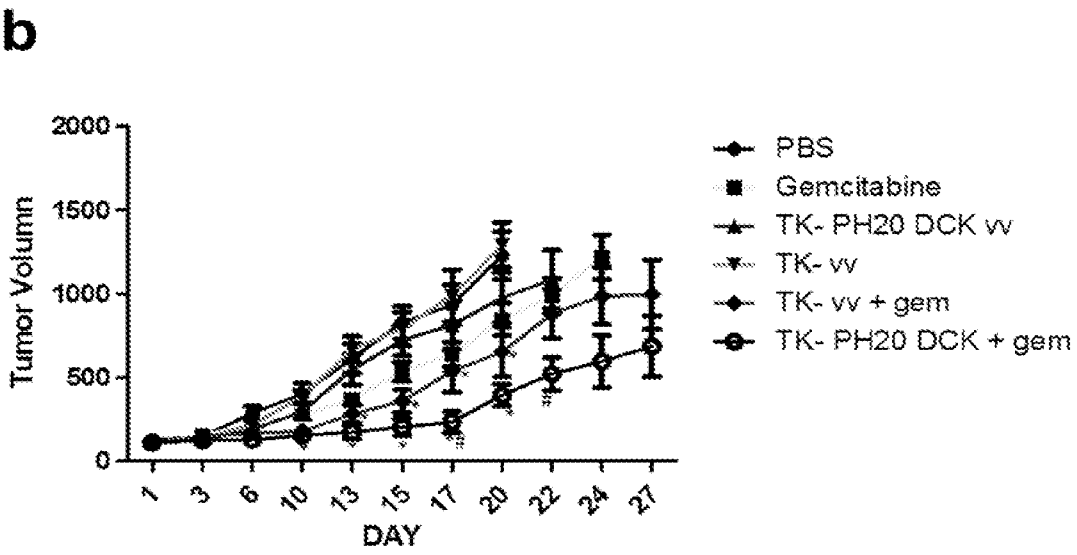

b
Tumor Volumn
2000
1500
1000
500
0
1
3
6
10
13
15
17
20
22
24
27
DAY
PBS
Gemcitabine
TK- PH20 DCK vv
TK- vv
TK- vv + gem
TK- PH20 DCK + gem

FIG. 13C

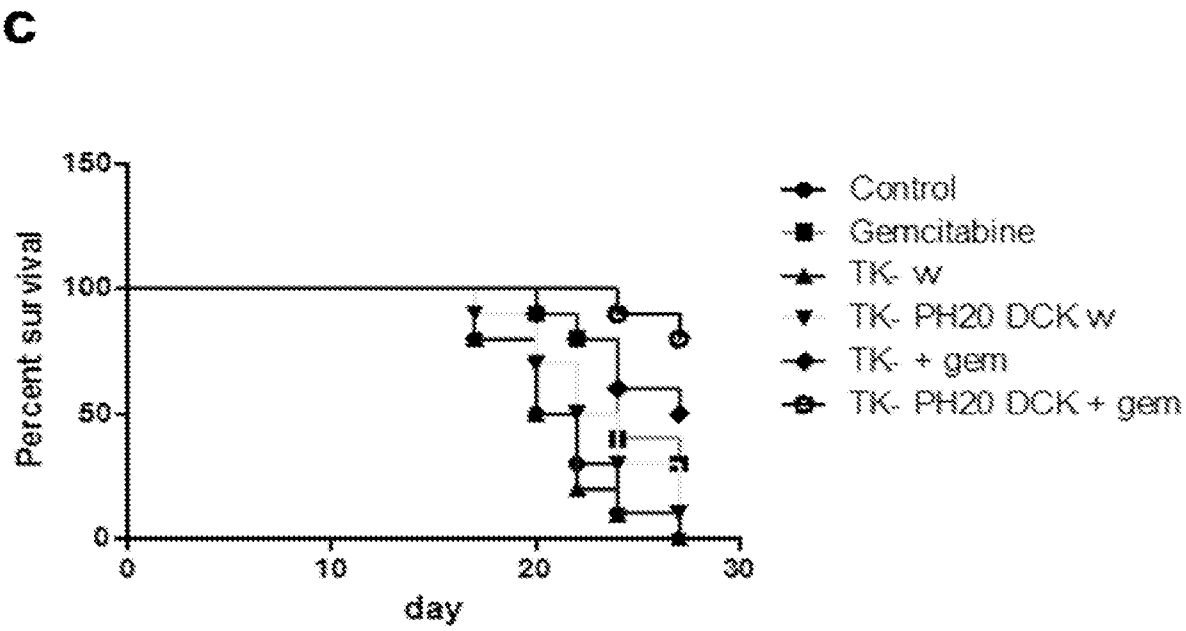

C
Percent survival
150
100
50
0
0
10
20
30
day
Control
Gemcitabine
TK- w
TK- PH20 DCK w
TK- + gem
TK- PH20 DCK + gem

FIG. 14

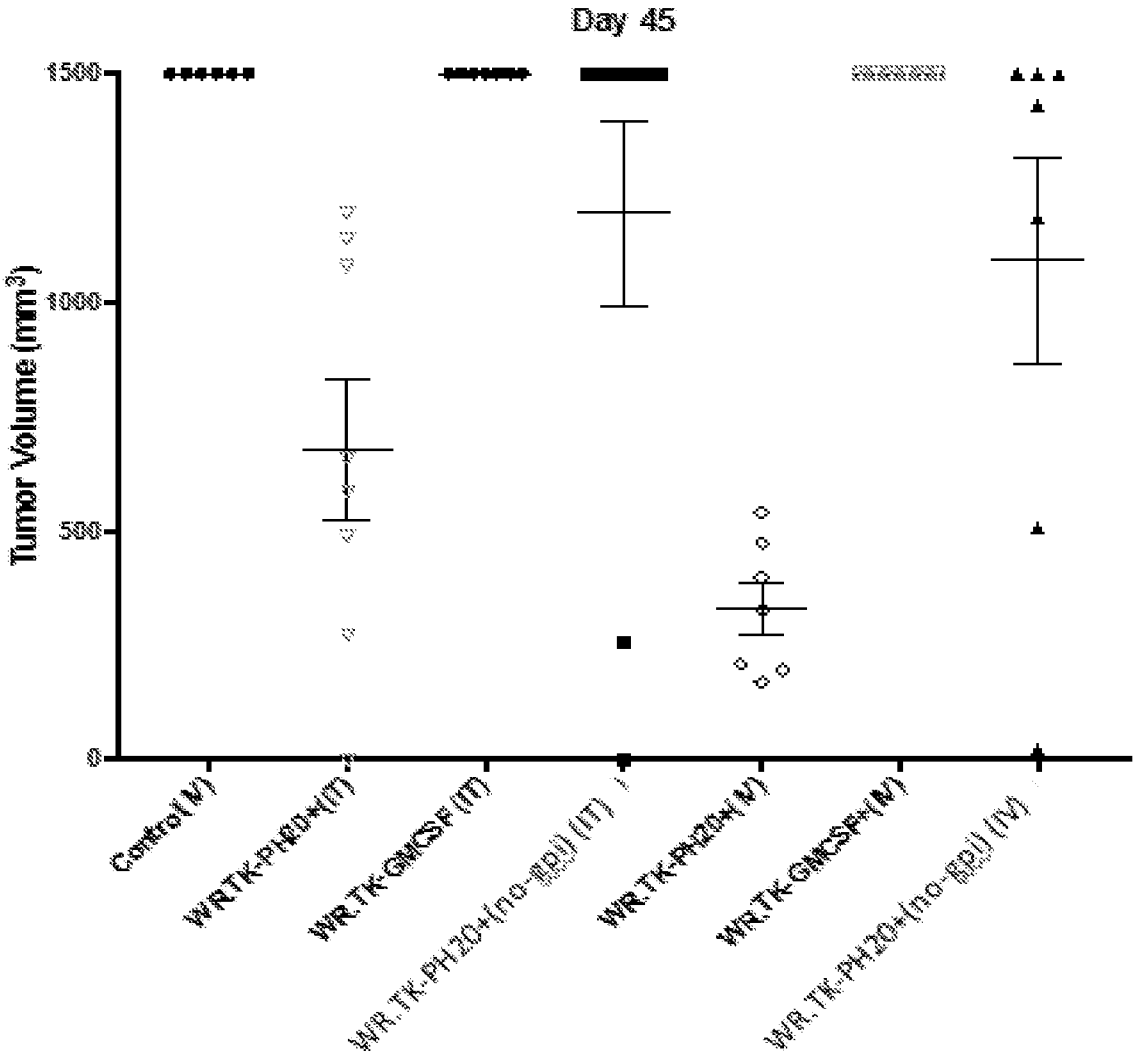

Day 45
Tumor Volume (mm3)
1500
1000
500
0
Control (IV)
WR.TK-PH20+(IT)
WR.TK-GMCSF (IT)
WR.TK-PH20+(IT)
WR.TK-PH20+(IV)
WR.TK-GMCSF+(IV)
WR.TK-PH20+(IV)

FIG. 15

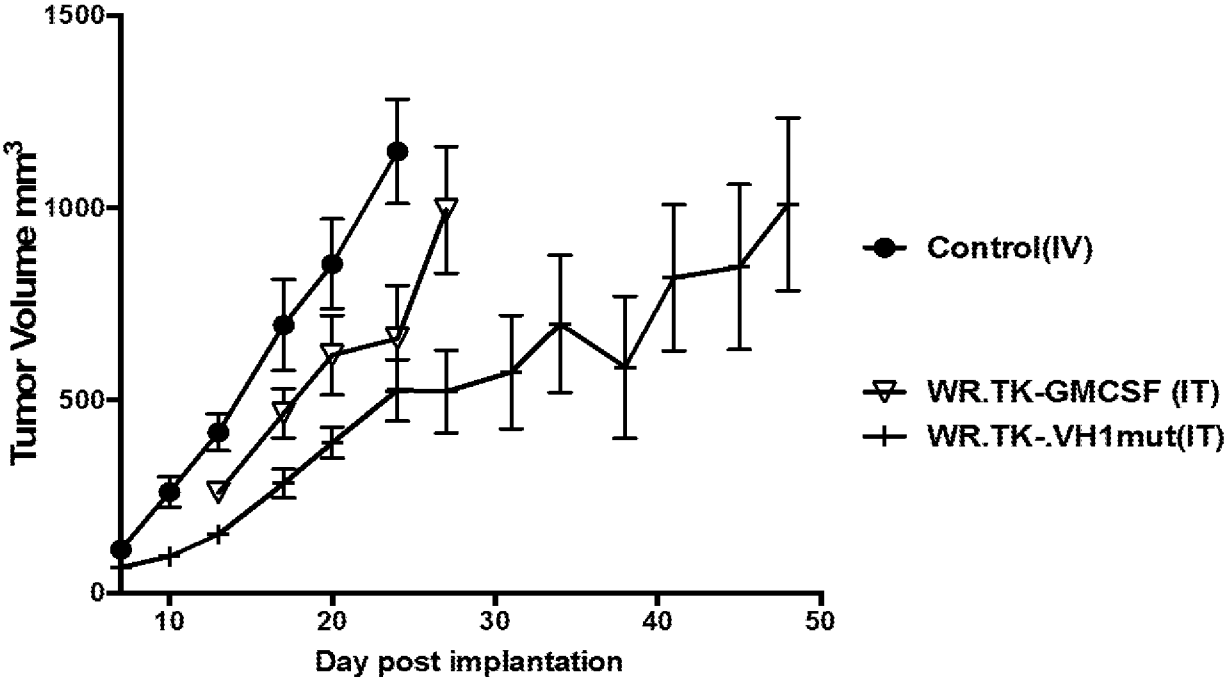

1500
1000
500
0
Tumor Volume mm3
10
20
30
40
50
Day post implantation
Control(IV)
WR.TK-GMCSF (IT)
WR.TK-.VH1mut(IT)

FIG. 16

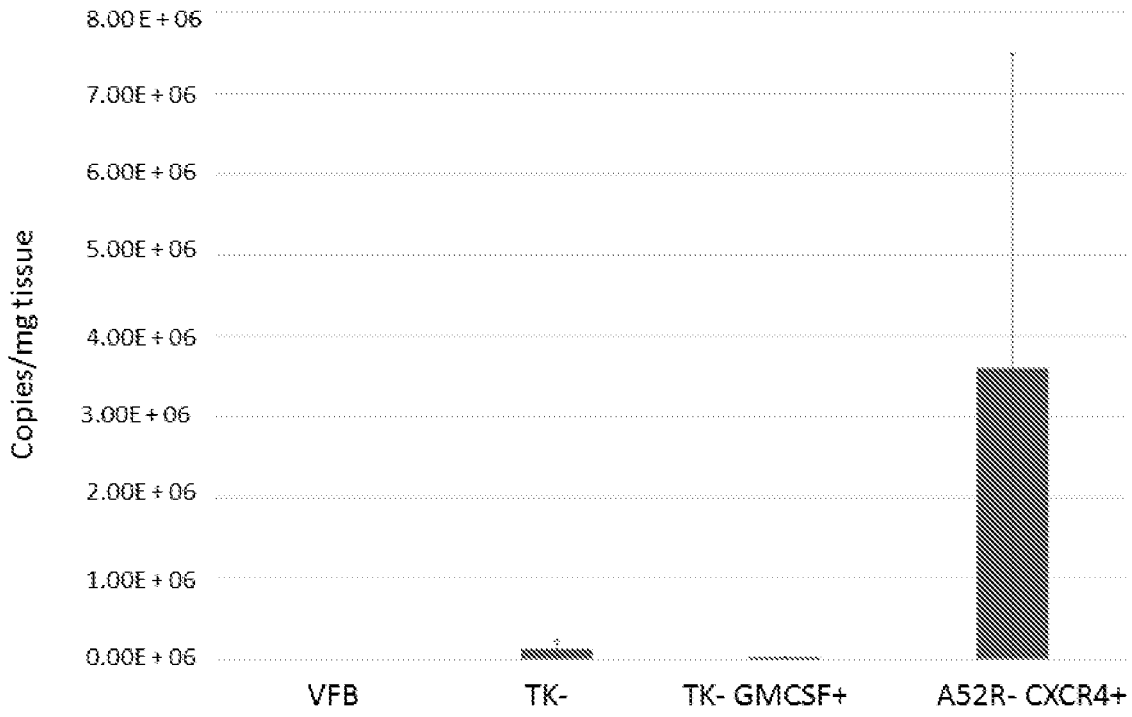

8.00E+06
7.00E+06
6.00E+06
5.00E+06
4.00E+06
3.00E+06
2.00E+06
1.00E+06
0.00E+06
Copies/mg tissue
VFB
TK-
TK- GMCSF+
A52R- CXCR4+

FIG. 17

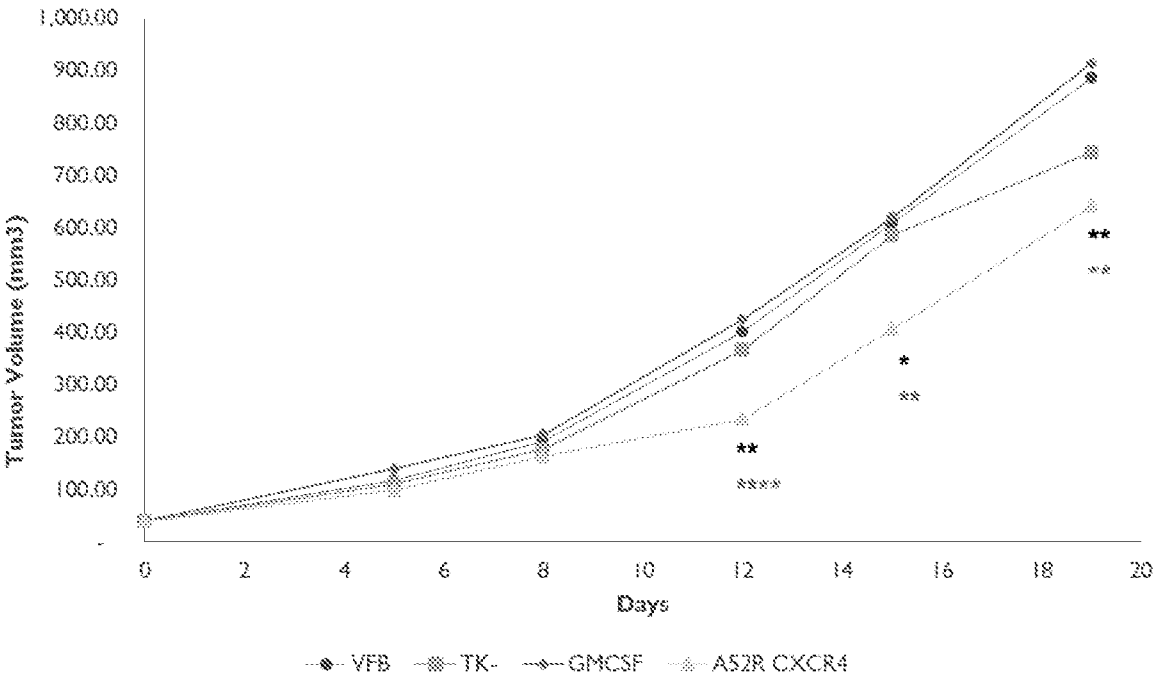

1,000.00
900.00
800.00
700.00
600.00
500.00
400.00
300.00
200.00
100.00
-
Tumor Volume (mm3)
0
2
4
6
8
10
12
14
16
18
20
Days
**
****
*
**
**
**
VFB
TK-
GMCSF
A52R CXCR4

FIG. 18A

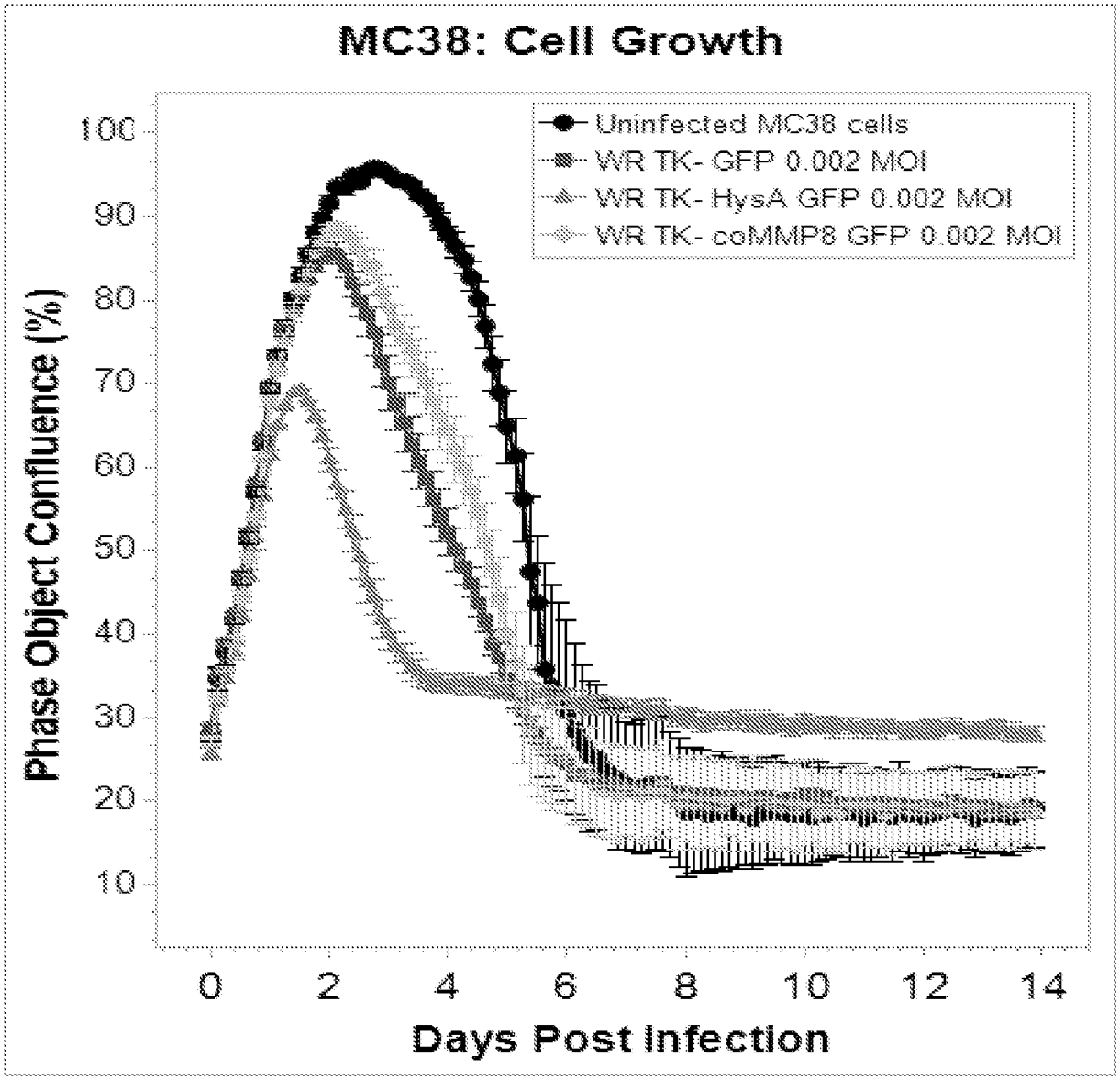

MC38: Cell Growth
Uninfected MC38 cells
WR TK- GFP 0.002 MOI
WR TK- HysA GFP 0.002 MOI
WR TK- coMMP8 GFP 0.002 MOI
Phase Object Confluence (%)
100
90
80
70
60
50
40
30
20
10
0
2
4
6
8
10
12
14
Days Post Infection

FIG. 18B

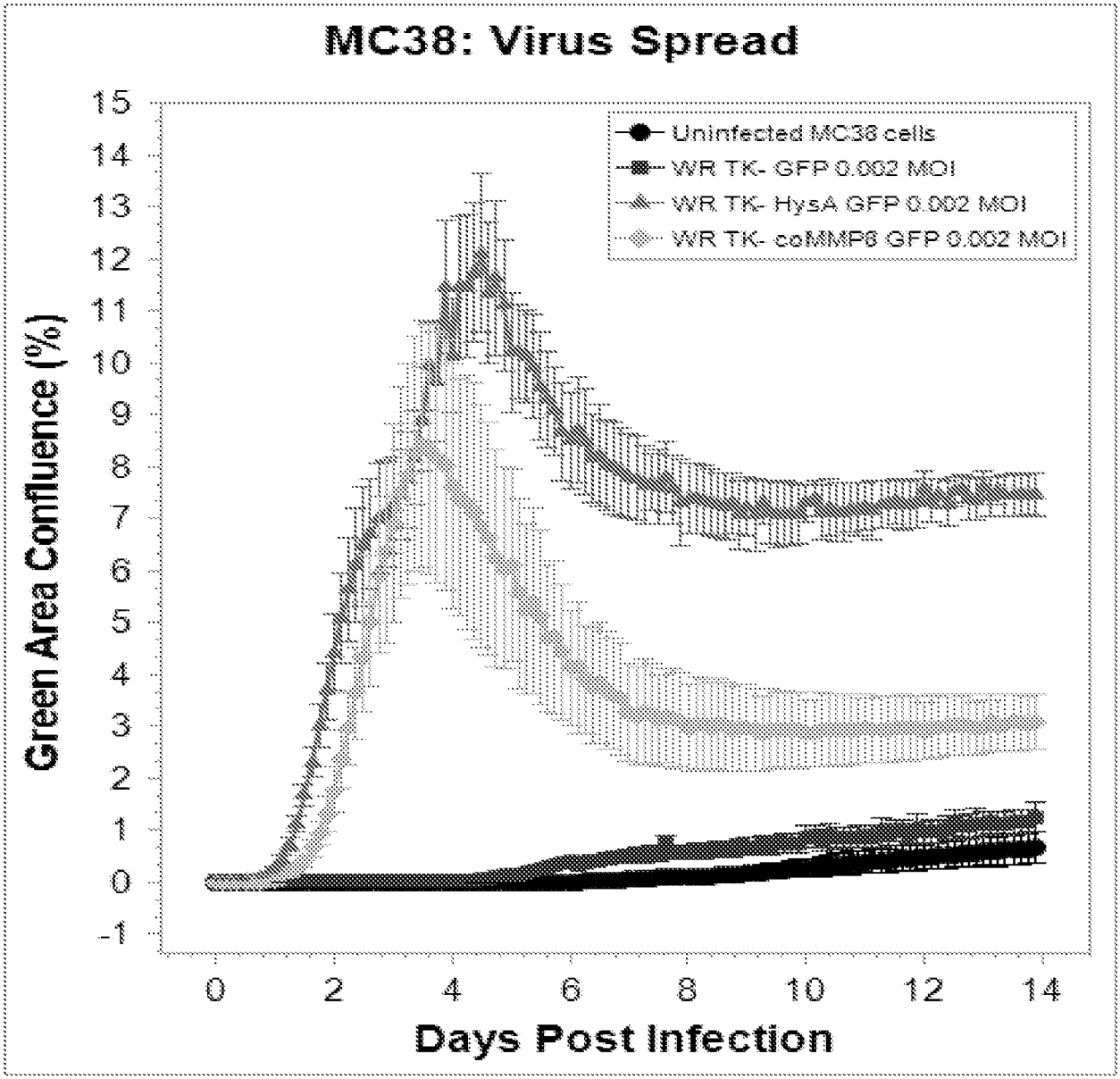

MC38: Virus Spread
Green Area Confluence (%)
Days Post Infection
Uninfected MC38 cells
WR TK- GFP 0.002 MOI
WR TK- HysA GFP 0.002 MOI
WR TK- coMMP8 GFP 0.002 MOI
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
0
2
4
6
8
10
12
14

FIG. 18C

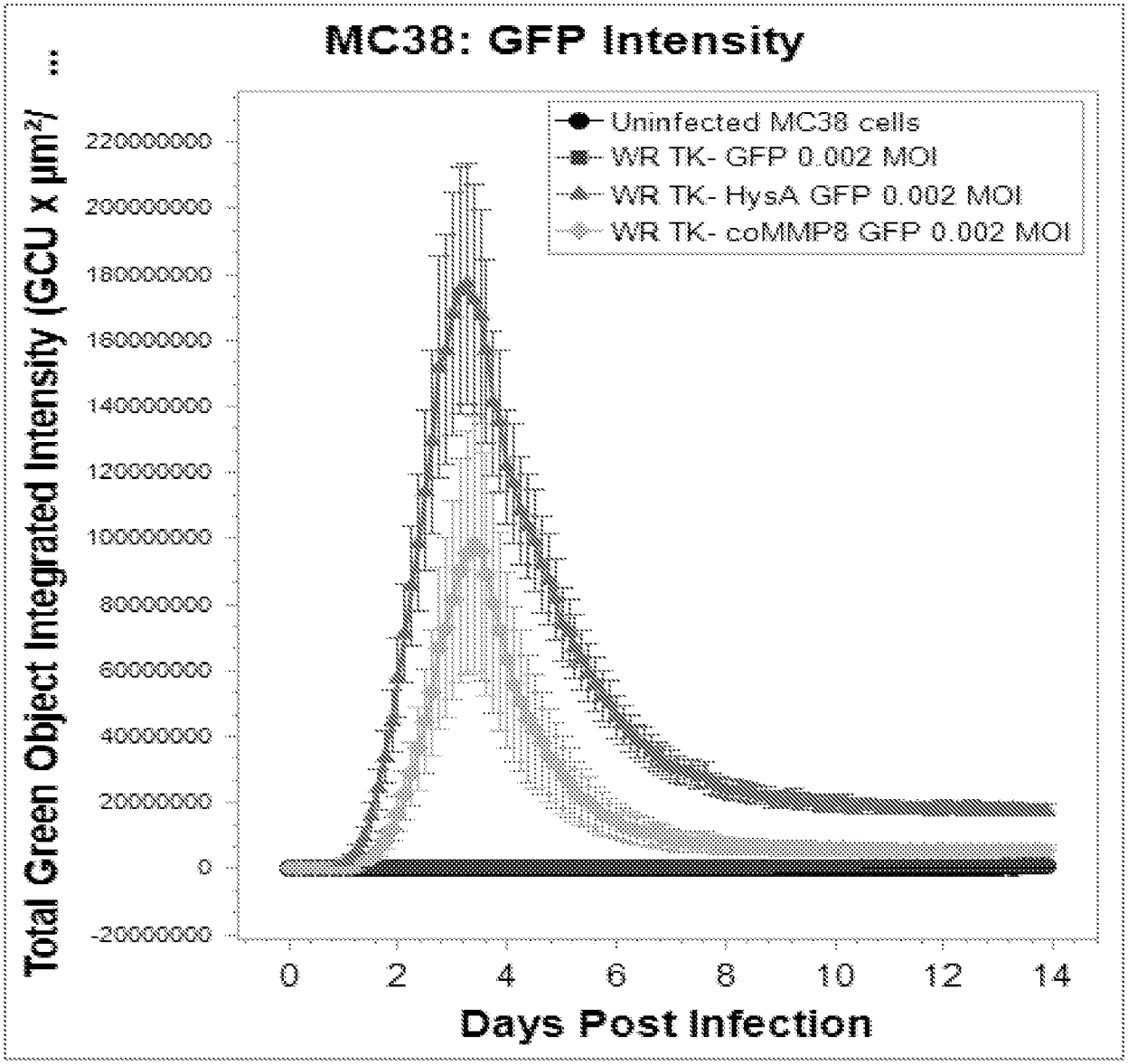

MC38: GFP Intensity
Total Green Object Integrated Intensity (GCU x µm²/ ...
220000000
200000000
180000000
160000000
140000000
120000000
100000000
80000000
60000000
40000000
20000000
0
-20000000
0
2
4
6
8
10
12
14
Days Post Infection
Uninfected MC38 cells
WR TK- GFP 0.002 MOI
WR TK- HysA GFP 0.002 MOI
WR TK- coMMP8 GFP 0.002 MOI

FIG. 18D

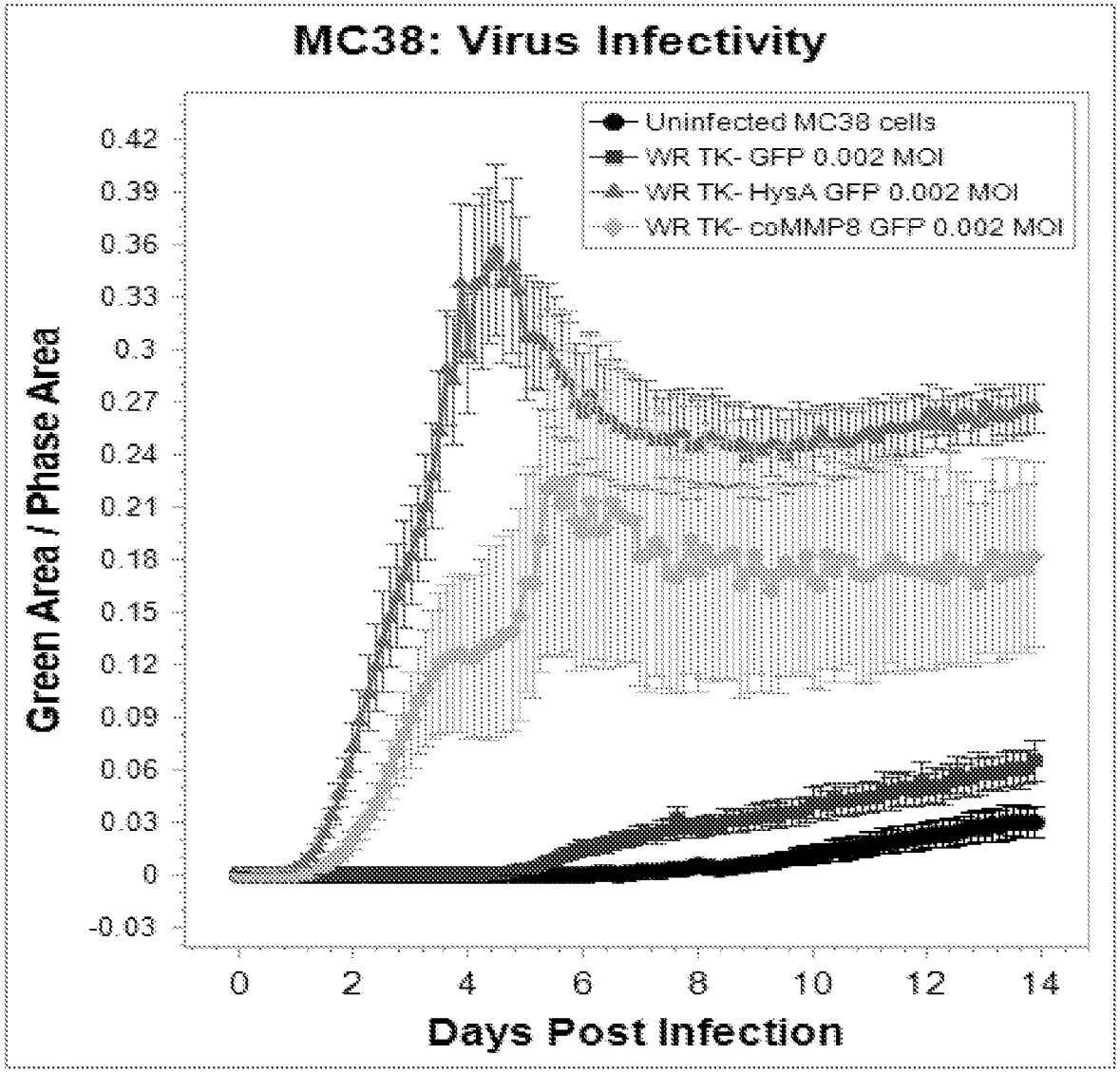

MC38: Virus Infectivity
Uninfected MC38 cells
WR TK- GFP 0.002 MOI
WR TK- HysA GFP 0.002 MOI
WR TK- coMMP8 GFP 0.002 MOI
Green Area / Phase Area
0.42
0.39
0.36
0.33
0.3
0.27
0.24
0.21
0.18
0.15
0.12
0.09
0.06
0.03
0
-0.03
0
2
4
6
8
10
12
14
Days Post Infection

FIG. 19A

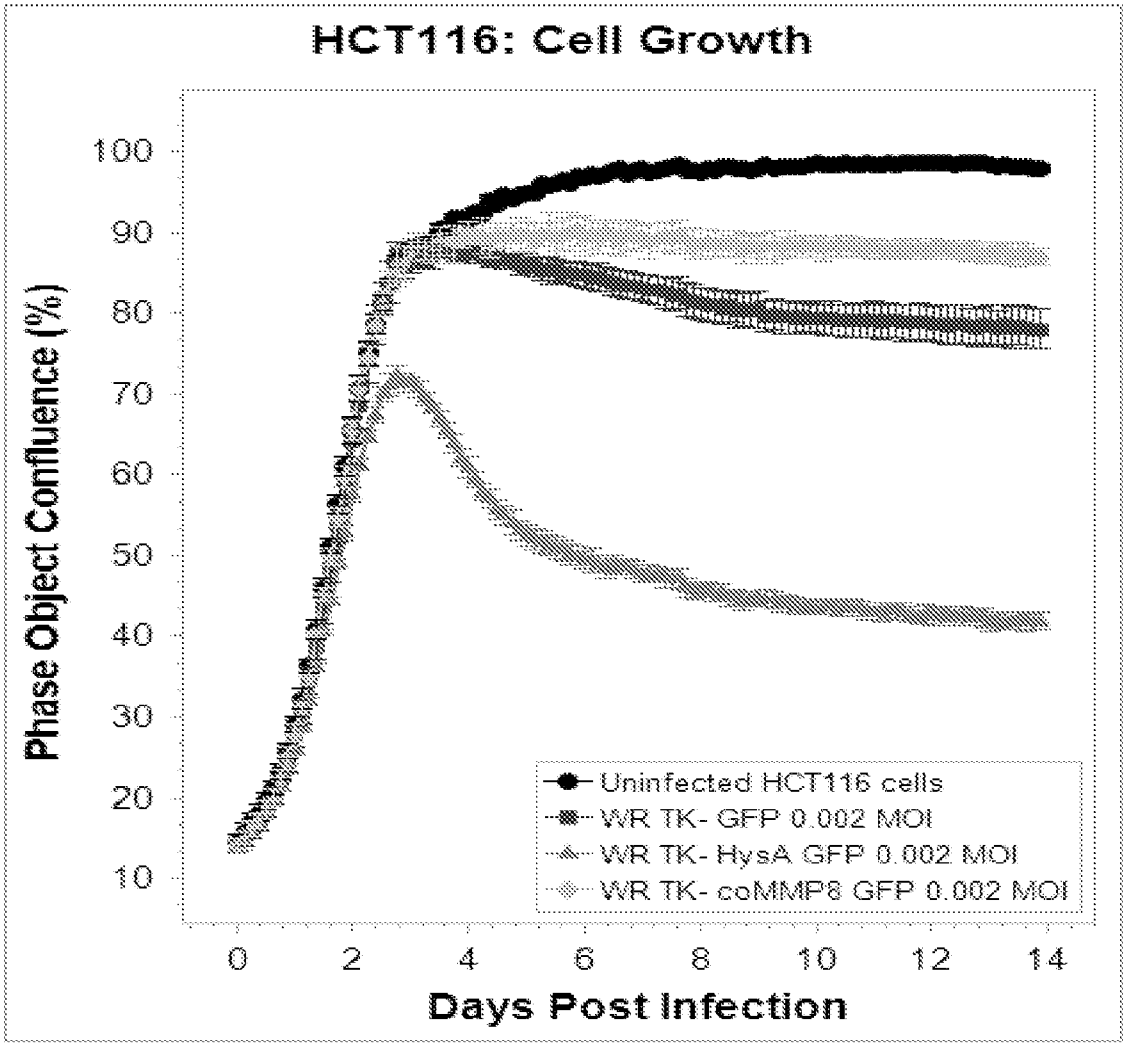

HCT116: Cell Growth
Phase Object Confluence (%)
100
90
80
70
60
50
40
30
20
10
0
2
4
6
8
10
12
14
Days Post Infection
Uninfected HCT116 cells
WR TK- GFP 0.002 MOI
WR TK- HysA GFP 0.002 MOI
WR TK- coMMP8 GFP 0.002 MOI

FIG. 19B

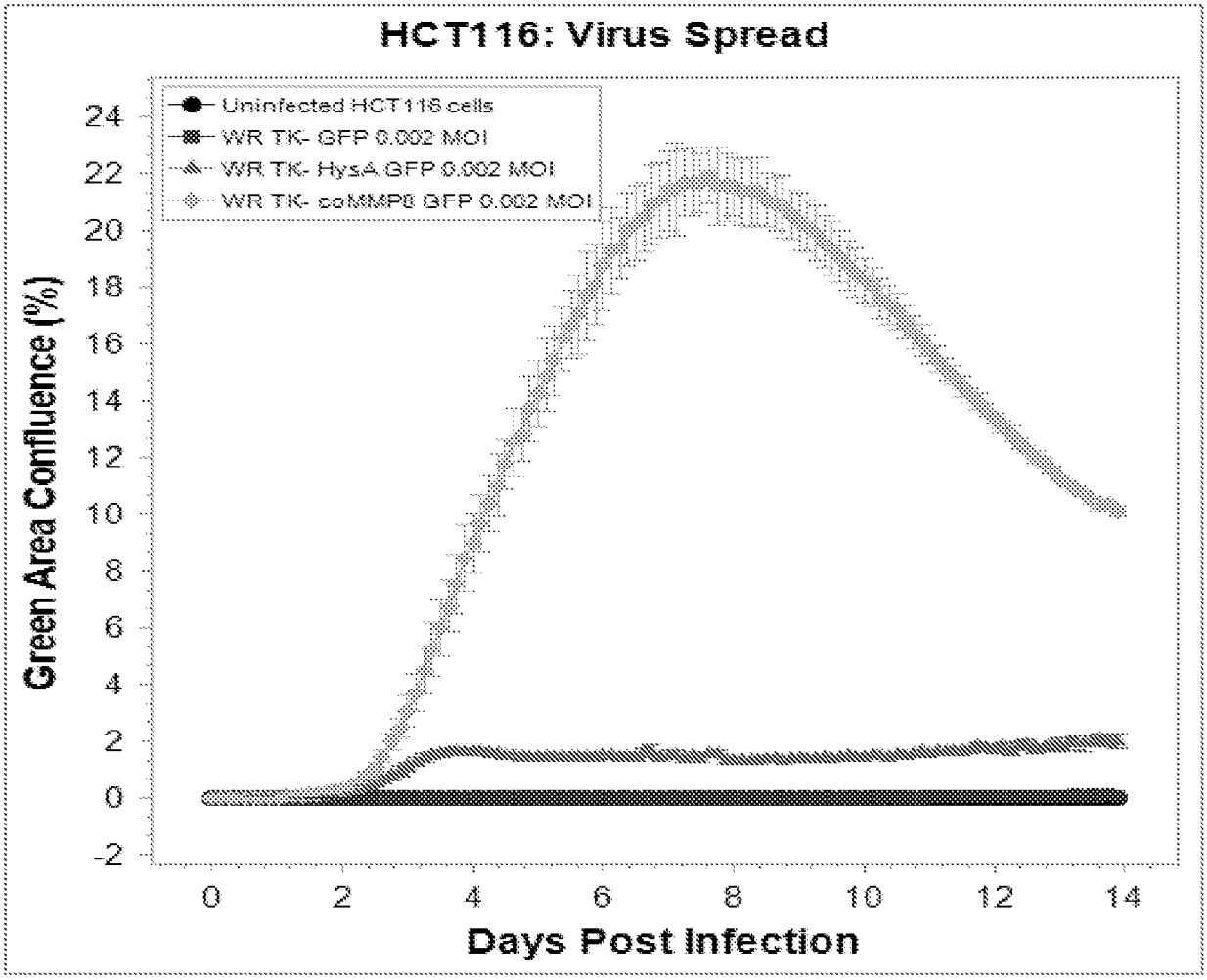

HCT116: Virus Spread
Uninfected HCT116 cells
WR TK- GFP 0.002 MOI
WR TK- HysA GFP 0.002 MOI
WR TK- coMMP8 GFP 0.002 MOI
Green Area Confluence (%)
24
22
20
18
16
14
12
10
8
6
4
2
0
-2
0
2
4
6
8
10
12
14
Days Post Infection

FIG. 19C

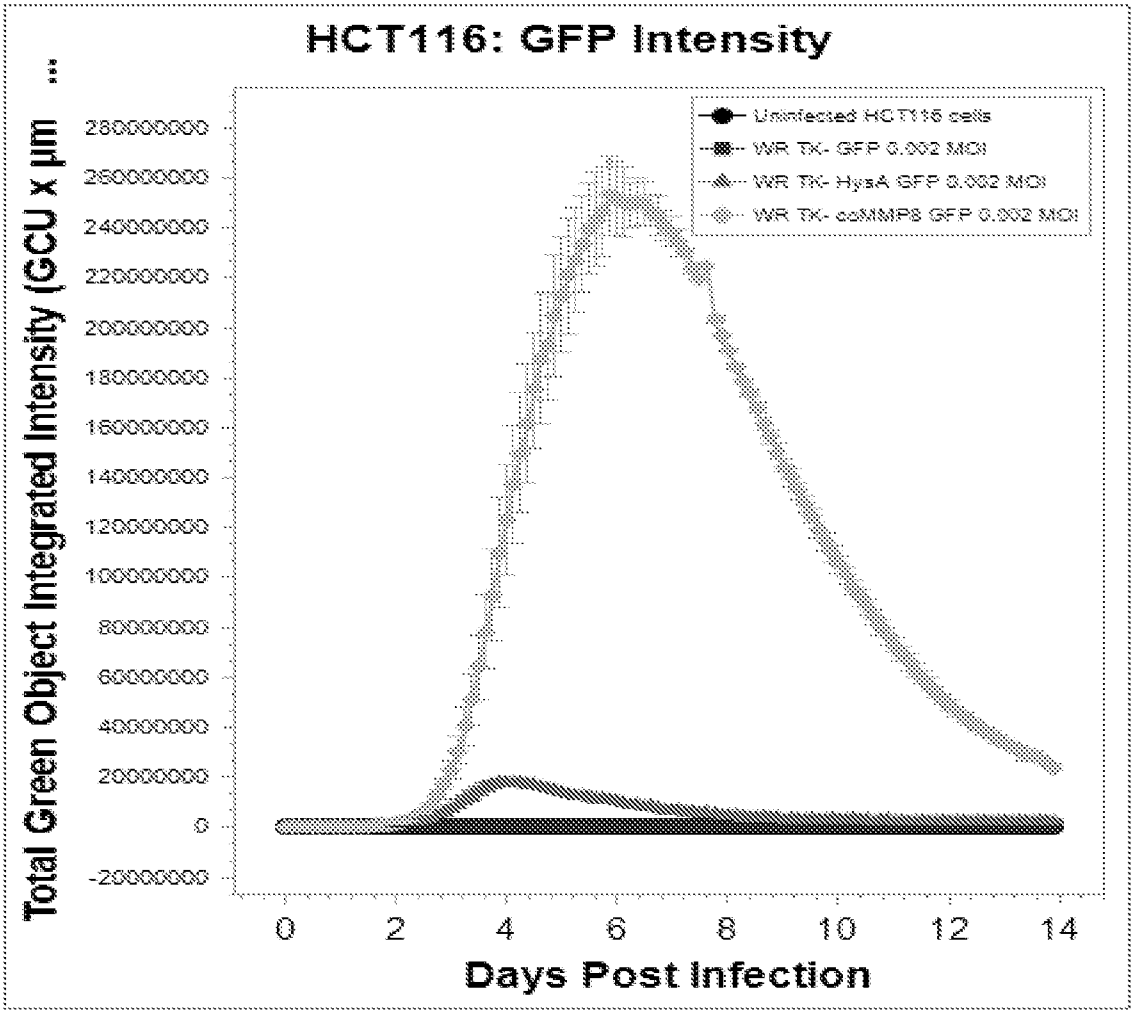

HCT116: GFP Intensity
Total Green Object Integrated Intensity (GCU x µm ...
280000000
260000000
240000000
220000000
200000000
180000000
160000000
140000000
120000000
100000000
80000000
60000000
40000000
20000000
0
-20000000
0
2
4
6
8
10
12
14
Days Post Infection
Uninfected HCT116 cells
WR TK- GFP 0.002 MOI
WR TK- HysA GFP 0.002 MOI
WR TK- coMMP8 GFP 0.002 MOI

FIG. 19D

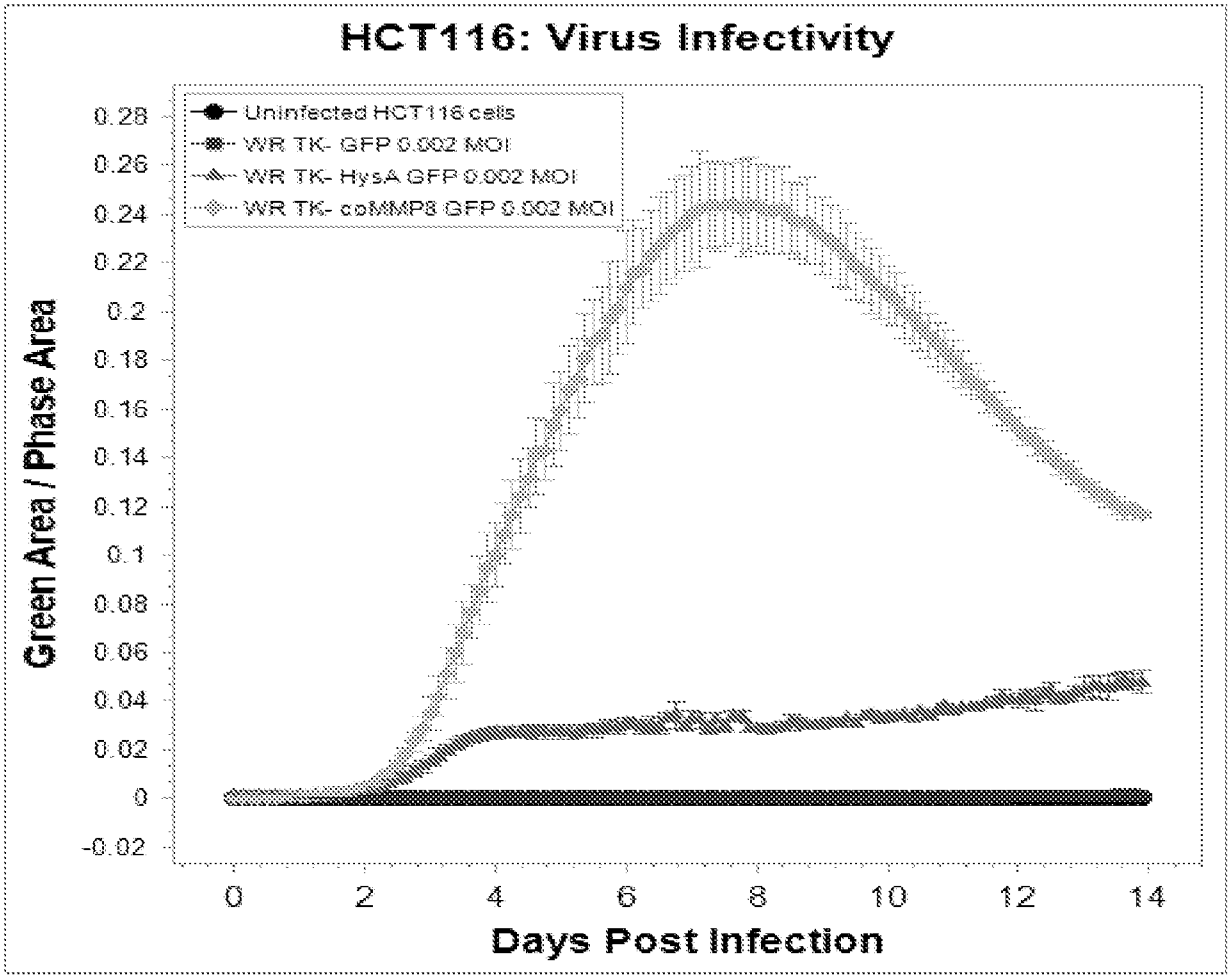

HCT116: Virus Infectivity
Uninfected HCT116 cells
WR TK- GFP 0.002 MOI
WR TK- HysA GFP 0.002 MOI
WR TK- coMMP8 GFP 0.002 MOI
Green Area / Phase Area
0.28
0.26
0.24
0.22
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
-0.02
0
2
4
6
8
10
12
14
Days Post Infection

FIG. 20

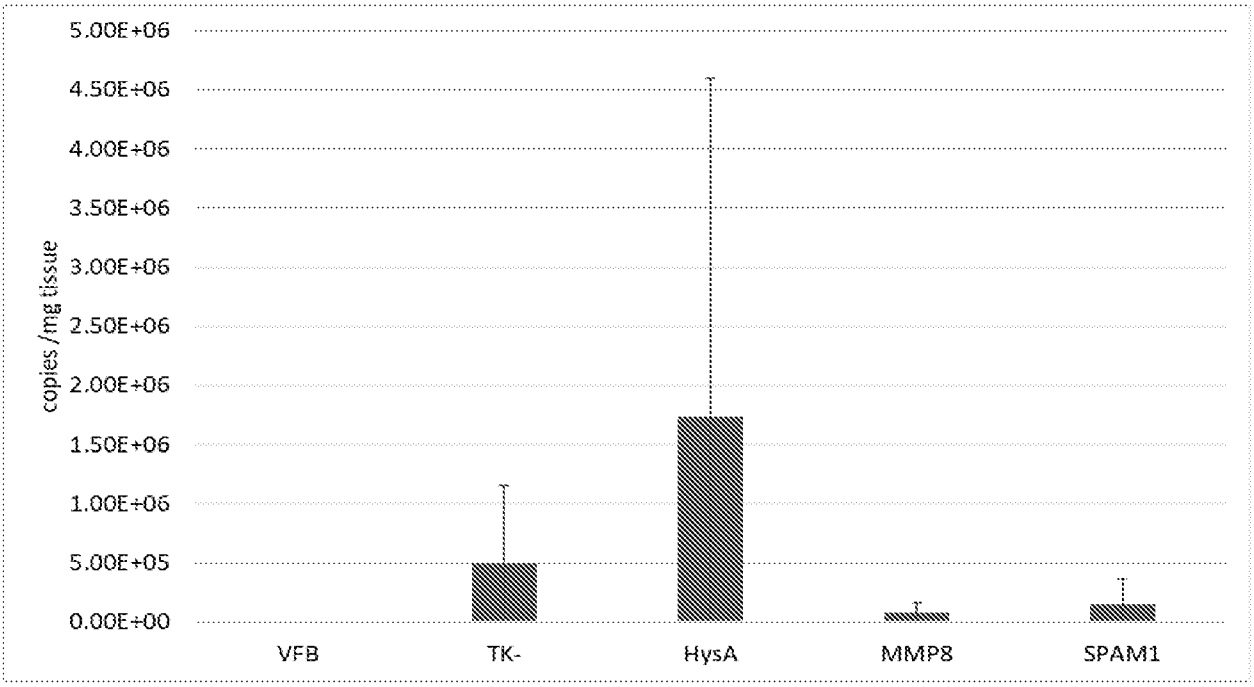

5.00E+06
4.50E+06
4.00E+06
3.50E+06
3.00E+06
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
copies /mg tissue
VFB
TK-
HysA
MMP8
SPAM1

FIG. 21

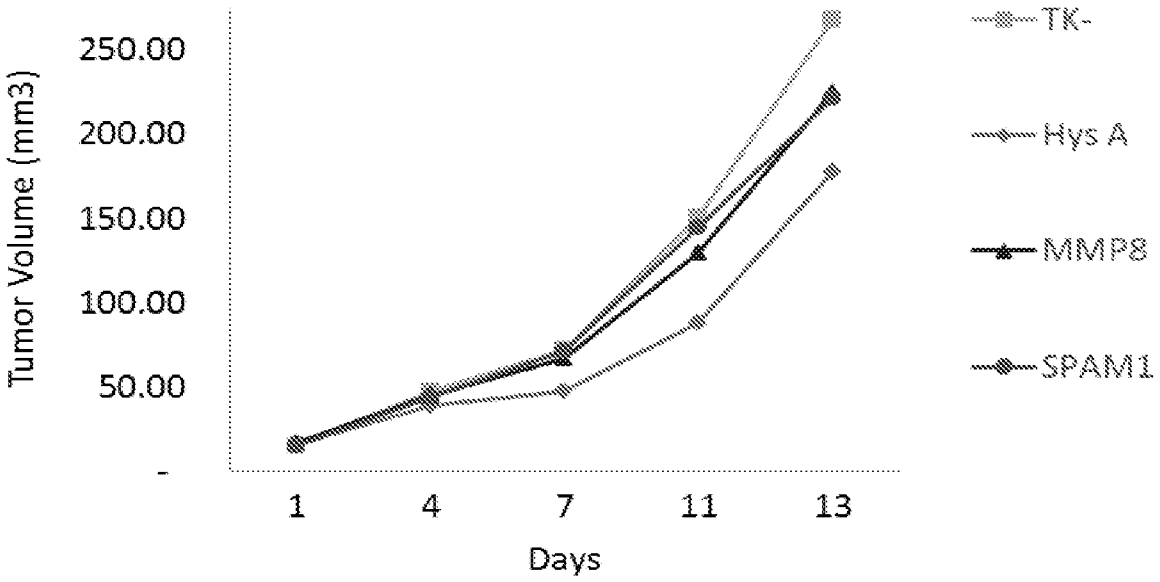

Tumor Volume (mm3)
250.00
200.00
150.00
100.00
50.00
-
1
4
7
11
13
Days
TK-
Hys A
MMP8
SPAM1

FIG. 22A

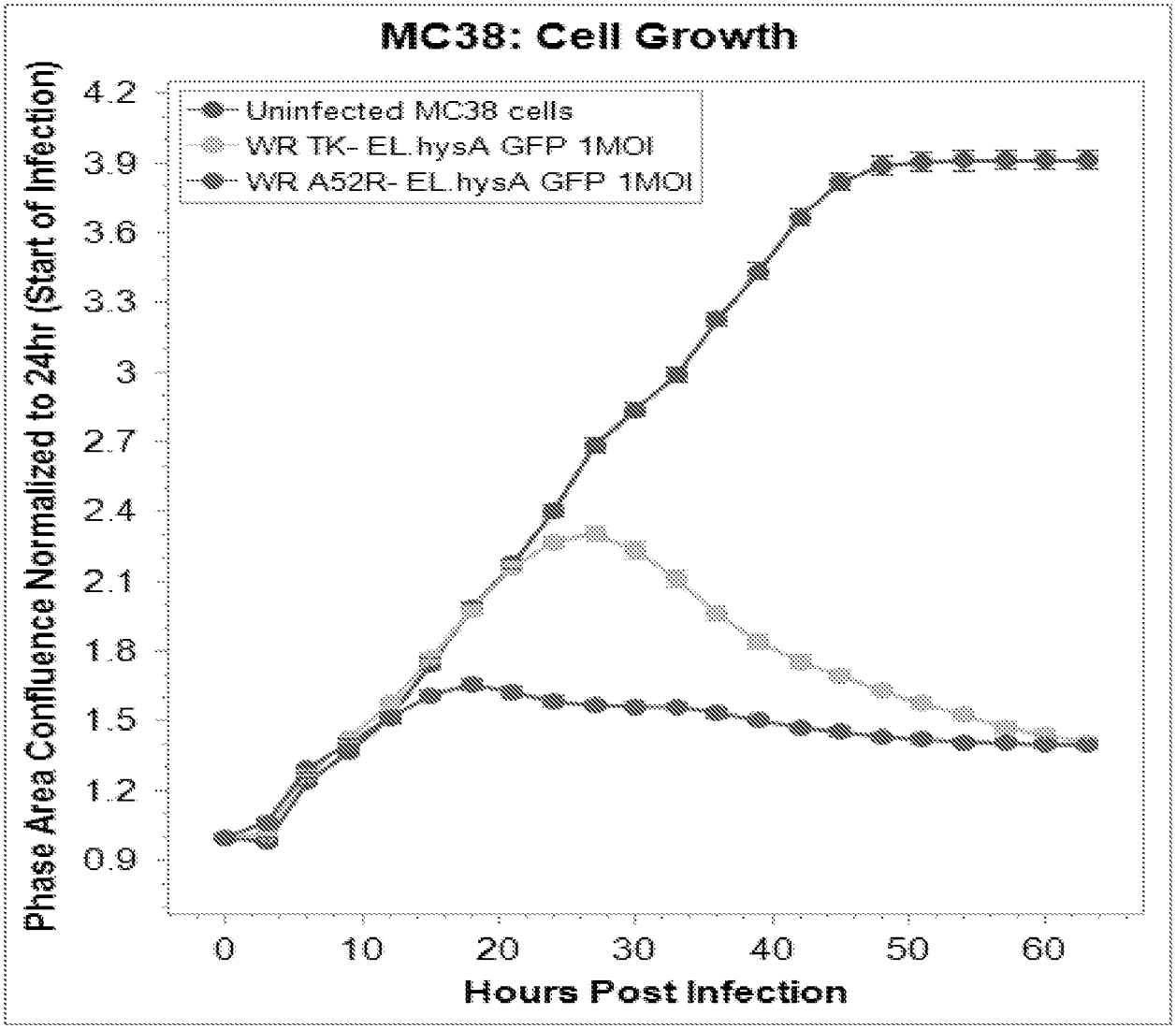

MC38: Cell Growth
Uninfected MC38 cells
WR TK- EL.hysA GFP 1MOI
WR A52R- EL.hysA GFP 1MOI
Phase Area Confluence Normalized to 24hr (Start of Infection)
4.2
3.9
3.6
3.3
3
2.7
2.4
2.1
1.8
1.5
1.2
0.9
0
10
20
30
40
50
60
Hours Post Infection

FIG. 22B

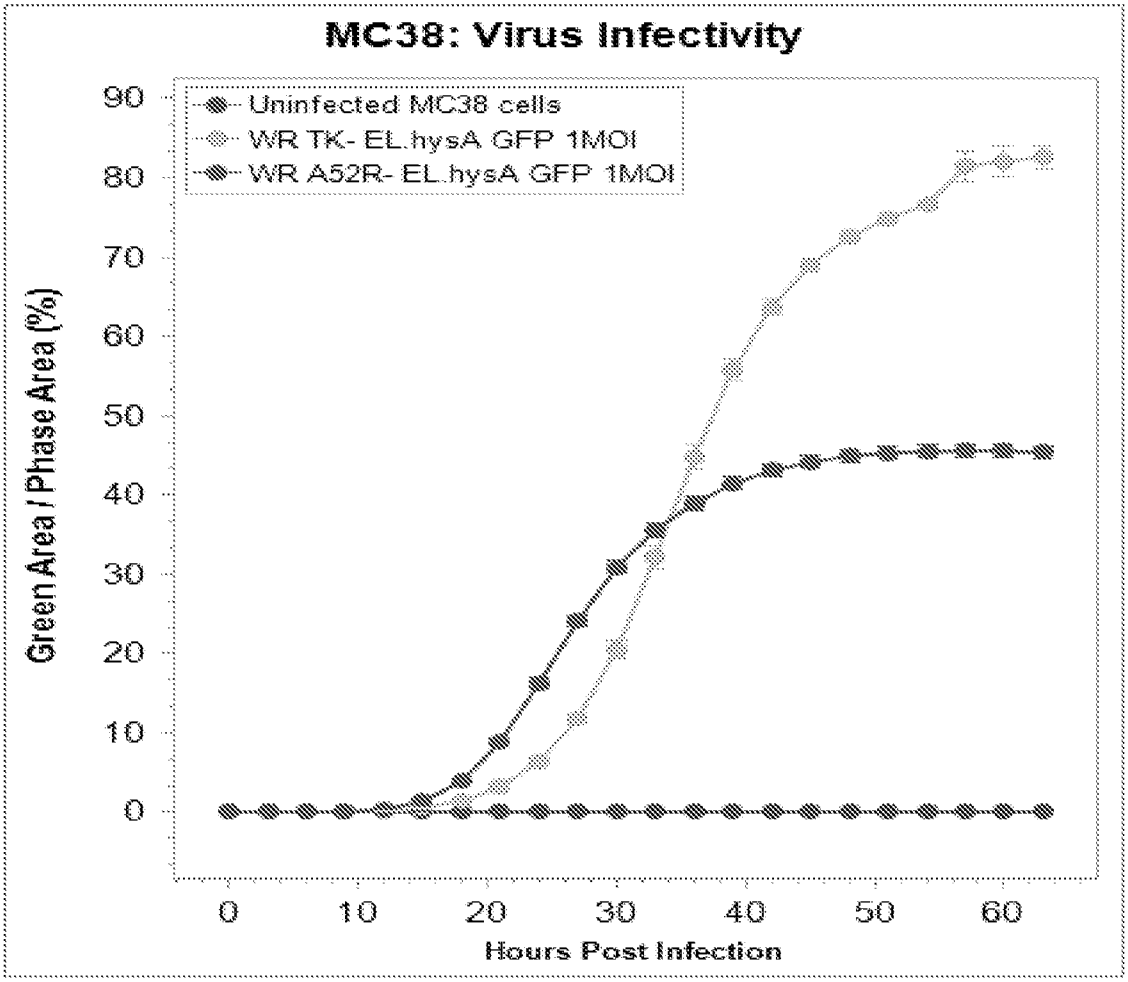

MC38: Virus Infectivity
Uninfected MC38 cells
WR TK- EL.hysA GFP 1MOI
WR A52R- EL.hysA GFP 1MOI
Green Area / Phase Area (%)
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
Hours Post Infection

FIG. 22C

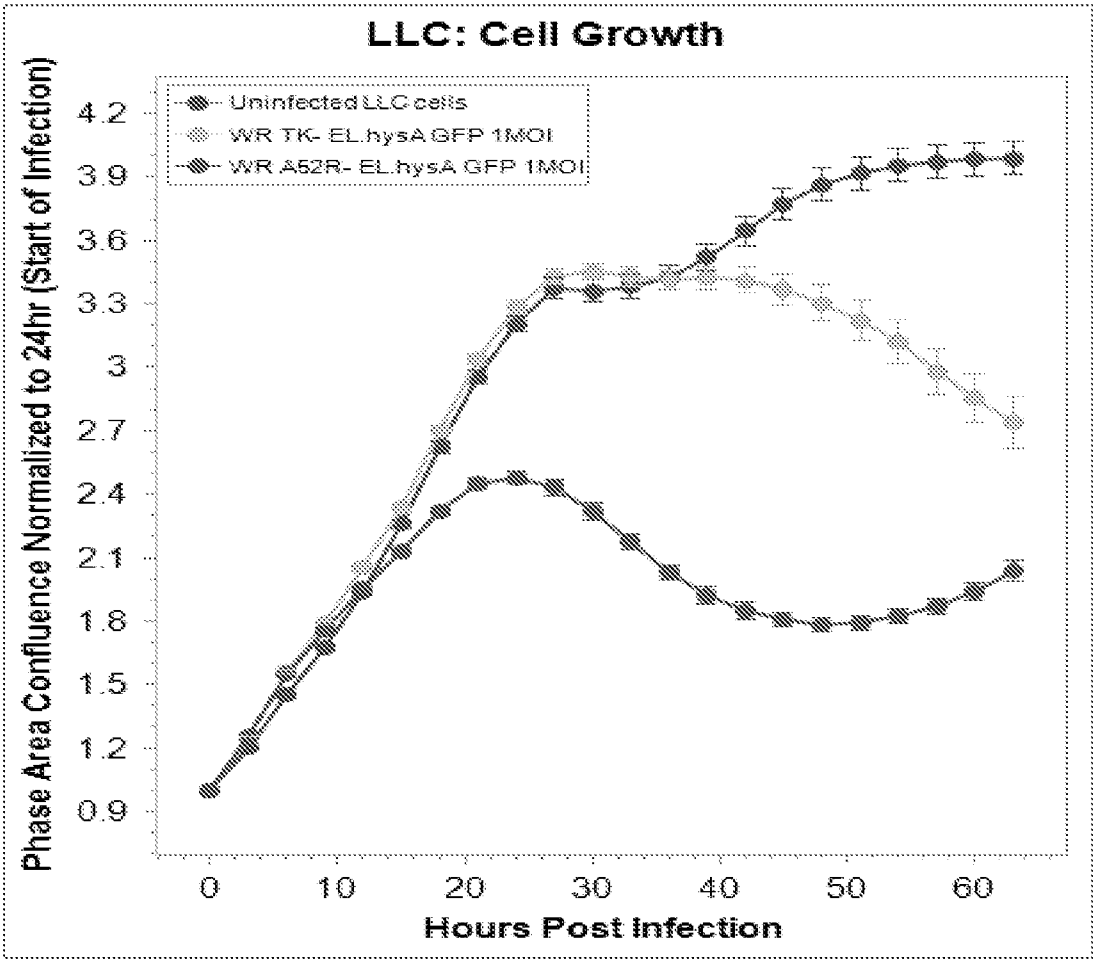

LLC: Cell Growth
Uninfected LLC cells
WR TK- EL.hysA GFP 1MOI
WR A52R- EL.hysA GFP 1MOI
Phase Area Confluence Normalized to 24hr (Start of Infection)
4.2
3.9
3.6
3.3
3
2.7
2.4
2.1
1.8
1.5
1.2
0.9
0
10
20
30
40
50
60
Hours Post Infection

FIG. 22D

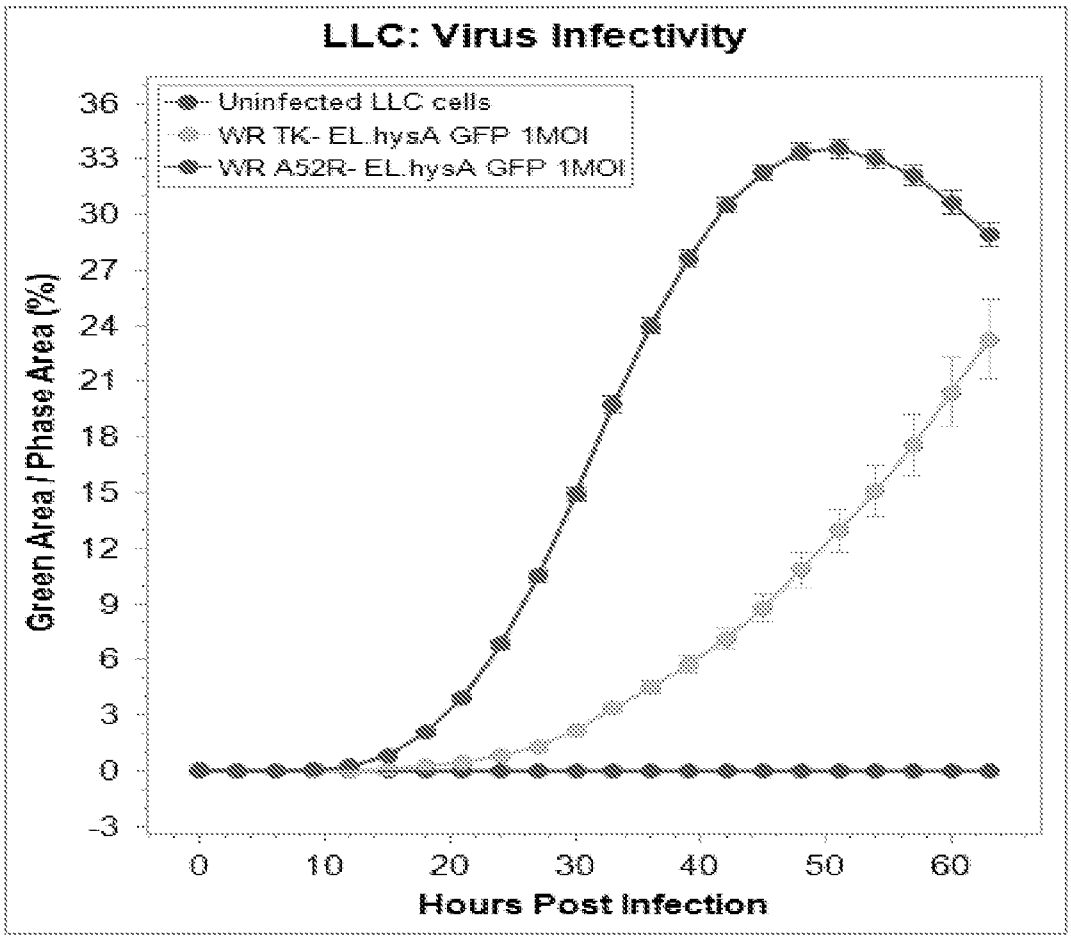

LLC: Virus Infectivity
Uninfected LLC cells
WR TK- EL.hysA GFP 1MOI
WR A52R- EL.hysA GFP 1MOI
Green Area / Phase Area (%)
36
33
30
27
24
21
18
15
12
9
6
3
0
-3
0
10
20
30
40
50
60
Hours Post Infection

FIG. 23

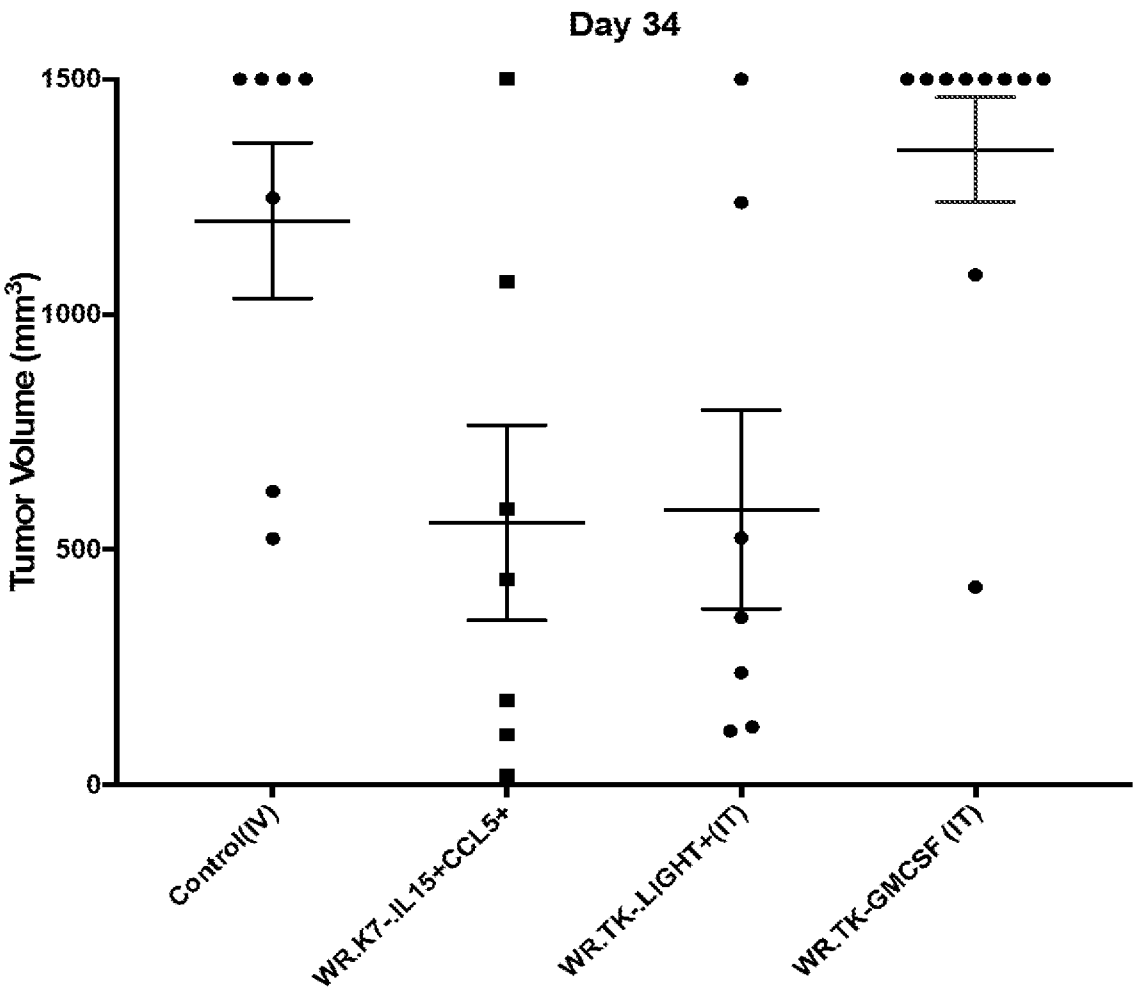

Day 34
Tumor Volume (mm3)
1500
1000
500
0
Control(IV)
WR.K7-.IL15+CCL5+
WR.TK-.LIGHT+(IT)
WR.TK-GMCSF (IT)

FIG. 24

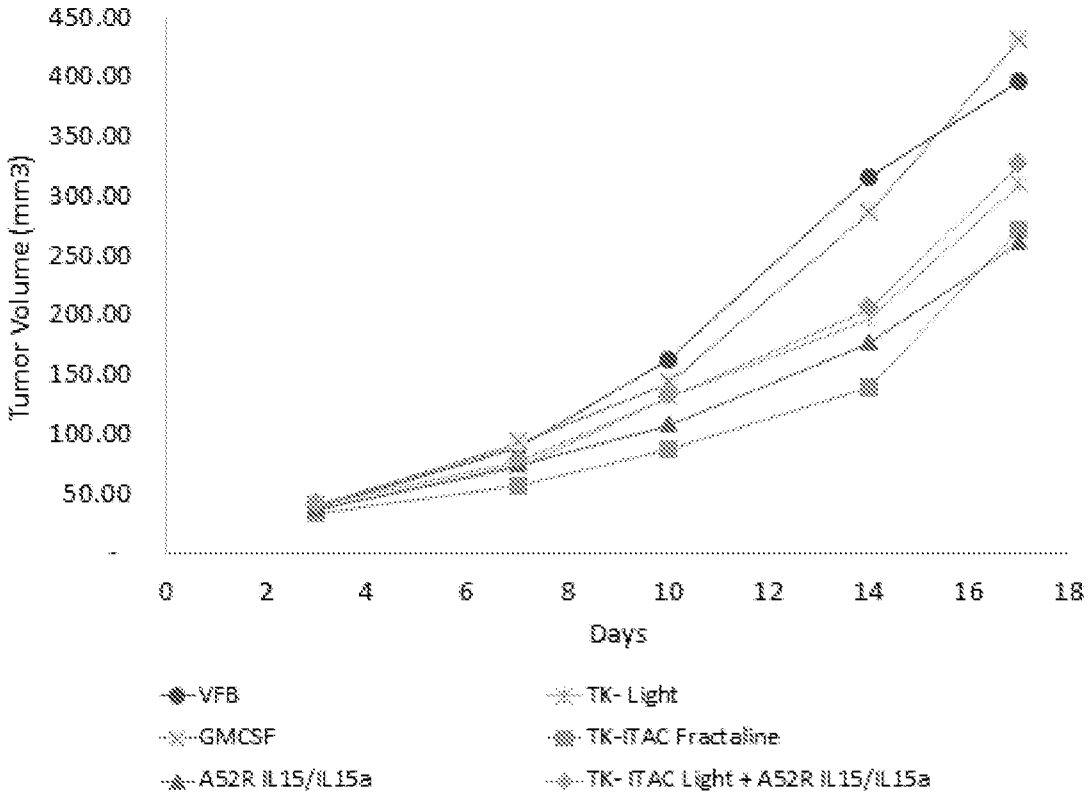

450.00
400.00
350.00
300.00
250.00
200.00
150.00
100.00
50.00
-
Tumor Volume (mm3)
0
2
4
6
8
10
12
14
16
18
Days
VFB
GMCSF
A52R IL15/IL15a
TK- Light
TK-ITAC Fractaline
TK- ITAC Light + A52R IL15/IL15a

FIG. 25A

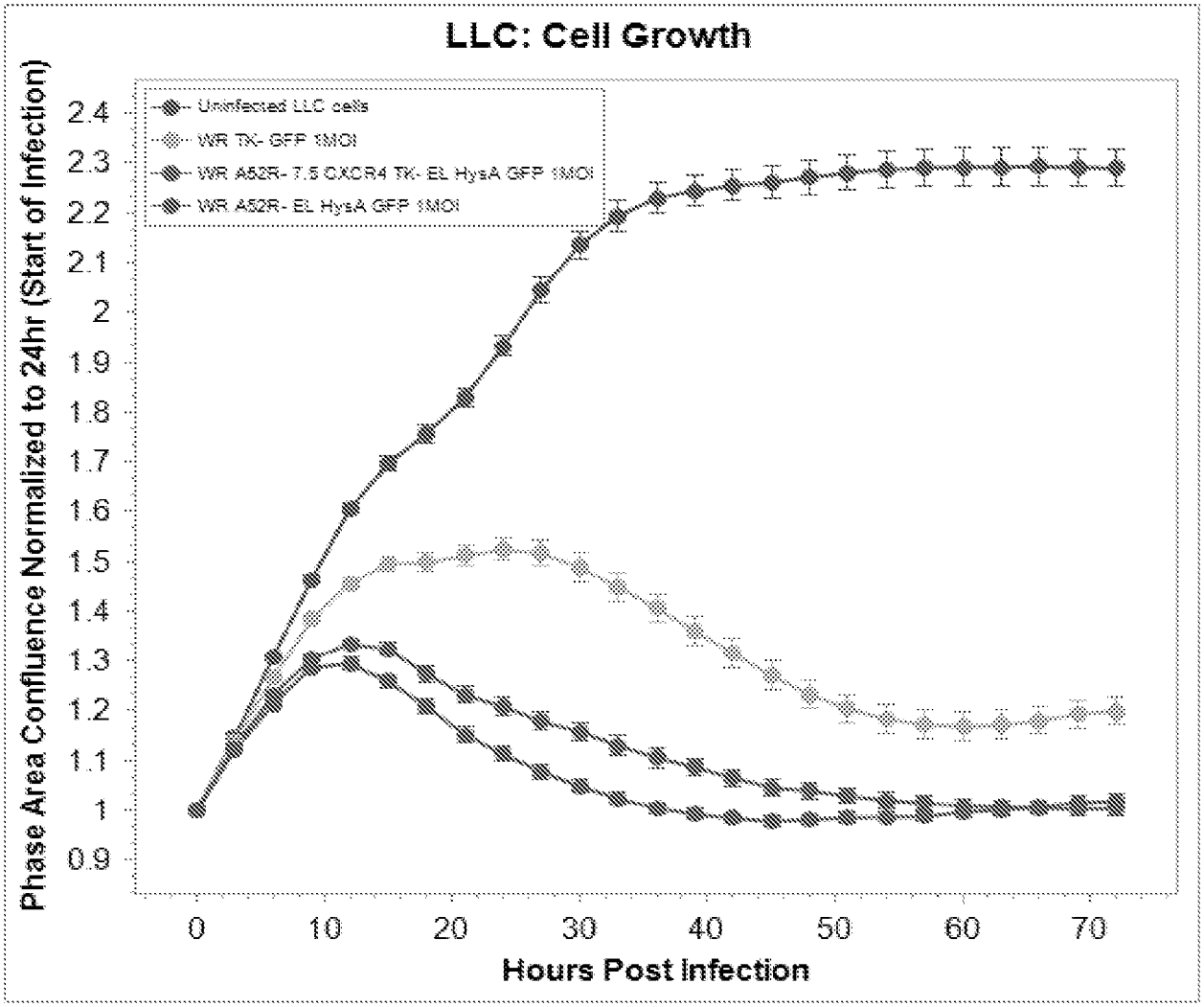

LLC: Cell Growth
Uninfected LLC cells
WR TK- GFP 1MOI
WR A52R- 7.5 CXCR4 TK- EL HysA GFP 1MOI
WR A52R- EL HysA GFP 1MOI
Phase Area Confluence Normalized to 24hr (Start of Infection)
2.4
2.3
2.2
2.1
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0
10
20
30
40
50
60
70
Hours Post Infection

FIG. 25B

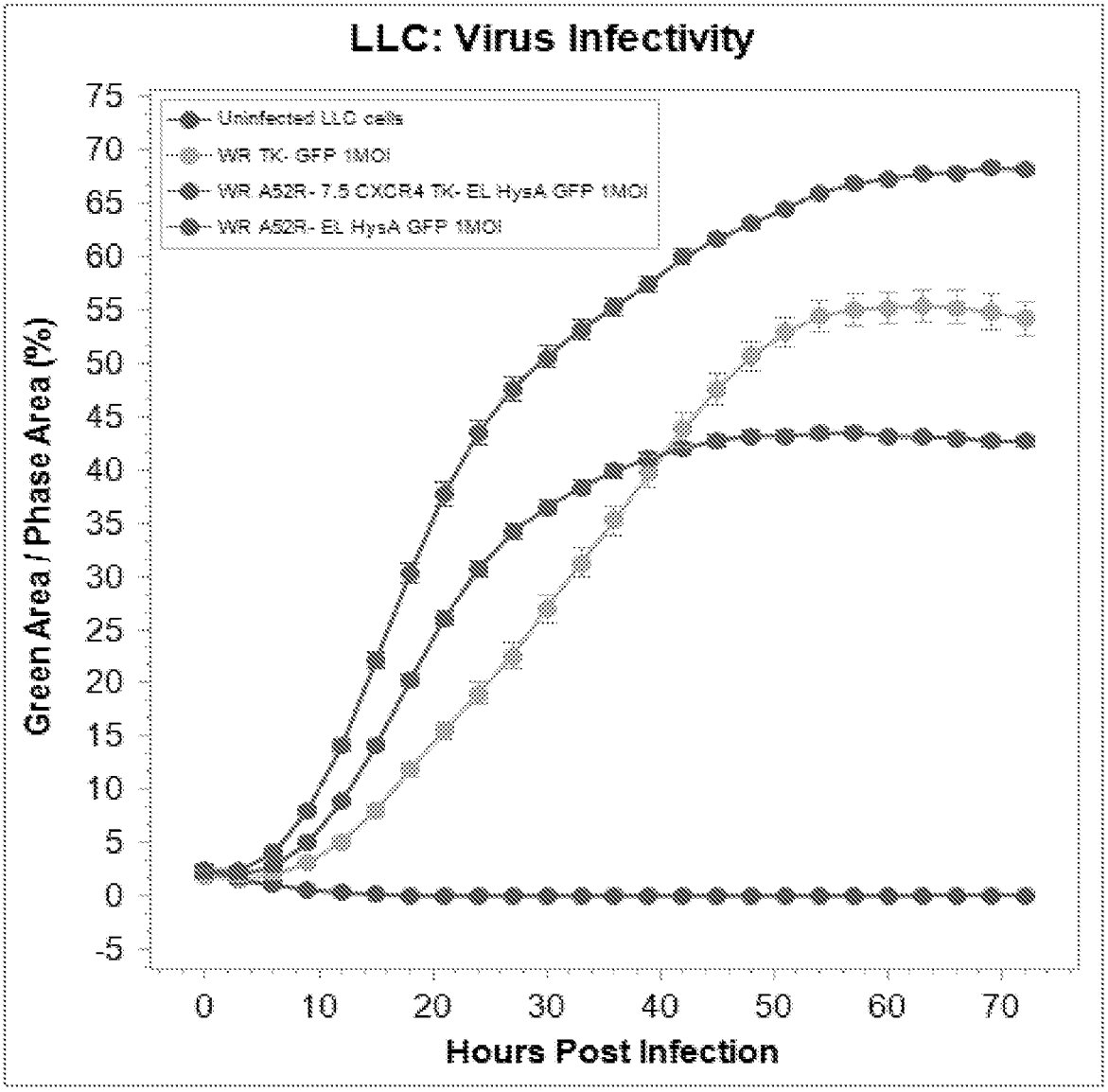

LLC: Virus Infectivity
Uninfected LLC cells
WR TK- GFP 1MOI
WR A52R- 7.5 CXCR4 TK- EL HysA GFP 1MOI
WR A52R- EL HysA GFP 1MOI
Green Area / Phase Area (%)
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
-5
0
10
20
30
40
50
60
70
Hours Post Infection

FIG. 26A

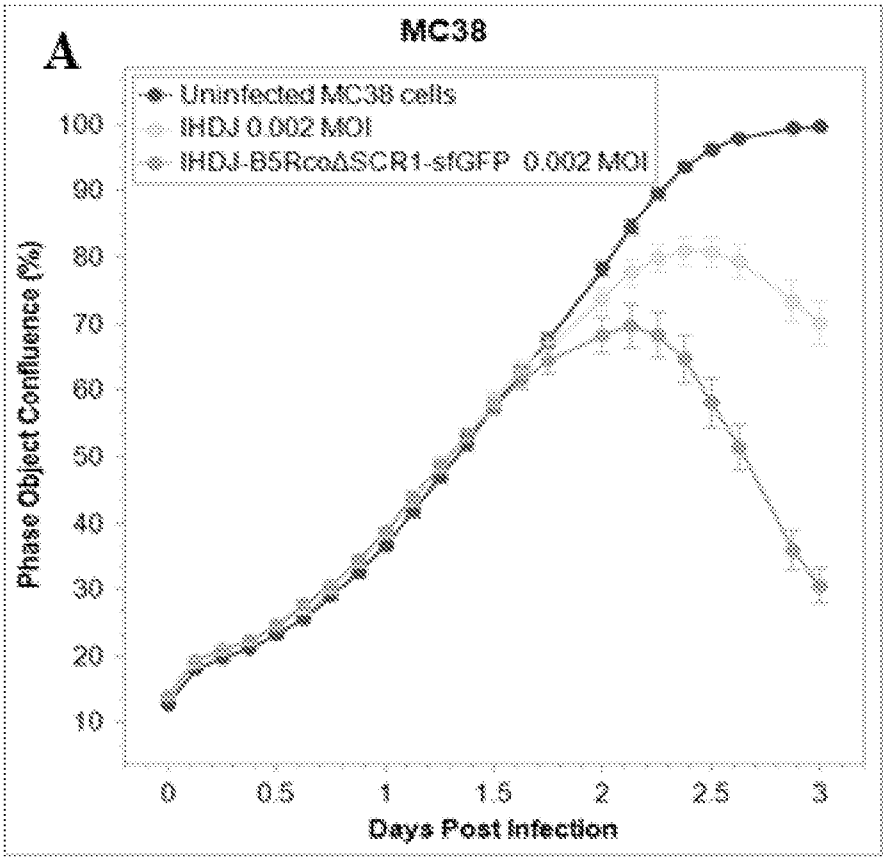
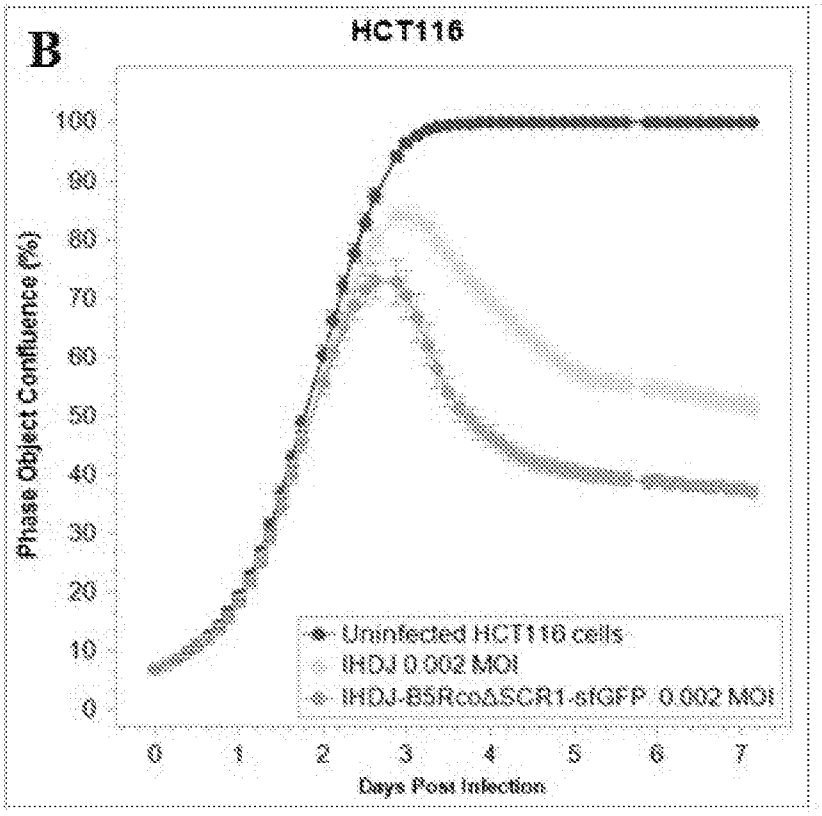
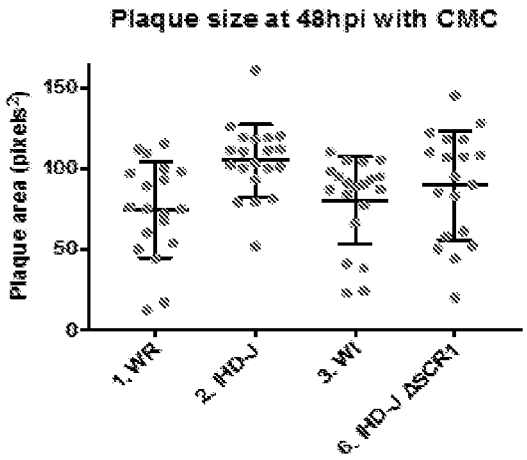

A
MC38
Uninfected MC38 cells
IHDJ 0.002 MOI
IHDJ-B5Rcoa&SCR1-sfGFP 0.002 MOI
Phase Object Confluence (%)
100
90
80
70
60
50
40
30
20
10
0
0.5
1
1.5
2
2.5
3
Days Post Infection

FIG. 26B


B
HCT116
Phase Object Confluence (%)
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
Days Post Infection
Uninfected HCT116 cells
IHDJ 0.002 MOI
IHDJ-B5Rcre&SCR1-sfGFP 0.002 MOI

FIGS. 26C-26D

C



D

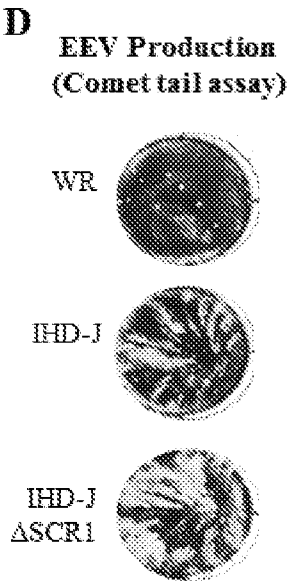
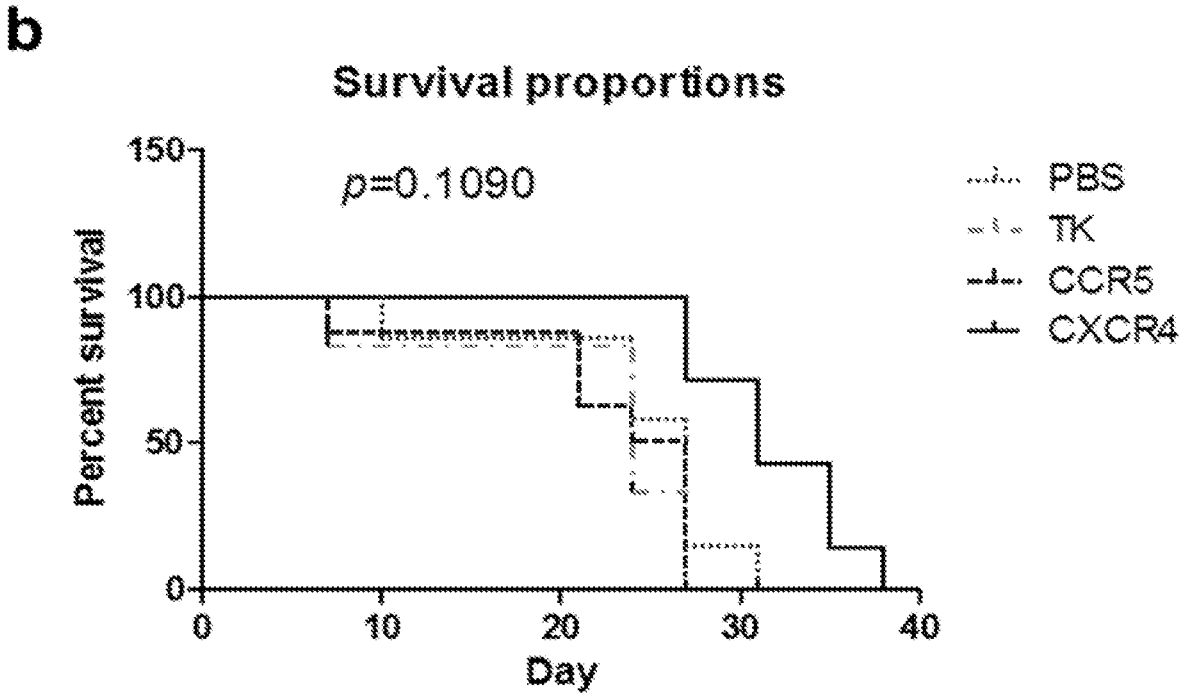


FIG. 5B


b
Survival proportions
p=0.1090
Percent survival
150
100
50
0
0
10
20
30
40
Day
PBS
TK
CCR5
CXCR4